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Contributions of CLASSICAL BIOLOGICAL CONTROL to the U.S. Food Security, Forestry, and Biodiversity

Edited by
Roy G. Van Driesche, Rachel L. Winston, Thomas M. Perring, and Vanessa M. Lopez

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Cover Images: (a) Vineyard surrounded by natural areas; (b) adult *Spathius agrili*, a parasitoid of emerald ash borer, *Agrilus planipennis*; (c) *Diorhabda* spp., a biocontrol agent of *Tamarix* spp.; (d) citrus psyllid (*Diaphorina citri*) nymphs parasitized by *Diaphorencyrtus aligarhensis* (left) and *Tamarixia radiata* (right). (a: Stan the Jeep, Wikipedia.org CC BY-NC-SA 2.5; b: Jian Duan, USDA-ARS; c: James L. Tracy USDA-ARS; d: Mike Lewis, UC Riverside)

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CONTRIBUTIONS OF CLASSICAL BIOLOGICAL CONTROL TO THE U.S. FOOD SECURITY, FORESTRY, AND BIODIVERSITY

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PREFACE

Classical biological control, which reunites invasive insects or plants that have spread in new, invaded areas with their natural enemies, is the only means of reducing permanently and over large areas the harm such invaders cause. Short of eradication, which is rarely practical once an invader has spread widely, this method is the only solution that does not depend on endless control activities and their associated costs.

The purpose of this book is to highlight the value that programs of classical insect and weed control have provided to the United States over roughly the last 40 years (1985–2022). Benefits have accrued to various sectors, which form the basis for the divisions of this book: (1) protecting crops (without expanding the use of pesticides), (2) protecting the landscaping plants used around cities and homes, (3) protecting shade trees, native trees, and forests, and (4) preserving rangeland, grasslands, and natural areas. We wish to acknowledge that there have been several other biocontrol projects that have been successful and beneficial in the United States during the period covered by this book, in addition to all the projects featured in this volume. In particular, chapters planned for the weeds, tropical soda apple and giant salvinia, and for borers and psyllids of eucalyptus trees, were ultimately ommitted from this book due to time constraints for the completion of those chapters.

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Chapter 1

History of Classical Biological Control in the United States

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INTRODUCTION

Each year in the United States, pest insects and plants cause millions of dollars of damage to agriculture, forestry, and infrastructure; they also threaten native biodiversity, alter ecosystem services, and affect human health (DiTomaso, 2000; Pimentel et al., 2005; Pyšek et al., 2012). While some pest species are native to the United States, many of the worst pests are not. Insect pests are frequently introduced inadvertently as stowaways in or among imported material, especially wood or living plants. Plant pests (weeds) can be introduced as contaminants of soil, hay, or seed mixes, but many were, and still are, introduced intentionally for agriculture, horticulture, or the aquarium trade, with their subsequent establishment and invasion of natural systems. Several factors may contribute to an introduced species becoming invasive, including favorable habitat and climatic conditions, less competition for resources, and fewer natural enemies to keep the pest population in check compared to its native range (Williamson, 1996).

In the United States, invasive pests are managed in a variety of ways, including treating insects and weeds with pesticides, physically controlling weeds though cutting, mowing, or roguing, suppressing insects and weeds by manipulating the crop or surrounding vegetation or using livestock for targeted grazing (known as cultural control), or manipulating their natural enemies (known as biological control). One type of biological control is called classical biological control, which is the regulation of an invasive pest's population by importing its specialized natural enemies (e.g., parasitoids, predators, herbivores, or pathogens) from the pest's native range for this purpose. The hope and expectation of classical biocontrol is for introduced natural enemies to establish in their new environment and increase in abundance to levels that inflict sufficient damage to target pests to reduce the target's impact and abundance until they are a less important pest or a non-pest. For example, in the last 20–30 years, birch trees in Alaska began being attacked by several invasive leaf-mining sawflies. These sawflies reduce the leaf area in the tree canopy, causing tree growth rates to drop. One such sawfly illustrates the process of classical insect control. The amber-marked

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birch leafminer (*Profenusa thomsoni*) was first noticed in Southeast Alaska in the late 1990s. By 2006, 70% of the leaves on Alaskan white birch (*Betula neoalaskana*, the dominant forest tree around Anchorage) were mined by *P. thomsoni*. This caused tree growth to decline by ca 25% (Van Driesche et al., unpub data). In 2004, the parasitoid *Lathrolestes thomsoni* was introduced to Alaska from Alberta, although it is likely native to Europe. Within 15 years, the parasitoid had increased leafminer mortality from approximately 30 to 70%, and the percentage of leaves mined dropped from 70% in 2006 to 9% in 2019 (Andersen et al., 2021). At this latter low level, the leafminer has only a small effect on tree growth. Most importantly, this change is permanent and does not require any ongoing control efforts to maintain leafminer suppression. Classical biological control has ecologically reconstructed the food web of this pest in Alaska with the addition of a new parasitoid that strongly attacks the pest.

Control of invasive insects and weeds through classical biological control can benefit crop production (Nechols et al., 1995), rangeland forage (Sheley et al., 2011), forests (Van Driesche and Reardon, 2016), native biodiversity (Van Driesche et al., 2010; Van Driesche and Reardon, 2017), infrastructure (Coetzee et al., 2009), and human health (Van den Bosch, 1976). Classical biological control moves through well-established steps to identify and introduce natural enemies likely to be both effective against their target pests and present little risk to native and/or desirable species (Harley and Forno, 1992; Van Driesche and Reardon, 2004; Van Driesche et al., 2008, see pp. 127–133). In the remainder of this chapter, we discuss the history of classical biological control in the United States, how biocontrol programs are currently executed, and some of the benefits obtained from classical biocontrol in the United States. Throughout the rest of this book, we use case studies to document the beneficial contributions of classical biocontrol to the economy and environment of the United States.

HISTORY OF CLASSICAL BIOLOGICAL CONTROL IN THE UNITED STATES

The five most active countries carrying out classical biological control programs (for both insects and weeds) have long been the United States, Australia, South Africa, Canada, and New Zealand. Classical biocontrol projects affect not only the country initiating the work, but they often lead to adoption in other regions as well (Day et al., 2020). Consequently, documenting the history and outcomes of classical biocontrol programs is important for the successful and safe implementation of this discipline worldwide. Australia, Canada, New Zealand, and South Africa have periodically produced summaries of the classical biocontrol programs undertaken in their countries (e.g., Anon., 1971; Kelleher and Hulme, 1984; Cameron et al., 1989; Waterhouse and Sands, 2001; Mason and Huber, 2002; Julien et al., 2012; Mason and Gillespie, 2013; Ferguson et al., 2021; Zachariades, 2021). The United States lags in this effort, with most of its program reviews either being outdated (Clausen, 1978) or only regional in coverage (e.g., Funasaki et al., 1988; Nafus and Schreiner, 1989; Frank and McCoy, 1993, 2007; Barbosa et al., 1994; Nechols et al., 1995). The content presented in the remainder of this book partially fills this gap for the United States by highlighting the successful use of classical insect and weed biological control in selected projects since 1985.

Insect Biological Control

Classical biocontrol of insects in the United States stretches from 1888 with the successful suppression of cottony cushion scale (*Icerya purchasi*) that saved the fledgling citrus industry of the new state of California (Caltagirone and Doutt, 1989), to the current efforts against emerald ash borer (*Agrilus planipennis*) to restore ash stands across two thirds of the country to a better condition, protecting forest productivity and biodiversity (Duan et al., 2014; Bauer et al., 2015; Duan et al., 2021).

For the United States as a whole, the number of projects of classical insect biocontrol declined sharply from 1985 through 2018 (Van Driesche et al., 2018, 2020a) (**Fig. 1**). Despite this decline, classical biocontrol of insects has made a significant contribution in combating a series of invasive species and reducing the damage they caused to crops, forests, and/or biodiversity. The purpose of this book is to illustrate that value and suggest that this decline in use of classical biological control runs counter to the continued invasions of many high-profile insect pests that have occurred in this same period, such as emerald ash borer (*A. planipennis*), spotted wing drosophila (*Drosophila suzukii*), brown marmorated stink bug (*Halyomorpha halys*), and spotted lantern fly (*Lycorma delicatula*), among others.

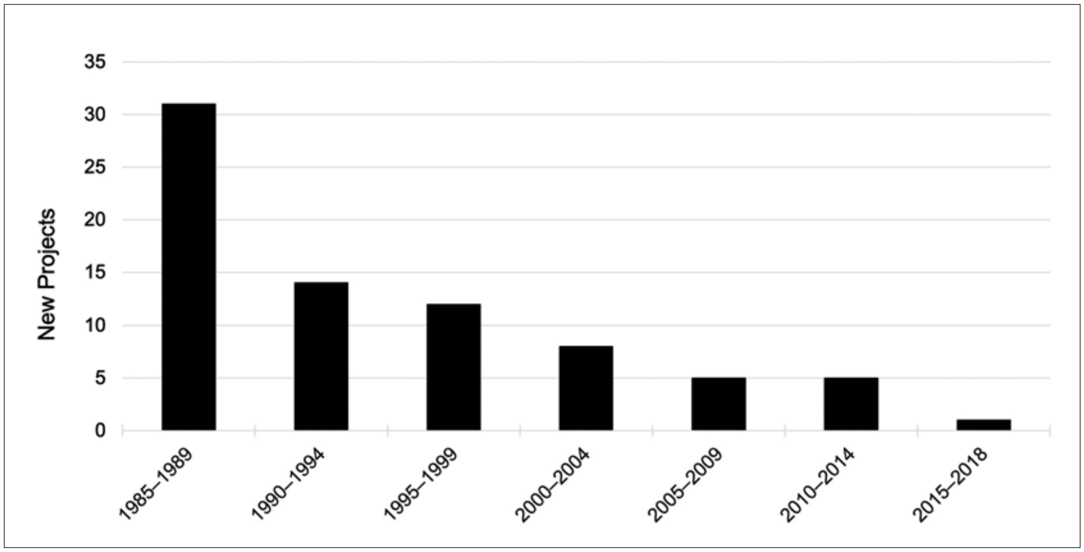

Figure 1. Number of projects of classical biological control of invasive insects from 1985 to 2018, in 5-year periods (apart from final interval), showing an 83.9% decline through 2014. (modified from Van Driesche et al., 2020a)

Weed Biological Control

The first weed targeted for biological control in the United States was *Lantana camara* in Hawaii. Beginning in 1902, 14 natural enemies were released throughout the islands to reduce the damage this species caused in pastures and native forests (Conant et al., 2013). Most of these natural enemies either failed to establish or had only slight impacts on *L. camara*, and the weed continues to be a problem at many sites (Winston et al., 2021). In the continental United States, the first weed biocontrol releases were made in 1945 when *Chrysolina* beetles were released against common St. Johnswort, *Hypericum perforatum*, in six western states (Holloway and Huffaker, 1952). In California, these beetles were highly effective at reducing *H. perforatum* infestations, and this program became one of the most famous examples of successful classical weed biocontrol (**Fig. 2**). Grateful landowners even erected a monument in honor of the beetles that saved their rangelands, and the weed is still considered under complete control in California 75 years later (Pitcairn, 2018).

Figure 2. *Hypericum perforatum* invasion in California (a) before *Chrysolina* spp. introductions; (b) after *Chrysolina* spp. introductions; (c) monument in honor of *Chrysolina* spp. (a–c: USDA-ARS European Biological Control Laboratory, Bugwood.org CC BY 3.0 US)

Through the year 2020, 211 species of natural enemies had been released against 83 weed species in the United States (including the continental U.S., Hawaii, and overseas U.S. territories). The number of new weed biocontrol agent species introduced to the United States and its territories peaked from 1985 to 1999 (**Fig. 3**). Similar to the pattern in insect classical biological control, the number of new species introduced as weed biocontrol agents has decreased steadily since 1994 (Winston et al., 2021).

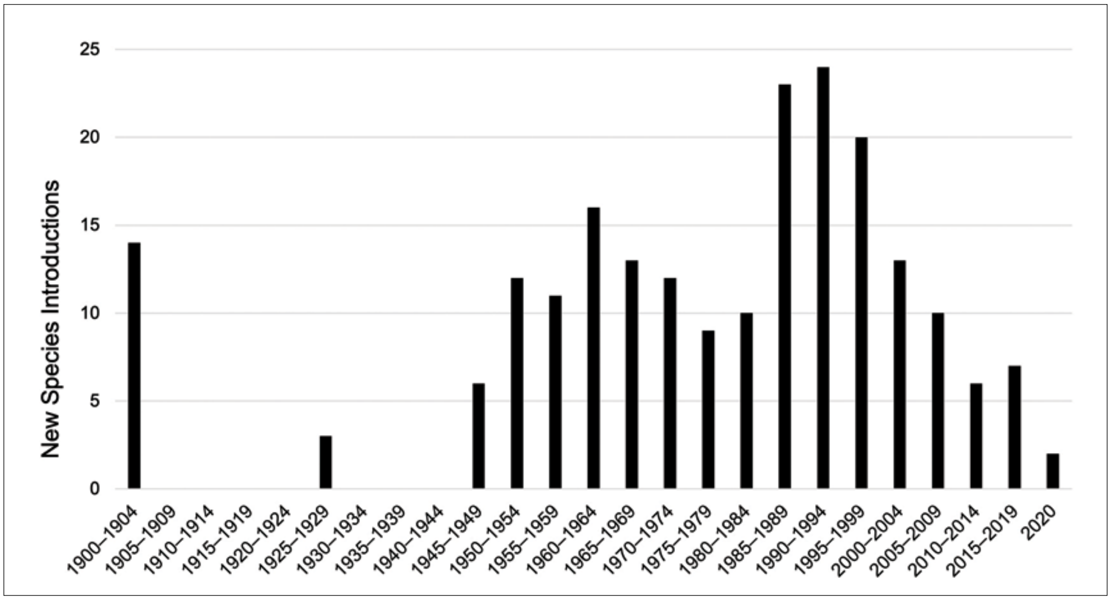

Figure 3. Numbers of new species of weed biocontrol agents introduced against weeds in the United States and its overseas territories from 1902–2020, in 5-year periods (apart from final interval). (Winston et al., 2021)

WHO RUNS CLASSICAL BIOLOGICAL CONTROL PROGRAMS?

A Description of the Process

Classical biological control programs begin with exploration for specialized natural enemies affecting an invasive pest in its area of origin. This process includes physical surveys for natural enemies attacking the target pest in its native range, as well as any relevant published literature. In both activities, emphasis is placed on natural enemies that feed on or develop on only the target pest or a few related species; such agents are said to show high host specificity and have narrow host ranges. Potential biocontrol agents identified during foreign exploration are then subjected to testing to show they are relatively host specific (Van Driesche and Reardon, 2004; USDA-APHIS, 2019) and potentially effective. The results of this testing are reviewed at several stages during the agent approval process, which are described in greater detail below. After a biocontrol agent is approved for introduction into the United States, it is released in the field. In ideal situations, biocontrol agent release sites are monitored before, during, and for several years after releases to gather data demonstrating the impacts of the release.

Introducing new classical biocontrol agents to the United States is a lengthy (3–5 years for parasitoids and predators, and 5–10 years for weed biocontrol agents) and expensive process, and many groups are often involved in the various stages of this process. Funding for many programs is obtained from federal or state government agencies because the pests targeted affect several sectors, and the development of safe biocontrol agents is for the general good of society. In some programs, however, funding has been provided entirely by private groups (e.g., ranchers or grower associations looking for solutions to their worst pest problems). Although some foreign exploration and host-specificity testing are conducted by scientists working for federal or state government agencies, there are also several private science firms and individual scientists worldwide who contribute to this part of the process. After an agent is approved for release in the United States, it is most often released in the field by the individual or agency who successfully petitioned for its approval. If the agent successfully establishes and/or large populations are easily propagated in the field or laboratory, surplus individuals are then shared with other federal, state, or private groups or individuals to increase the release and distribution of the new natural enemy over a wider area. While there are some private companies that charge for the collection or propagation of biocontrol agents, most federal, state, and country programs make every effort to distribute these natural enemies free of charge. Monitoring of biocontrol agent releases is initially conducted by the individual or agency who made the first release, but as the natural enemy becomes more widely established, other agencies and citizen scientists are frequently included in monitoring efforts to track establishment, spread, and impact on the target pest.

Regulations for Approving Classical Biocontrol Agents

Federal laws and regulations are in place to identify and avoid potential risks to native or desirable plants or animals that could result from organisms introduced to manage insect pests and weeds (USDA-APHIS, 2019; Van Driesche et al., 2020b). The U.S. Department of Agriculture's Animal and Plant Health Inspection Service–Plant Protection and Quarantine (USDA-APHIS-PPQ) is the federal agency responsible for authorizing the importation of biocontrol agents into the United States (under 7 CFR 330) as well as providing host specificity testing guidelines (USDA-APHIS, 2019). The regulation of classical biocontrol agents in the United States proceeds in two steps. The first step is the issuance of an importation permit to move the discovered natural enemies to a certified quarantine facility where their host specificity and efficacy can be studied. In phase two, following the completion of quarantine host specificity and efficacy studies, the concerned person or agency submits a written petition to USDA-APHIS-PPQ to request permission or approval of environmental releases of the candidate agent.

Upon receipt of a release petition, USDA-APHIS-PPQ proceeds down two different pathways, dependent upon whether the biocontrol agent being petitioned is for an insect or weed pest. For insect classical biocontrol agents, USDA-APHIS-PPQ will first seek reviews and comments from the North American Plant Protection Organization (NAPPO), which is a phytosanitary standard-setting organization whose mission is to provide a forum for public and private sectors in Canada, the United States, and Mexico to collaborate in the development of science-based standards intended to protect agricultural, forest and other plant resources against regulated plant pests, while facilitating trade. Based on comments and reviews from NAPPO, USDA-APHIS-PPQ will conduct an Environment Impact Assessment (EA), in which the characteristics of the potential agent, its host range, the need for the introduction, and any likely side effects are discussed. If the conclusions of the EA are favorable, USDA-APHIS-PPQ then submits the release petition for publication in the Federal Register for public comments. The agency then responds to public comments and reaches its decision, which, if positive, is called a FONSI (Finding of No Significant Impact), and a release permit is issued.

For weed classical biocontrol agents, USDA-APHIS-PPQ first seeks reviews and comments from the Technical Advisory Group (TAG) for Biological Control Agents of Weeds, which is an expert committee with representatives from U.S. federal regulatory, tribal, resource management, and environmental protection agencies, as well as regulatory counterparts from Canada and Mexico. Following positive recommendations from the TAG, USDA-APHIS-PPQ then prepares a Biological Assessment that is shared with the U.S. Fish and Wildlife Service. If the latter agency agrees that there are sufficient data to demonstrate the candidate weed biocontrol agent is safe to threatened and endangered species, and if they concur with the proposed release, they provide a Letter of Concurrence to that effect. USDA-APHIS-PPQ then prepares an EA (similar to the process for insect biocontrol agents), but they then share the EA with Native American tribes in areas within the range of the target plant as a Tribal Consultation. Following this step, the process is identical to that of insect biocontrol agents in that USDA-APHIS-PPQ publishes the petition in the Federal Register for public comments, prepares a FONSI in the event of a positive decision, and issues a release permit. In addition, some states have their own approval process to permit field release of weed biocontrol agents.

Regulations for the introduction of new species for classical biocontrol have increased over time and are now the most stringent and comprehensive for any species introduced to the U.S. for any purpose, including species introduced for use commercially for horticulture, as pets, or in aquaculture (Van Driesche et al., 2020b).

HOW SUCCESSFUL HAS CLASSICAL BIOLOGICAL CONTROL BEEN?

Complete control of an invasive pest occurs when the pest population is reduced below an ecologically or economic threshold and is maintained at that level with no additional controls (Coetzee et al., 2009). However, even partial control of a pest population may be considered a success if the pest's negative impacts on society or nature are reduced, along with a reduced frequency or need for other control methods (Schwarzlaender et al., 2018). Worldwide up to about 2007, Van Driesche et al. (2008) determined from multiple sources that about 60% of all insect biocontrol projects had a positive effect, reducing the pest either partially or completely. Control was complete in 17% of the projects targeting insects, and partial control was achieved in 43% of the projects. More recently for the United States alone, Van Driesche et al. (2020a) found that for 75 projects against invasive insects carried out from 1985 to about 2015, 50% had positive effects (partial or complete control), fluctuating between 40 and 60% per 5-year period (**Fig. 4**).

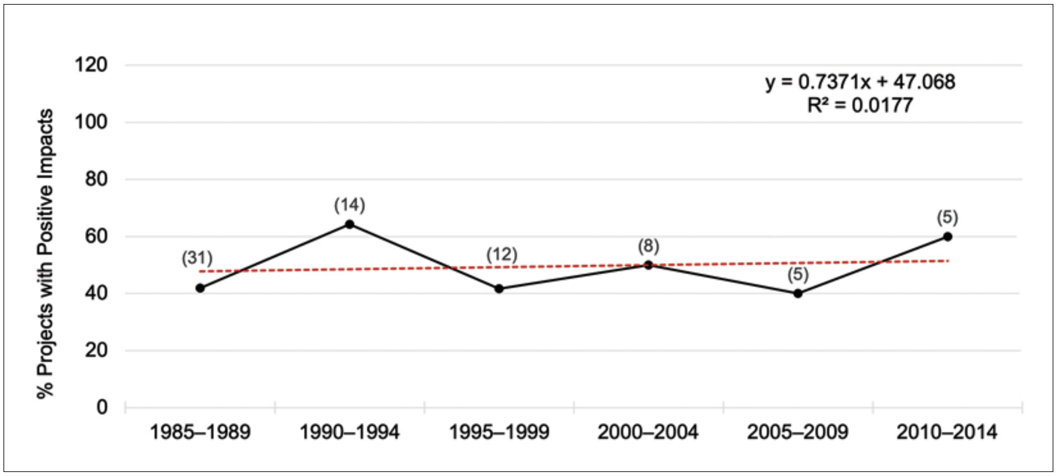

Figure 4. Trend in the percentage of U.S. insect classical biocontrol projects that, summed over each 5 year period, had a positive impact on their target pest in the United States and U.S. overseas areas. (modified from Van Driesche et al., 2020a)

For weed biocontrol, the most recent worldwide analysis of biocontrol programs (Schwarzlaender et al., 2018) further separated partial control into 'slight' and 'medium'. Of all weed species for which impact could be categorized, 65.7% of release programs worldwide resulted in medium to complete control. For weed biocontrol in the United States (and U.S. overseas territories), the percent of new biocontrol agent species introductions (not projects, but single agents) resulting in medium to complete control of their target weed averaged 44.6% per 5-year period since 1902 (**Fig. 5**; Winston et al., 2021).

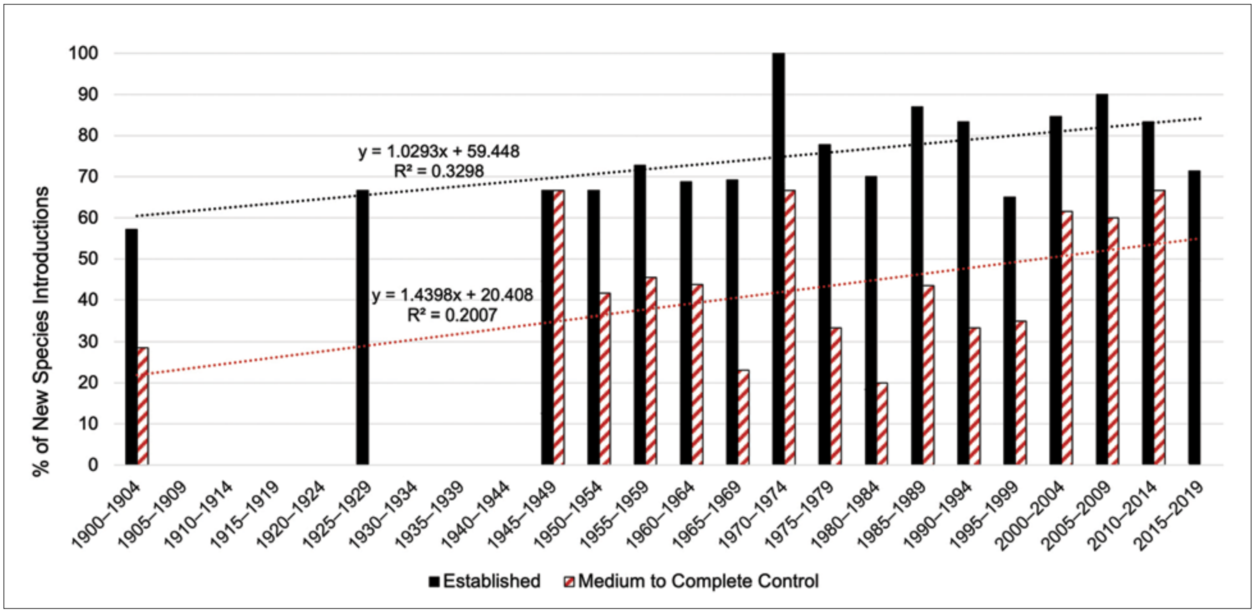

Figure 5. Trend in the percentage of U.S. weed biocontrol agent species introductions resulting in medium to complete control of their target weed per 5-year period (including overseas areas). (Winston et al., 2021)

For both insects and weeds, many advancements have been made in the discipline of classical biocontrol in recent decades. These include an increased awareness of the potential of negative direct and indirect effects of natural enemy releases, tightened introduction regulations, increased emphasis on demonstrating natural enemy impacts before release, advances in pre-release host-specificity testing methodologies, and development of molecular tools that clarify evolutionary relationships of target pests. Some of these changes have led to fewer releases of new natural enemy species in recent years (**Fig. 3**). Comparing **Figures 3** and **5** demonstrates an important trend that while the number of weed biocontrol agent species has decreased since 1999, the percent of species establishing and causing medium to complete control of their target weeds has increased since then.

ECONOMIC AND ECOLOGICAL BENEFITS

Economic Benefits

Measurement of the economic benefits of classical biocontrol is most common for pests affecting agriculture or horticulture because benefits are readily expressed in terms of dollars saved (e.g., increases in productivity or yield, lower control costs, lower expenses for other work such as removal of dead landscape trees, etc.). The ratio of project benefits to project costs is the commonly used measure of the economic value of classical biocontrol projects against agricultural pests. If pests are strongly suppressed, benefits continue to grow indefinitely as more years of reduced pest pressure accumulate. Estimates for benefit/cost ratios have ranged from 1.4:1 for white wax scale (*Ceroplastes destructor*) in Australia (Tisdell, 1990) to 4331:1 for the golden wattle (*Acacia pycnantha*) project in South Africa (van Wilgen et al., 2004). Some examples specifically for the United States include the project against mole crickets (Gryllotalpidae spp.) in Florida, which had a benefit: cost ratio of 52:1 (Mhina et al., 2016), and the project against the ash whitefly (*Siphoninus phillyreae*) in California, which had a ratio of 270:1 (Jetter et al., 1997). In Oregon, biological control of the weed tansy ragwort (*Jacobaea vulgaris*) produced a benefit: cost ratio of 13:1 (Coombs et al., 1996), though the overall savings across the United States and Canada is likely an order of magnitude higher considering the number of states and provinces initially heavily infested with the weed. The biological control of the weed leafy spurge (*Euphorbia virgata*) across the United States and Canada has delivered a benefit: cost ration of between 8.6:1 and 56:1 (Cock et al., 2015).

More recent studies demonstrate that worldwide, the economic benefits of classical biocontrol projects are very large relative to costs and that these returns grow substantially over time as the benefits of avoided negative impacts or costs accumulate (van Wilgen et al., 2020). This allows agencies engaged in classical biological control to have strong, positive net effects on society even though only roughly 60% of classical biocontrol programs have succeeded in partially or completely controlling their target pest. For comparison, many municipal infrastructure projects are funded if they have benefits that only slightly exceed costs (in the range of 1.1:1 to 2:1), yet these activities are seen as good public investments.

Ecological Benefits

Ecological benefits of classical biocontrol of insects (see Van Driesche et al., 2010 for review) and weeds (e.g., de Lange and van Wilgen, 2010; van Wilgen et al., 2020) are very real and significant but are harder to quantify, and less attention has been paid to this matter than for agricultural pests. In some cases, the benefits can be measured in terms of dollars, as for example the impacts on South Africa of control of woody acacias and other tree species (van Wilgen et al., 2004; Moran and Hoffmann, 2012), but this is often not the case. Impacts are often studied in terms of restoration of affected native species or degraded habitats or an increase in biodiversity (de Lange and van Wilgen, 2010; Coetzee et al., 2020). In some cases, benefits can be measured as conservation of valuable ecosystem services (see pp. S21–23, Van Driesche et al., 2010) or resources such as water (van Wilgen et al., 2004; Arp et al., 2017). However, it is not always possible to assign monetary or ecological units of value to the environmental benefits achieved through classical biocontrol. For example, control of erythrina gall wasp in Hawaii has likely saved the endemic wiliwili tree, *Erythrina sandwicensis*, from extinction, thus preserving the dominant tree of native Hawaii dry forests (Kaufman et al., 2020), but there is no obvious way to measure those benefits in terms of dollars. Similarly, biocontrol control of the invasive swamp tree *Melaleuca quinquenervia* has been an important part of the efforts to restore the Everglades National Park and other areas of south Florida, preserving the open glades that define many of the area's most characteristic habitats (Center et al., 2012). In the Galapagos National Park, a world conservation interest of the highest order, the successful biological control of the polyphagous invasive scale *Icerya purchasi* protected many native plants, including both rare endemics and foundational species that create key habitats such as mangrove swamps (Calderón Alvarez et al., 2012; Hoddle et al., 2013). As our understanding and ability to quantify the ecological harm of invasive pests increase (Vilà and Hulme, 2017), so too will our ability to quantify the ecological benefits of classical biocontrol in natural environments.

CONCLUSIONS

In the United States, invasive insects and plants cause millions of dollars of damage annually both economically and ecologically. Continuing globalization and increasing international trade will likely result in more invasive species becoming established, exacerbating their impacts and the need for management solutions. Classical biological control is one important tool for reducing the negative impacts caused by invasive pests. Since 1888 when it was first applied in the United States, the discipline of classical biocontrol has grown and evolved. Under strict regulations and with improvements in testing practices, classical biocontrol has emerged as an effective and sustainable method for the long-term management of invasive pests. Subsequent chapters of this book illustrate the important role biocontrol has played in the control of invasive pests over the past 30 years. Its benefits are illustrated with a series of detailed case studies of invasive insects and weeds in the United States.

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Chapter 2

How Classical Biological Control Fits into Key Issues

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INTRODUCTION

Invasive insects and plants can cause problems that matter. They increase the cost of food and the use of pesticides, reduce the productivity of grazing land, damage and kill the ornamental plants we use in urban landscapes, kill trees in forests, and hurt native habitats and species. Some of these invasions' effects can be permanently ended through classical biological control. In this chapter, the benefits of some of the many projects of classical biocontrol that have been carried out in the United States are pointed out, in general terms, and linked specifically to the various chapters in this book where the details are presented.

PROTECTING CROP YIELDS WITHOUT INCREASING PESTICIDE USE

Crop production systems must suppress, avoid, or manage all the key diseases and insect or mite pests of the crop, while also providing the crop with the basics (adequate water, soil nutrition, weed control, and effective cropping, harvesting, and marketing systems). Pest-control and production systems vary locally with climate and soils and take time for growers and scientists to develop. New important pests invading a cropping region can throw the crop protection/production system out of balance, either causing production losses or requiring new investments in pesticides or other means to suppress the invader. Pest invasions are wild cards that often arrive with no pre-planning, causing a scramble to figure out how to cope with the new problem. Some recent examples of this process include efforts to mitigate losses associated with spotted wing drosophila (*Drosophila suzuki*) in blueberries, raspberries, and other fruits (e.g., Lee et al., 2019) as well as efforts against brown marmorated stink bug (*Halyomorpha halys*) (Krawczyk et al., 2017), and spotted lantern fly (*Lycorma delicatula*) (Lee et al., 2019).

Tools that can be activated to reduce damage from new invaders include development of new pesticides or new registered uses, breeding of more resistance crop varieties, better use of existing natural enemies, and programs to introduce new species of natural enemies. The last of these approaches is what we

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mean by classical biological control. As discussed in Chapter 1, this approach seeks to permanently reduce the pest's density. In the last 40 or so years, at least ten invasions of new crop pests have been resolved partly or completely through introductions of natural enemies, mostly from the pest's native range.

Sometimes a biological control program is developed and carried out rapidly after the invasion, as was the case for ash whitefly (*Siphoninus phillyreae*, Chapter 17) (Pickett and Pitcairn, 1999), leading in that case to the rapid disappearance of the pest as an object of concern within a few years. In other cases, pests may have been spread with the crop itself into new parts of the world and thus have 'always' been part of the cropping system, as is the case of codling moth (*Cydia pomonella*) in apple, pear, and walnut orchards in the western United States. Sometimes, new information or new ideas lead scientists to develop biocontrol programs against pests of this type decades or even centuries after their original invasion. This was the case for codling moth (Chapter 7) (Mills, 2005) and the imported cabbageworm (*Pieris rapae*, a pest of cabbage and other brassica crops, Chapter 5) (Van Driesche, 2008).

Some invasive pests affect a single crop or small group of similar crops. For example, among many other pests, the U.S. citrus industry has been affected by the damaging but not profoundly destructive citrus leafminer (*Phyllocnistis citrella*, Chapter 8) (Hoy and Nguyen, 1997) as well as the potentially industry-destroying citrus psyllid (*Diaphorina citri*, Chapter 12), which is the vector of the lethal pathogen causing greening disease, *Candidatus* Liberibacter asiaticus (Hoddle and Hoddle, 2013). A similarly narrowly focused pest affecting another California crop (olives) is the olive fruit fly, *Bactrocera oleae* (Chapter 11) (Daane et al., 2015). In contrast, some other invasive pests that have been partly or completely controlled with biological control introductions are very polyphagous, affecting a wide range of crops and other plants. One such invasive insect, variously known as the poinsettia whitefly, sweet potato whitefly, or tobacco whitefly (*Bemisia tabaci*, Chapter 6), was a billion-dollar invader affecting a wide range of crops from greenhouse flowers grown from Massachusetts to Florida to winter melons and vegetables in Arizona and California (Goolsby et al., 2005). Clearly for such multi-crop pests, development of one area-wide biological control program has many advantages over developing pesticide controls that must be designed, tested, adjusted, and registered separately for each affected crop in many different locations.

Some invasive pests occur in contexts where they are the only pest that needs to be managed. This greatly simplifies the implementation of classical biocontrol projects because the natural enemies being introduced will not immediately be killed by broad-spectrum pesticides applied against other pests. Ash whitefly (Chapter 17), for example, occurred on unsprayed street and yard trees of just one group (ash), and this setting presented a good habitat for the introduced parasitoid to forage, find, and attack the target pest. Similarly, cereal leaf beetle (*Oulema melanopus*, Chapter 10) is a pest of grains (mainly wheat and oats) and occurred in crops that received few if any foliage treatments with broad-spectrum insecticides. Consequently, the parasitoids introduced against this pest were not subjected to the destructive effects of pesticides (Pickett et al., 2021).

In contrast, other invasive pests are more amenable to suppression at the landscape level than inside fields of high value crops. The butterflies that produce the larvae known as imported cabbage worms, for example, are strong fliers and have many wild and home-garden host plants. Consequently, the pest pressure in commercial vegetable fields is strongly affected by the regional population of this insect, and these non-crop sites are more favorable for the biological control agents to act because they are not being harmed there by pesticides. In contrast, other invasive species are not the only pests in need of management on their host growing in a crop setting. For example, imported cabbage worms (Chapter 5) are just one of many pests that attack brassica crops, and augmentative releases of the egg parasitoid *Trichogramma evanescens* have been hindered by the application of pesticides used to treat the other pests. Two recent invaders in California—the glassy-winged sharpshooter (*Homalodisca vitripennis*, Chapter 9) and citrus psyllid (*D. citri*, Chapter 12)—have landscape-wide pest dynamics, meaning they attack host species both within a crop setting as well as more widely across the landscape when their hosts occur in urban or wild settings. For both pest species, biological control agents have substantially reduced the pests outside of their focal crops of concern, but pesticide programs in crops remain a barrier to the same natural enemies surviving long enough inside the focal crop to be effective. In the case of the glassy-winged sharpshooter, data from unsprayed citrus orchards show a >95% drop in sharpshooter density due to biological control (Banks et al., 2019), but in the focal crop (grapes), the potential risk of infection of the vines with sharpshooter-vectored Pierce's disease still causes growers to use pesticides intensely. For citrus psyllid, introduced parasitoids now attack the pest on 'backyard' citrus (which is rarely sprayed) in urban areas; in that setting, the introduced parasitoid has reduced psyllid numbers by 70%. The suppression of the psyllid on yard citrus means that the psyllid and its associated tree-killing pathogen are much less common in the general landscape, and consequently the pathogen has not yet infected commercial citrus orchards (see Chapter 12 for more details). Therefore, even though the action of the biological control program occurs mainly outside commercial orchards, the program's benefits reach into commercial citrus orchards. Similarly, biological control of western U.S. species in the genus *Lygus* (Chapter 4) is a program that suppresses the pest regionally in suitable habitats; however, in order for the parasitoid to be effective in commercial strawberries, habitat enhancement through the use of an alfalfa intercrop is required (Swezey et al., 2014).

REDUCING PESTICIDE USE AROUND HOMES TO CONTROL LANDSCAPE PESTS

Horticulture and landscaping are major industries that help homeowners and commercial building owners enhance the value of their properties and improve their appearance by surrounding buildings with attractive plants of many types and species. These urban plantings include some native plants, as well as many imported species selected for such use. Many insects that attack ornamental plants have been accidently introduced to the United States. Such species often become damaging to their host plants by reaching high densities due to a lack of specialized natural enemies outside their native ranges. The homeowner/property manager's response in such situations is quite often to either apply pesticides to infested plants themselves or hire commercial companies to do so. These reactions, understandably motivated by the desire to protect the health of valuable plants, increases pesticide use close to where people live, creating opportunities for pesticide exposures of potential health concern. For invasive urban landscape pests that are of foreign origin, classical biological control can help reduce pest densities without any action on the part of the property owner and without the use of pesticides. While individual invasive species affecting urban landscape plants may seem to be of limited importance, given that the homeowner has many landscaping choices and can often simply shift from susceptible plant species to ones that are not, in aggregate such pests increase pesticide use around homes and increase costs to replace susceptible plants. Also, some trees used in landscaping, such as eucalyptus in California or ash in the northcentral and northeastern United States, are high value plants that cannot be quickly or cheaply replaced. For this reason, most of the classical biocontrol projects against invasive urban horticultural pests have been against species affecting trees (see later in this section). However, this has not always been the case. In this book, we discuss two projects directed at horticultural plants that are not trees. The first of these is euonymus scale (*Unaspis euonymi*, Chapter 13) (Van Driesche et al., 1998), which became widespread in urban areas on shrubs and vines in the genus *Euonymus*, in large part because of the common and widespread use of variegated euonymus species for landscaping around homes and businesses. In northern states, lilies of various species are widely used in gardens, and their cultivation became more difficult and expensive after the invasion of the lily leafbeetle (*Lilioceris lilii*, Chapter 15). Indeed, the mess and bother created by this pest often led gardeners to abandon planting susceptible lily species during the height of the pest's invasion, before it was brought under control by several introduced parasitoids (Tewksbury et al., 2017). In warmer areas such as Florida, Hawaii and California, the invasive pink hibiscus mealybug (*Maconellicoccus hirsutus*, Chapter 14), a pest with a very broad host range that includes popular *Hibiscus* shrubs, increased the need for pesticide use in many kinds of urban plantings (Castle and Prabhaker, 2011).

In cities and towns, trees have high value for their beauty, shade, colored foliage, and flowers. Urban plantings of trees are important in reducing the temperatures in cities, a particularly valuable feature in times of a warming climate. The streets of California cities and towns make extensive use of plantings of many species of eucalyptus trees. Pest damage to these trees, which was very low for a long time, started to increase in the 1990s with the introduction of several borers and psyllids (Paine and Millar, 2005). Dramatic suppression of a species of tree-killing eucalyptus borer in the 1990s using natural enemies protected (and continues to protect) many eucalyptus species still widely planted in Californian cites (Hanks et al., 1996). Two invasive psyllids also damaged eucalyptus species in California—blue gum psyllid (*Ctenarytaina eucalypti*, and red gum lerp psyllid (*Glycaspis brimblecombei*). These psyllids were successfully suppressed by importing their specialized natural enemies (Dahlsten et al., 1998; Daane et al., 2012). Also in California cities, ash was (and still is) an important landscaping tree that became heavily damaged by ash whitefly, a pest that was totally suppressed by a classical biocontrol project (Chapter 17). At the time, ash whitefly infestations led to an increase in spraying of street and yard trees, but this use of pesticides near people's homes is no longer needed due to the biocontrol project. In Anchorage, Alaska, Alaska white birch trees (*Betula neoalaskana*) were heavily damaged in both urban and forested areas by the invasive ambermarked birch leafminer (*Profenusa thomsoni*, Chapter 21) (Soper et al., 2015), a pest whose level was suppressed by more than 87% (Andersen et al., 2021) through the importation of a single species of specialized parasitoid. Similarly, in the northeastern United States, another invasive birch leafminer (*Fenusa pumilla*, Chapter 16) was a ubiquitous urban pest of white birch (*Betula papyrifera*) in yards before the 1990s; it has since been highly suppressed by an introduced parasitoid (Van Driesche et al., 1997). No single tally has even been made of all the protection extended to urban landscaping from these and other biological control programs, but it is clearly large and, most importantly, permanent.

PROTECTING GRAZING LAND

A less obvious but very real problem caused by invasive species is their impact on producing grass or other forage for livestock, particularly in low-value rangelands in the drier parts of the western United States. Many invasive range weeds are often toxic to cattle, exclude desirable vegetation, and lower forage yield, therefore reducing the carrying capacity of rangeland. In this book, we discuss the biological control of two such weed problems (of a much larger group): leafy spurge (*Euphorbia virgata*, Chapter 24) and two species of toadflax (*Linaria vulgaris* and *L. dalmatica*, Chapter 26). While the losses per acre may seem modest, the vast areas affected make these weeds big problems to ranchers.

In some instances, invasive insects can also be factors lowering the productivity of pastures. In Florida, which remains an important ranching state despite intense coastal development, several invasive mole crickets (*Neoscapteriscus* species, Chapter 25) lowered pasture productivity across large areas by consuming the roots of forage grasses. A biological control program successfully suppressed mole cricket populations and continues to protect the productivity of Florida pastures and croplands (Mhina et al., 2016).

Because rangeland values are typically low, especially in the arid parts of the western United States, few interventions against such invasive species are economically justified. Classical biocontrol, because it is self-spreading and naturally sustained, is one of the very few technologies that function well under such limitations.

REDUCING DAMAGE TO TREES IN FORESTS

Protecting forests from damage by invasive insects has long been a focus of classical biocontrol efforts in the United States, and several important pests have been suppressed (Van Driesche and Reardon, 2016).

Forests are of immense importance as sources of natural resources such as lumber, and the national forest system is also a major component of the nation's system for protecting native biodiversity. In the past few decades, several new important forest invasive pests have been suppressed by biocontrol programs. The project against winter moth (*Operophtera brumata*, Chapter 20) has been highly successful (Elkinton et al., 2021), ending defoliation of mixed deciduous forests in the northeastern United States. Similarly, in Alaska, a program against ambermarked birch leafminer (Chapter 21) reduced the level of leaf mining by ~85%, eliminating the stress from that species on forest birch (Soper et al., 2012; Andersen et al., 2021). Currently, the USDA is attempting to suppress what has been characterized as the most damaging forest invasion in the nation's history, emerald ash borer (*Agrilus planipennis*) (Duan et al., 2022). While as yet incomplete, this project has achieved a great deal in its nearly 20-year history (see Chapter 22). In North America, invasive plants have not yet been as damaging in forests as in some other biomes, such as grasslands. Nevertheless, some invasive vines have been recognized as threats to forest regeneration due to their ability to smother seedlings. One such species that has been the target of a successful biocontrol program is mile-a-minute weed (*Persicaria perfoliata*, Chapter 19) in the Mid-Atlantic states (Lake et al., 2011).

PROTECTION OF BIODIVERSITY

In natural ecosystems, effects of individual invasive species can be highly focused, as when specialized pests like emerald ash borer (Chapter 22) and cycad scale (*Aulacaspis yasumatsui*, Chapter 18) cause great harm, but only for a specific species or small group of species. In other cases, the effects of invasive species may be much broader. For example, winter moth (Chapter 20) defoliated many species of deciduous trees and shrubs in eastern deciduous forests. Similarly, invasive fire ants (*Solenopsis* spp., Chapter 23) have broad effects on native ants in invaded areas.

The effects of invasive plants tend to be very broad because they act through competition for resources and through alteration of habitat-defining processes such as fire, flooding, or grazing regimes (Foxcroft et al., 2014). Invasive plants affect a variety of habitats in the United States, and biological control has been used successfully in many of these. In addition to grassland and forest weed biocontrol programs described earlier, air potato (*Dioscorea bulbifera*, Chapter 32) has been successfully targeted with biocontrol in forests and shrubland in south Florida (Rayamajhi et al., 2010). In wetland habitats, biocontrol has helped suppress purple loosestrife (*Lythrum salicaria*, Chapter 27) throughout the northern United States along freshwater rivers and marshes (Blossey et al., 2001), giant salvinia (*Salvinia molesta*) in southern regions from Texas to Florida (Tipping et al., 2008), and melaleuca (*Melaleuca quinquenervia*, Chapter 29) in the Everglades and surrounding parts of southern Florida (Center et al., 2012). In riparian habitats, arundo (*Arundo donax*, Chapter 31) and salt cedar (*Tamarix* spp., Chapter 28) have both been targeted for biological control along desert rivers throughout the arid Southwest (Goolsby et al., 2016; Bean and Dudley, 2018).

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Chapter 3

Risks of Classical Biological Control

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INTRODUCTION

The public view of biological control introductions has changed markedly over time, from a 'green' technology in the 1970s that ideally should replace pesticides (DeBach, 1974), which were clearly polluting the environment (Carson, 1962), into a potentially dangerous activity that many felt should be highly regulated (Howarth, 1991). If biological control introductions are indeed highly dangerous as claimed, the question is 'compared to what?' and the logical response is 'to introductions of new species made for other purposes and to alternative forms of pest control.'

Risks of New Species Introductions Made for Other Purposes

When the risks of biological control introductions are compared to introductions made for horticultural, the pet industry, aquaculture, and fish and game introductions, risks of biocontrol are, as we will show here, small in comparison. Over the 500+ years since Europeans colonized North America, many new species have arrived (Crosby, 2003). Some arrivals were accidental invasions, but many new species were deliberately introduced for agriculture, forestry, fisheries, and other commercial activities. Some introduced species were familiar from the colonists' mother countries. Community attitudes on the safety and benefits of these introduced species depended on this familiarity and on the pleasure or value associated with the species in question. Attractive plants and edible fish were considered good to import if they were not already present. Risks were largely unrecognized or ignored unless obvious damage resulted.

Species have been intentionally introduced for many reasons, and Van Driesche et al. (2020) grouped introductions around 17 motives: (#1) use as forestry, forage, and fodder plants, (#2) ornamental plants, (#3) crop plants, (#4) terrestrial mammals for ranching or viewing, (#5) fish used for aquaculture, (#6) commercially produced invertebrates, (#7) predatory mammals farmed for fur, (#8) herbivorous mammals farmed for fur, (#9) birds and mammals for hunting, (#10) non-native fish for sport or commercial fishing, (#11) animals sold as pets, (#12) animals introduced for medical uses, (#13) vertebrates for pest control, (#14) parasitoids for insect biocontrol, (#15) predatory insects and mites for insect biocontrol, (#16) insects or mites for weed biocontrol, and (#17) pathogens introduced for weed or insect biocontrol (**Table 1**). While

Van Driesche, R. G. and R. L. Winston. 2022. Risks of classical biological control, pp. 19–35. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

Table 1. Summary of degree of regulation for 17 groups (purpose of introduction) versus six risks. Biological control agents, groups 13 to 17, in boxed portion of figure. (modified from Van Driesche et al., 2020)

GROUP	RISK 1 Non-target attacks	RISK 2 Vector insects, mites, ticks	RISK 3 Vector pathogens	RISK 4 Damage crops	RISK 5 Degrade habitats	RISK 6 Change food webs
#1 Forestry, forage, or fodder plants	Not Applicable	Regulated	Partially Regulated	Partially Regulated	Partially Regulated	Not Regulated
#2 Ornamental Plants	Not Applicable	Regulated	Partially Regulated	Partially Regulated	Partially Regulated	Not Regulated
#3 Crop plants	Not Applicable	Regulated	Partially Regulated	Partially Regulated	Partially Regulated	Not Regulated
#4 Terrestrial mammals for ranching or viewing	Not Regulated	Regulated	Regulated	Not Regulated	Not Regulated	Not Regulated
#5 Fish used for aquaculture	Partially Regulated	Not Applicable	Regulated	Not Applicable	Not Regulated	Not Regulated
#6 Commercially produced invertebrates	Not Applicable	Not Applicable	Partially Regulated	Partially Regulated	Partially Regulated	Not Regulated
#7 Predatory mammals farmed for fur	Not Regulated	Regulated	Regulated	Not Applicable	Not Regulated	Not Regulated
#8 Herbivorous mammals farmed for fur	Not Regulated	Regulated	Regulated	Not Regulated	Not Regulated	Not Regulated
#9 Birds and mammals for hunting	Not Regulated	Regulated	Regulated	Not Regulated	Not Regulated	Not Regulated
#10 Fish for sport or commercial fishing	Partially Regulated	Not Applicable	Partially Regulated	Not Applicable	Not Regulated	Not Regulated
#11 Animals sold as pets	Not Regulated	Regulated	Partially Regulated	Partially Regulated	Partially Regulated	Not Regulated
#12 Animals introduced for medical uses	Not Regulated	Not Applicable	Not Regulated	Not Applicable	Not Regulated	Not Regulated
#13 Vertebrates used for pest control	Regulated	Regulated	Regulated	Not Applicable	Regulated	Regulated
#14 Parasitoids for insect biocontrol	Regulated	Regulated	Regulated	Not Applicable	Regulated	Regulated
#15 Predatory Insects and Mites for biocontrol	Regulated	Regulated	Regulated	Regulated	Regulated	Regulated
#16 Insects or mites for weed biocontrol	Regulated	Regulated	Regulated	Regulated	Regulated	Regulated
#17 Pathogens for weed or insect biocontrol	Regulated	Not Applicable	Not Applicable	Regulated	Regulated	Regulated

risks of species introductions were gradually recognized over time, from a modern perspective, risks that should have been considered before introducing any new species span six major categories: (1) direct attack on non-target species, (2) the potential to harbor pest insects, mites, or nematodes, (3) the potential to vector pathogens, (4) the potential to become pests in crops, (5) the potential to change or degrade native habitats or ecosystems, and (6) the potential to greatly disturb food webs in natural communities.

When one examines how the United States government has regulated the above six risk categories across all the species introduced for the 17 different reasons listed above, the first obvious conclusion is that the only groups for which all six risks are considered before introduction of a new species are biocontrol agents (groups #14–17) (**Table 1**). For all other groups, regulation has controlled only some risks, and these are most often risks to crops and valuable plants. The pattern of disparity in risk regulation (**Table 1**) is further illustrated in **Figure 1**, which marks regulated (green striped), partially regulated (yellow dotted), and unregulated (red) risks for all 17 species use-groups. Only for biocontrol agents are all risks regulated, while species introduced for commercial reasons conspicuously fail to regulate important risks.

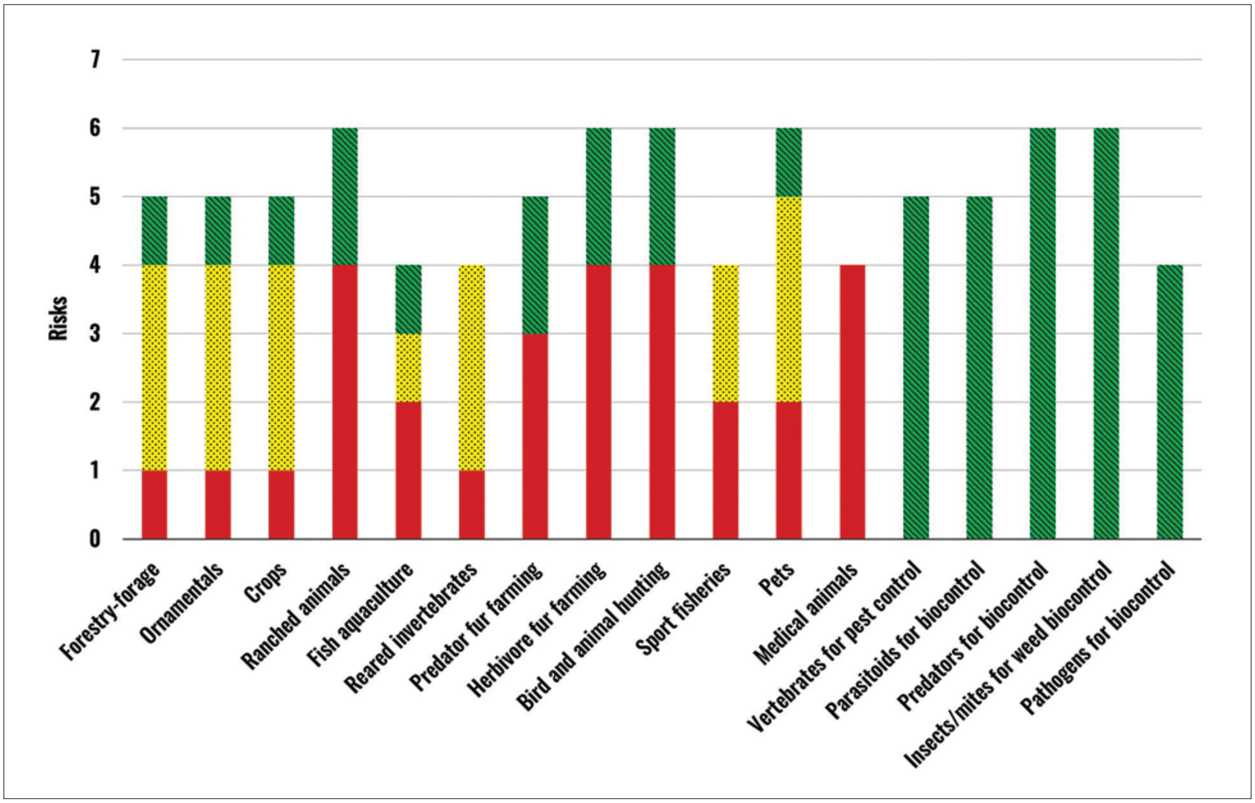

Figure 1. Number of risks that are regulated (green striped), partially regulated (yellow dotted), or not regulated (red) for each of the 17 categories of novel species, under U.S. law (risks judged "not applicable" make up the remainder in each group). (reprinted with permission from Van Driesche et al., 2020)

Because biocontrol introductions are the most highly regulated, they are the introduction group least likely to cause problems because strict testing regulations identify problematic introductions before they occur. Indeed, the use of vertebrates (predatory mammals and fish, group #13) to control pest species has not been practiced in the United States for 30 years because vertebrate biocontrol agents are insufficiently host-specific. In later sections of this chapter, we examine what risks may occur for each of the four groups of biological control agents still currently used in the United States, including: (1) insect parasitoids, (2) predaceous insects or mites, (3) herbivorous insects or mites, and (4) pathogens of either pest insects or weeds.

Risks of Alternative Forms of Pest Control

As discussed in Chapter 1, classical biological control is just one of many forms of pest management currently practiced in the United States. The most common alternative pest control methods are chemical, physical, and cultural. Although much attention has been given to the notion that biological control is dangerous and too risky (e.g., Simberloff and Stilling, 1996; Simberloff, 2012), it must be emphasized that all forms of pest management have been shown to impact one or more of the six major risk categories described in the previous section (DiTomaso, 1997).

Chemical control

Most pesticides are not selective for only their target pest, and pesticides are often broadcast over large areas, including agricultural fields and rangeland, resulting in contact with humans and many non-target species (Culliney, 2005; Stemele, 2017). As such, there are many examples of pesticides causing immediate and direct damage to non-target species, including plants, invertebrates, and vertebrates, in both terrestrial and aquatic ecosystems (Herman and Bulger, 1979; Pearce et al., 1979; Colborn, 1992; Freemark and Boutin, 1995; Burn, 2000; Culliney, 2005; Sheley and Denny, 2006; Sheley et al., 2011). Chemical control has also been shown to cause long-term effects through accumulation in the soil, leaching into water systems, and incorporation into the food chain, affecting several aspects of natural ecosystem function as well as human health (Reimold and Shealy, 1976; DiTomaso, 1997; Jaga and Dharmani, 2003; Pfeuffer and Rand, 2004; Gasnier et al., 2009; Helander et al., 2012; Mesnage et al., 2013; Séralini et al., 2014; Wagner and Nelson, 2014). Widescale use of pesticides has led to resistance in many weeds (Culliney, 2005; Heap, 2019) and insects (Georghiou and Legunes-Tejeda, 1991; Nayak et al., 2015; Fujii et al, 2020).

Physical control

Physical control measures for insect pests can include traps of various types, flooding of water tolerant crops, and use of barriers, among other methods (Flint, 2012). These approaches are relatively benign to the environment but are often specific to individual pests in particular crops, limiting their application. Traps are mostly used for pest monitoring to time pesticide applications, rather than as a mean of control themselves.

Physical weed control methods, such as mowing and tilling, are non-selective and directly impact non-target species in the treatment area. These practices have also been shown to increase weed infestations in some instances by spreading seeds and propagules and resulting in compensatory growth and increased reproductive output (DiTomaso, 1997; Hackett and Monfils, 2014). Tilling as a form of weed management has been shown to increase soil erosion and alter soil structure, impacting ecosystem function (DiTomaso, 1997).

Cultural control

Cultural controls for management of pest insects include crop rotation, community-level dates for crop planting or destruction of crop residues, and the use of resistant crop varieties (Flint, 2012). Like physical control methods for insects, these cultural controls are relatively benign to the environment.

Cultural control methods for weeds have had much greater negative environmental consequences. Because grazing by cattle, sheep, and goats is not selective, in some instances grazing as a form of weed management has been shown to target competing or more desirable species, making the weed problem worse (DiTomaso, 1997). Many infestations on rangeland in the United States are the result of intensive overgrazing (Billings, 1994), and several weed species that were introduced in livestock hay were later spread in livestock wool or fur or in manure used in fields (DiTomaso, 1997). Suppressing insects or weeds by manipulating the crop or surrounding vegetation has also resulted in problems, including the alterations of natural ecosystems and food webs. The planting of corn and cotton genetically modified to be resistant to insects, and the planting of soybeans modified to tolerate herbicides, have resulted in a reduction of the amount of insecticide applied to those crops, but it has led to emergence of new pest insects and an increase in the amount of herbicide applied (Perry et al., 2016). Greater herbicide use on genetically modified cropland has led to a decline of susceptible native weeds, including common milkweed, *Asclepias syriaca* (Pleasants and Oberhauser, 2012), which is the primary host of the monarch butterfly, *Danaus plexippus*. Fire is sometimes intentionally applied to large weed infestations to burn off thatch and reduce weed seeds in the soil (DiTomaso et al., 1999). This form of cultural pest control has lowered air quality in urban areas (Campbell and Cahill, 1996), fires escaping control, harm to small animals and desirable insects unable to escape the burn, and an increase in soil erosion (DiTomaso, 1997).

Although all forms of pest management have been shown to have some adverse impacts, the ‘do nothing’ approach is not a suitable alternative. In the United States, pest insects and plants cause millions of dollars of damage annually to agriculture, forestry, and infrastructure, while simultaneously threatening native biodiversity, altering ecosystem services, and affecting human health (DiTomaso, 2000; Pimentel et al., 2005; Pyšek et al., 2012). Without intervention, the negative impacts of established invasive species are expected to grow (Pimentel et al., 2005).

WHAT ARE THE POTENTIAL RISKS OF PARASITOIDS?

History of Introduction and Regulation of Parasitoids as Biocontrol Agents

Parasitoids are tiny stingless wasps or flies that attack insects or, in a few groups, other invertebrates. They play key roles in all terrestrial food webs because they limit the density of many plant-eating insects, commonly protecting native plants from high levels of damage. Consequently, they are extremely useful in lowering the damage that invasive insects (especially aphids, whiteflies, scales, stinkbugs, mealybugs, leafminers, and leafhoppers) cause to crops and native plants. The control of cottony cushion scale (*Icerya purchasi*) in California in the 1880s, included a parasitoid (*Cryptochaetum iceryae*) as one of the two introduced species that saved the young state’s citrus industry (Caltagirone and Doutt, 1989). Since 1985, 176 species of parasitoids have been introduced as biological control agents into North America and U.S. overseas territories (Van Driesche et al., 2021).

Before 1993, the primary regulation governing releases of parasitoids in the United States was the Plant Quarantine Act of 1912. Because the focus of that Act was on species that might pose a risk to plants, species of parasitoids for biocontrol projects were automatically approved because they do not pose any risk to plants. In 1993, a lawsuit led to the ruling that APHIS lacked authority to regulate parasitoids, as they were not plant pests. Later, the Plant Protection Act of 2000 provided USDA-APHIS-PPQ with explicit authority to regulate parasitoids and predaceous insects. This Act also charged APHIS with facilitating the use of biological control agents in solving invasive species problems.

As described in Chapter 1, the introduction of biocontrol agents to the United States is dependent upon results of a strict review process. For parasitoids, this process is based on host records from published studies and new data from quarantine laboratory tests of the parasitoid’s ability to lay eggs and develop successfully in species that are taxonomically or ecologically close to the target pest. In addition, the odors or other cues used by parasitoids to find hosts from a long distance in natural habitats may also be considered, although they are logistically more difficult to measure in a laboratory. Information from field surveys of parasitism of insects related to the target pest in its native range can provide information on what other insects a parasitoid may attack in the field, when all influences on host ranges are allowed to operate freely. See **Box 1** for an example of host range assessment of parasitoids introduced to control emerald ash borer.

Box 1. Risk assessments for three parasitoids imported to suppress emerald ash borer (*Agrilus planipennis,* Coleoptera: Buprestidae) (Van Driesche and Reardon, 2015).

#1. The egg parasitoid *Oobius agrili* (Hymenoptera: Encyrtidae). In laboratory no-choice tests, this egg parasitoid did not attack moth eggs or eggs of long-horned borers (Coleoptera: Cerambycidae). The parasitoid did attacks other species of *Agrilus* with eggs of similar size as *A. planipennis*, including the bronze birch borer (*A. anxius*), the two-lined chestnut borer (*A. bilineatus*), and the red-necked cane borer (*A. ruficollis*). There were no attacks on *Agrilus* species with dissimilar-sized eggs, including *A. cyanescens, A. egenus,* or *A. subcinctus*. In choice tests, this egg parasitoid preferred eggs of emerald ash borer over other *Agrilus* species (Bauer and Liu, 2007; Federal Register, 2007; Gould et al., 2015). Because there are no *Agrilus* species in ash trees with eggs similar in size to *A. planipennis*, the field host range is predicted to be narrower than these laboratory findings.

#2. The larval parasitoid *Tetrastichus planipennisi* (Hymenoptera: Eulophidae). In laboratory no-choice tests, this larval parasitoid did not attack larvae of two moths or one sawfly (non-beetles), nor five species of longhorned beetles or eight species of buprestids (five *Agrilus* and three *Chrysobothris*) (Federal Register, 2007; Liu and Bauer, 2007; Gould et al., 2015), suggesting that *T. planipennisi* attacks only the target pest.

#3. The larval parasitoid *Spathius galinae* (Hymenoptera: Braconidae). In laboratory no-choice tests, this larval parasitoid did not attack larvae of two non-beetle borers (one moth, one sawfly), nor eight beetle borers in different families. There were no attacks on four species of *Agrilus* or one of *Chrysobothris*. Attack in the laboratory did occur on the gold-spotted oak borer, *Agrilus auroguttatus*, an invasive pest of oaks in California (Duan et al., 2015; Federal Register, 2015; Gould et al., 2015). *Agrilus auroguttatus*, however, is not attacked in oak trees because the bark of its hosts is too thick for *S. galinae* females to penetrate and contact larvae for egg laying.

Possible Risks of Parasitoids

Attack on native insects

Evaluations of parasitoid introductions focus mainly on risks to non-target species. From 1880 to 2000, the database BIOCAT (Cock et al., 2016) reports approximately 5,000 species-by-country introductions of parasitoids worldwide. Of these, only 17 (0.34%) introductions also report harm to native insects (van Lenteren et al., 2006). Although rare, harm to populations of non-target insects due to attacks by introduced parasitoids have occurred in North America. For example, the tachinid fly *Compsilura concinnata* (introduced in 1906 into the northeastern United States against the gypsy moth, *Lymantria dispar*) has attacked larvae of several giant silk moths, including *Hyalophora cecropia* (Boettner et al., 2000; Elkinton and Boettner, 2012). Since that time, a highly developed evaluation system has been created (Van Driesche and Hoddle, 1997; Van Driesche and Reardon, 2004) and is routinely applied to each species of parasitoid proposed for introduction before it is approved for release (see **Box 1** for example of results).

Accidental co-introduction of pest insects, mites, or nematodes

Parasitoid introductions potentially pose some risk of accidentally introducing unrecognized hyperparasitoids able to attack beneficial parasitoids (e.g., *Encarsia pergandiella* [Bográn and Heinz, 2002]) This risk, however, is rare in practice because these unwanted species are routinely and efficiently eliminated in quarantine (e.g., Hoddle et al., 2013).

Accidental co-introduction of plant pathogens

A parasitoid might conceivably spread a plant pathogen if its ovipositor became physically contaminated during oviposition into the pathogen's insect vector. For example, for the pathogen causing citrus greening disease, there was concern that parasitoids being introduced against the pathogen's vector (citrus psyllid, *Diaphorina citri*) might unknowingly introduce the pathogen. However, screening parasitoids in quarantine for the pathogen's DNA showed that no parasitoids were contaminated (Hoy et al., 2001).

Damage to crops

Insect parasitoids are not biologically capable of damaging crop plants.

Harm to wildlife habitats or ecosystems

Because introduced parasitoids are unlikely to harm populations of native insects, harm to wildlife habitats could only occur indirectly if parasitoids were introduced to suppress an invasive insect that was attacking an invasive plant that damaged a native habitat. For example, biocontrol of two species of invasive *Pulvinaria* scales that attack introduced ice plants in California dunes (Tassan et al., 1982; Washburn and Frankie, 1985) could damage dunes habitats by preventing scale impacts on ice plant. Successful biological control of these scales could be seen as harmful given that ice plants outcompete native dune vegetation (Magnoli et al., 2013). In other cases, parasitoids can greatly improve habitats for native wildlife if they control invasive pests that attack native plants and thus damage the habitat (e.g., *Ceroplastes* wax scales in rain forests in Queensland, Australia [Waterhouse and Sands, 2001; Van Driesche et al., 2010]).

Changes to native food webs

Harm to native food webs is possible from introductions of parasitoids through a mechanism termed 'apparent competition,' in which the introduced parasitoid attacks both the target pest and a native species related to the pest. For example, the parasitoid *Cotesia glomerata*, introduced to North America in the 1880s (as *Apanteles glomeratus*) to control the imported cabbage worm, *Pieris rapae*, contributed to the decline of the native butterfly *Pieris oleracea* in Massachusetts, which it also attacked (Benson et al., 2003). The stringent testing requirements currently in place ensure that this particular introduction would not be acceptable today.

WHAT ARE THE POTENTIAL RISKS OF PREDACEOUS INSECTS?

History of Introduction and Regulation of Predaceous Insects as Biocontrol Agents

The first dramatic success in biological control of insects in the United States was largely due to the lady beetle *Rodolia* (now *Novius*) *cardinalis*, which saved the young citrus industry of California in the late 1880s by controlling an invasive species, the cottony cushion scale (*Icerya purchasi*) (Caltagirone and Doutt, 1989). Over time, the advantages of the greater specialization of parasitoids caused biocontrol introductions to shift away from predators. Today, only the most specialized predators as still used, as for example *Laricobius nigrinus*, which was moved from the western United States to eastern states to control the hemlock woolly adelgid, *Adelges tsugae* (Mausel et al., 2010). Specialized predators are most useful in programs against pests that have no specialized parasitoids. Since 1985, only 28 species of predatory insects have been introduced into North America and the U.S. overseas territories (Van Driesche et al., 2021). Regulation of predaceous insects or mites for biocontrol in the United States is the same as that for parasitoids, as described in the previous section.

Possible Risks of Predaceous Insects

Attack on native insects

All predatory insects must on occasion include non-target insects in their diet, but no predaceous insects used as biocontrol agents have been reported to cause population-level effects on native prey through direct consumption. Some introduced lady beetles have been observed feeding on native insects of conservation interest such as the woolly alder aphid (*Prociphilus tesselatus*) (which is the only food of the harvester butterfly, *Feniseca tarquinius*) and some lacewings. These lady beetles were *Harmonia axyridis* (Butin et al., 2004) and *Propylea quatuordecimpunctata* (Wheeler, 1990). However, no changes in the aphid's field density have been reported due to such feeding.

Accidental co-introduction of pest insects, mites, or nematodes

Any parasitoids, mites, or nematodes found attacking beneficial predaceous insects being considered for introduction as biocontrol agents are eliminated while the colony is held in quarantine for testing.

Accidental co-introduction of plant or animal pathogens

Pathogens attacking predaceous insects are not pathogens of plants. While pathogens of insects may occur in populations of predaceous arthropods intended for introduction, colonies of these predators are screened for pathogenic fungi and microsporidian during the evaluation process, and laboratory colonies are cleared of such pathogens before introduction.

Damage to crops

Some predatory insects (such as mirid bugs) have the potential to be crop pests by feeding on crop foliage during periods when there are few pests (Hall et al., 2021). If plant-feeding/predaceous mirids, which are used in European greenhouses for pest control, were proposed for use in the United States in the future, their possible effects on crops would have to be assessed. Risks of this type are recognized and regulated in the United States. Predatory insects may also damage crops indirectly. For example, the lady beetle *H. axyridis* has become a minor pest of wine vineyards because even a few beetles mixed with the harvested grapes can impart an off taste to the wine (Linder et al., 2009).

Harm to wildlife habitats or ecosystems

This risk is regulated in the United States, but no clear examples of such effects are known for introduced predatory insects.

Changes to native food webs

Two important, well-documented examples of food-web effects on native insects exist in the United States. The introduced lady beetles *H. axyridis* and *Coccinella septempunctata* have reduced populations of pest aphids (mostly invasive species) in crop fields, but this drop in prey numbers has indirectly reduced the density of some native lady beetles that were formerly common in some crops, especially *Adalia bipunctata* and *Coccinella novemnotata* (Wheeler and Hoebeke, 1995; Elliott et al., 1996; Turnock et al., 2003; Harmon et al., 2007; Losey et al., 2007; Fothergill and Tindall, 2010). Such food web effects are regulated under current rules, and the key to preventing them is to be able to conceive of what potential effects might exist in particular cases.

WHAT ARE THE RISKS OF HERBIVORES USED FOR WEED BIOLOGICAL CONTROL?

History of Introduction and Regulation of Weed Biocontrol Agents

Natural enemies used in classical biological control of weeds include different organisms, such as insects, mites, nematodes, and pathogens. Plant pathogens are discussed in the following section. In North America, most weed biological control agents are plant-feeding insects, of which beetles, flies, and moths are the groups that have been used most. Weed biological control in the United States began in Hawaii in 1902. The first introductions in the continental United States were made in 1945 when *Chrysolina* beetles were released against common St. Johnswort, *Hypericum perforatum*, in six western states (Holloway and Huffaker, 1952). As discussed in Chapter 1, these first St. Johnswort introductions proved highly effective against their target weed, and this success stimulated U.S. institutions to develop additional weed biocontrol programs, causing the United States to become a world leader in this area. Since 1902, 222 species of herbivorous insects and mites have been introduced to North America and the U.S. overseas territories for weed biological control (Winston et al., 2021).

Insects used for control of invasive weeds were regulated for certain risks from their earliest use in the United States because the specialized weed-eating insects used for biocontrol were perceived to be like the pest insects attacking crops. To ensure that weed biocontrol insects did not attack valuable plants, their introduction was regulated under the Plant Quarantine Act (1912). These early risk avoidance efforts were based on testing locally important crops to determine if any might be attacked. In 1973, the first Endangered Species Act was passed in the United States, with the goal of protecting rare or threatened native plants and some groups of animals. Concurrently, screening programs designed earlier to ensure that introduced plant biocontrol agents do not damage economic plants were expanded to protect all native plants. This expansion led researchers to seek the boundaries of the agent's host range, so the test data would be predictive of risk even for untested species. To do that, plants at various taxonomic distances from the target weed were tested (Wapshere, 1974). Later improvements in host range estimation included efforts to define the role of preference among accepted plants (preferred vs. acceptable but not preferred) and the role of plant chemicals in attracting foraging females for egg laying (Briese, 2005).

Possible Risks of Weed Biocontrol Agents

Attack on other plants

The principal risk for which new weed biocontrol agents are currently assessed is direct attack on crops (described in a later subsection), valuable horticultural plants, or native plants. Introduced (non-native) plants of limited economic importance are not protected. Pre-release screening of weed biocontrol agents (Wapshere, 1974) has been highly effective in preventing damage to native or valuable plants. Of 457 species introduced worldwide against invasive weeds through 2008, only two insects have had negative population-level impacts on native plants (Hinz et al., 2020). These two are the cactus moth *Cactoblastis cactorum* (Stiling et al., 2004; Pemberton and Liu, 2007) and the thistle weevil *Rhinocyllus conicus* (Louda, 1998; Louda et al., 2005). Of these, only the thistle weevil was intentionally introduced into the United States, as the cactus moth was either accidentally moved from Caribbean islands to Florida via cacti imported for the nursery trade (Pemberton, 1995) or spread naturally in storm events (Andraca-Gómez et al., 2015). As described above, current testing requirements would prevent the introduction of either of these species as weed biocontrol agents today.

Accidental co-introduction of pest insects, mites, or nematodes

Although rare, pest species have occasionally been introduced to the United States along with weed biological control agents. In one case, a single release of the peacock fly, *Chaetorellia australis*, against yellow starthistle, *Centaurea solstitialis*, was unknowingly contaminated with the morphologically similar false peacock fly, *Chaetorellia succinea* (Winston et al., 2021). *Chaetorellia succinea* proved to be more effective on yellow starthistle than *C. australis*. Later, *C. succinea* larvae were found feeding in the heads of an uncommon variety of commercial safflower, *Carthamus tinctorius*, causing minor damage (Randall et al., 2017). In addition, a second yellow starthistle agent, *Larinus curtus*, was found to be infected with a *Nosema* species after release. *Nosema* species can reduce survivorship and fecundity of infected insects; however, no population-level impacts on *L. curtus* adults in the field have been observed (Randall et al., 2017). Advancements in the evaluation process now ensure that all cryptic herbivores, parasitoids, and pathogens associated with weed biocontrol insects are eliminated during quarantine (Hinz et al., 2020).

Accidental co-introduction of plant pathogens

To the best of our knowledge, only one instance has been documented in which a plant pathogen was accidentally introduced as a hitchhiker on an insect biocontrol agent. The fungus *Passalora ageratinae* was likely unknowingly present in shipments of the gall fly *Proccidochares utilis* collected in Mexico and released in Hawaii in 1945 against crofton weed, *Ageratina adenophora* (Muniappan et al., 2009). The fungus went undetected and was subsequently unknowingly redistributed along with *P. utilis* from Hawaii to Australia, New Zealand, and India (Winston et al., 2021). To date, *P. ageratinae* has not been documented attacking any other plant species, and it has had a slight to moderate impact on crofton weed (Winston et al., 2021). This potential risk is now prevented by improved procedures in the evaluation process for weed biocontrol agents. Molecular technologies allow plant pathogens to be identified and then eliminated from quarantine colonies with DNA screening of weed biocontrol herbivores.

Damage to crops

This is one of the main risks for which new weed biocontrol agents are currently assessed. Pre-release screening of weed biocontrol agents has been highly effective in preventing damage to crop plants. Of 457 species introduced worldwide against invasive weeds through 2008, 16 insects (3.5%) have been documented feeding on crop species; however, in all cases, the damage was minor and temporary spillover with no important or continuing effect (Hinz et al., 2020). In the United States, most cases of damage to crop species occurred in Hawaii before passage of the Plant Quarantine Act of 1912 or the implementation of centrifugal phylogenetic testing (Wapshere, 1974). Under current testing requirements, even a slight potential to become a crop pest results in the agent's rejection (e.g., Cristofaro et al., 2013). This filter is now so conservative that some introduced agents that have proven to be safe and effective would be rejected (Hinz et al., 2014).

Harm to wildlife habitats or ecosystems

Because weed biocontrol agents introduced in modern times do not cause important damage to native plants, habitats created by native plants are unaffected. However, invasive plants often suppress and displace native plants and form new habitats, on which some native wildlife species come to depend as their preferred plants are missing. If invasive plants are suppressed by an effective biocontrol project, some temporary effects on wildlife may occur during the period between the control of the invasive plant and the regrowth of the native plants originally making up the habitat. For example, invasive saltcedars (*Tamarix* spp.) in the southwestern United States have displaced native willow/cottonwood habitats used by the endangered southwestern willow flycatcher, a subspecies of *Empidonax traillii*. As a result, this flycatcher sometimes uses saltcedar thickets for nesting, even though they are a poorer quality habitat for rearing offspring. Biocontrol of saltcedar aims to restore the native willow/cottonwood habitat along rivers, but there is a potential for some reduction in flycatcher nesting success unless activities promoting regrowth of native plants are paired with the biocontrol program in areas where flycatcher nesting is concentrated (Dudley and Bean, 2012). This interaction is discussed in detail in Chapter 28.

Changes to native food webs

Harm to native species via food web changes induced by an introduced weed biocontrol agents are conceivably possible, but few cases have been demonstrated. One example that has been identified is that of the highly host-specific seed fly *Mesoclanis polana* that was introduced into Australia to control the weed *Chrysanthemoides monilifera*. Because this fly shares natural enemies (predators and parasitoids) with native seed-feeding insects on native plants, increases in the biocontrol agent populations were associated with declines of some native seed predators due to the impact of their shared natural enemies (Carvalheiro et al., 2008). However, it remains unclear whether these localized events have occurred across the entire range of the native seed predators (Fowler et al., 2012).

Similarly, in the western United States, increases in populations of the biocontrol agents *Urophora* spp., which form galls on the pest weed *Centaurea stoebe*, were associated in time with increases in the abundance of *Peromyscus maniculatus*, a mouse that feeds on the gall flies (Pearson and Callaway, 2008). Food web interactions are difficult to predict in advance, but the discipline of weed biological control has increasingly acknowledged the potential for indirect impacts from biocontrol agent introductions, and efforts are made to forecast potential indirect impacts in pre-release evaluations (e.g., López-Núñez et al., 2017).

WHAT ARE RISKS OF PATHOGENS USED FOR BIOLOGICAL CONTROL?

History of Introduction and Regulation of Pathogens Used for Biocontrol

In the United States, pathogens used as biological control agents have been introduced against both weed and insect pests. Plant and insect pathogens have different regulatory histories in the United States. Plant pathogens have always been recognized as potential threats to plants and have been regulated in a consistent manner, first by the Plant Quarantine Act (1912) and then by the Plant Protection Act of 2000. Thirteen species of plant pathogens have been introduced to North America or the U.S. overseas territories as weed biocontrol agents, including two nematodes and 11 fungi (Winston et al., 2021). No plant-pathogenic viruses, bacteria, or microsporidia (now considered fungi) have been intentionally introduced as weed biocontrol agents.

Insect pathogens have been regulated under several different laws historically. Insect pathogens that have been introduced as biocontrol agents include nematodes, viruses, microsporidia (now considered fungi), bacteria, and fungi (Hajek et al., 2016). These pathogens have been regulated differently by group. Before 2000, insect pathogens (except nematodes) were regulated by the U.S. Environmental Protection Agency (EPA) under laws designed for pesticides (Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA), even when used as classical biocontrol agents rather than as formulated products. Nematodes, however, were treated differently and were regulated by APHIS in a manner similar to parasitoids and predators used in augmentative biocontrol. The review of insect pathogens (except nematodes) under rules designed for chemical pesticides ended in 2000 when the North American Plant Protection Organization (NAPPO) endorsed a similar standard to that used by the European and Mediterranean Plant Protection Organization (EPPO, 2000), based on the FAO Code of Conduct (FAO/IPPC, 1996). EPA then ceased regulating this group of agents, and APHIS provided instructions on how to petition for the release of non-native entomopathogens and insect parasitic nematodes for biological control (Hajek et al., 2007).

Across the globe, 37 species of viruses, bacteria, fungi, microsporidia, or oomycetes and eight species of nematodes have been introduced to one or more countries for insect biocontrol, with fungi being the most frequently released group, followed by viruses and nematodes (Hajek et al., 2007). The species most frequently employed have been the *Oryctes rhinoceros* nudivirus, the fungi *Metarhizium anisopliae* and *Entomophaga maimaiga*, and the nematodes *Romanomermis culicivorax* and *Deladenus siricidicola* (Hajek et al., 2016)

Possible Risks of Pathogens

For plant pathogens, the main risks of concern have been the potential infection of native, economically valuable, or crop plant species. None of the other major risk categories are applicable to weed biocontrol pathogens. Of 457 species introduced worldwide against invasive weeds through 2008, only one species of fungus (and no nematodes) has been documented causing non-target attack (Hinz et al., 2020). The fungus *Puccinia spegazzinii* was released against mile-a-minute weed, *Mikania micrantha*, in Papua New Guinea in 2008. It was subsequently found infecting the very closely related native species *Mikania cordata*, although the attack was most likely temporary spillover and occurred only where the two *Mikania* species overlapped. In the United States, no weed biocontrol pathogens have been documented attacking native or economically valuable plants or crops (Hinz et al., 2020; Winston et al., 2021).

Similarly, introduced insect pathogens may pose risks of infection to native insects related to the target pest (e.g., Lockwood, 1993). Hajek et al. (2007), however, found no documented cases in the literature of substantial mortality to a non-target species caused by an introduced insect pathogen. None of the other major risk categories are applicable to insect biocontrol pathogens.

CONCLUSIONS

Species are intentionally introduced to the United States for a variety of reasons. When these introductions are grouped according to their motives, and the histories and current regulations governing each group are considered, it becomes readily apparent that species introduced for biological control are the most highly regulated of all groups introduced to the United States and the least likely to cause important environmental or economic damage. Compared to the risks from introducing new species for reasons other than biocontrol, the risks of introducing new biocontrol agents (parasitoids, predators, herbivorous insects, and insect or plant pathogens) are small and well-regulated (Van Driesche and Hoddle, 2017; Van Driesche et al., 2020). Although some non-target impacts have resulted from biocontrol agent introductions, it must be emphasized that all forms of pest management have been documented to cause some non-target impacts. The discipline of biological control has advanced markedly over the last century. Based on comprehensive regulations and improvements in host range testing, classical biocontrol is an effective, safe, and sustainable method for the long-term management of invasive pest insects and plants.

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GROUP 1
PROTECTING FOOD AND FORAGE

Alfalfa (right) intentionally planted adjacent to organic strawberries (left) to serve as a trap crop for pest species of *Lygus*. The alfalfa allows for continuity in the lygus bug population, sustaining populations of the parasitoid *Peristenus relictus* that was successfully introduced to control lygus bugs. (D. Nieto, Driscoll's Inc.)

CHAPTER
4

Importation Biological Control of *Lygus* Species in California

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NON-TECHNICAL SUMMARY

Lygus bugs (Hemiptera: Miridae) are serious pests of many crops, including forage, fiber, fruit, and seed crops. The bug's feeding with piercing-sucking mouthparts causes developing fruits to drop off plants (e.g., cotton), to have distorted shapes (e.g., strawberries), or damaged tissue (e.g., celery). Pest lygus bugs, such as *Lygus hesperus* and *Lygus lineolaris*, are native to North America. The impact of these pests began to worsen in the 1960s, stimulating a search for alternatives to insecticides by the USDA-ARS. Native parasitoids were seldom found attacking lygus bugs in the United States, and those that did had negligible impact. Consequently, efforts began in the 1960s to import parasitoids known to suppress species of lygus bugs in Europe. After the first successful permanent establishment of the lygus bug parasitoid *Peristenus digoneutis* (Hymenoptera: Braconidae) in the eastern United States in the 1980s, it was postulated that similar introductions into the western United States might also be successful. Beginning in 1998, *Peristenus relictus* and *Peristenus digoneutis*, both from Europe, were released in the Central Valley of California. In 2002, these same two parasitoids were also released on the Central Coast of California. Only *P. relictus* became permanently established, in either inland or coastal sites. High levels of parasitism, and subsequent suppression of lygus bugs, were recorded in alfalfa fields in Sacramento, as well as in wild vegetation and organically grown strawberries in Monterey County. Post-release monitoring in 2018 indicated that *P. relictus* populations had spread 500 km (310 mi) from their original release site in Sacramento to the southern end of the Central Valley of California, and from the Monterey Bay area to the southern coast of California (Santa Barbara and Ventura Counties), almost 400 km (249 mi) away. Integration of alfalfa trap crops, including the use of tractor-mounted vacuum machines to suppress lygus bugs, is credited with enhancing the impact of *P. relictus* in organically produced strawberries. Modeling efforts, validated by field data, showed that reduced lygus bug pressure lowered yield losses, saving strawberry growers $4,697/ha ($1,900/acre). This translates to over $3.75 million in annual savings for organic strawberry growers on the Central Coast of California.

Pickett, C. H. and D. J. Nieto. 2022. Importation biological control of *Lygus* species in California, pp. 37–48. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

HISTORY AND ECOLOGY OF THE PROBLEM

There are 29 species of *Lygus* (Hemiptera: Miridae) reported from North America (Schwartz and Foottit, 1998), of which seven are considered agricultural pests (Kelton, 1975). *Lygus lineolaris* is widely distributed throughout North America and is the principal agricultural pest *Lygus* species in the eastern United States and Canada. *Lygus hesperus* is widely distributed in the western United States, where it too is considered an important pest (**Fig. 1**). Along with *L. hesperus, L. elisus* and *L. shulli* can also be found damaging crops in California. In our surveys, *L. shulli* was found in the climatically mild Monterey Bay region (Pickett et al., 2009), whereas we collected *L. elisus* from the much warmer Central Valley (Pickett et al., 2007).

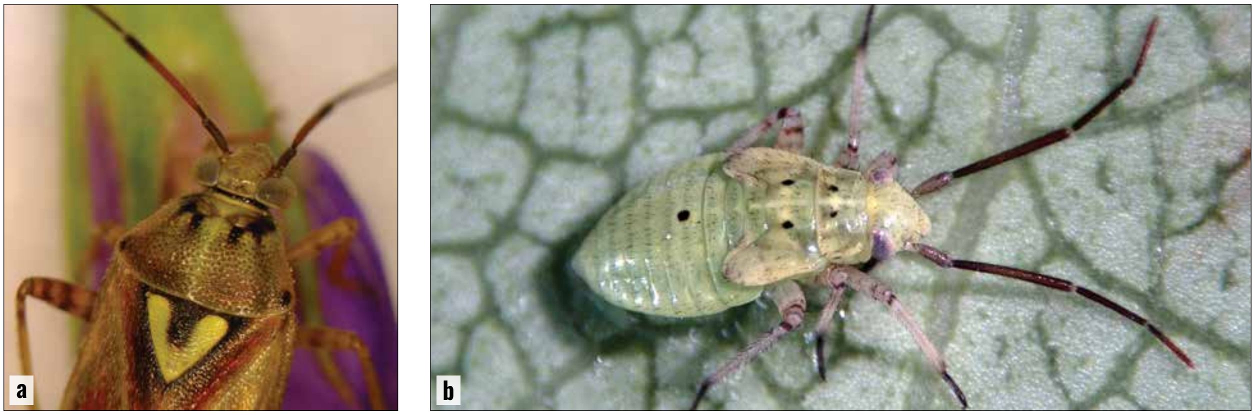

Figure 1. *Lygus hesperus* (a) adult and (b) nymph. (a,b: D. Nieto, Driscoll's Inc.)

Although some are pests, lygus bugs (e.g., *L. lineolaris*) are endemic to North America. In California, many of the cropping systems where these *Lygus* species feed are irrigated areas planted to non-native plants, and exploitation of these plants broadens the feeding opportunities for these bugs. Before the expansion of modern agriculture into California, the Central Valley was covered with native grasses that were dormant during the summer months. Today, production of crops such as alfalfa allows lygus bugs in summer to move from their overwintering sites into a very warm, lush community of flowering and fruiting agricultural crops (Carrière et al., 2012). Contemporary agriculture in California, which is irrigated, year-round, and spatially concentrated, is especially favorable to lygus bugs, which can feed on many crop types, such as forage crops, fruits, vegetables, and nut orchards. Lygus bugs readily migrate between crops based on relative feeding preferences and flowering times of different crops. Furthermore, Day (1996) speculated that native lygus bug parasitoids may not be attracted to the same introduced plants that these pest lygus bugs prefer to feed on.

WHY CONTROL THIS NATIVE PEST?

Lygus bugs in North America are polyphagous. For example, *L. hesperus* has been reported on over 100 plant host species across 24 plant families (Scott, 1977), while *L. lineolaris* has been reported on over 300 plant species within 55 families (Young, 1986). Consequently, *Lygus* species are considered serious pests of a broad range of agricultural commodities in North America, including beans (Nagalingam and Holliday, 2015), strawberries (Zalom et al., 1990; Day and Hoelmer, 2012), apples (Day et al., 2003), cotton (Leigh et al., 1988), and various seed crops, including canola (Turnock et al., 1995).

Lygus bugs use a 'macerate and flush' feeding strategy, injecting various salivary enzymes into plant tissue that break down the tissue's physical and chemical defenses via extraoral digestion, i.e., breaking down food outside of the gut. This feeding behavior allows lygus bugs to feed on a variety of plant host structures, including leaves, stems, meristematic tissue, flowers, and seeds. Plant damage from lygus bug feeding can be expressed in various ways, including localized tissue damage (e.g., on celery or lettuce), abscission of fruiting structures (e.g., on cotton or pistachio), fruit deformation (e.g., on strawberry or apple), altered vegetative growth (e.g., on blackberry or carrot), and tissue malformation (e.g., on cotton or sugar beet) (Tingey and Pillimer, 1977). Such opportunistic feeding by lygus bugs causes significant yield losses. For instance, estimates from Ontario, Canada show that lygus bugs annually reduce yields in fruit and vegetable crops by 5%, resulting in losses of Can $12 million; in the alfalfa seed crops of Saskatchewan, lygus bugs can cause Can $50 million in annual losses if left untreated (Broadbent et al., 2002). Insecticide resistance, which has been reported in populations of *L. lineolaris* (Dorman et al., 2020) and is presumed to be common in populations of *L. hesperus* (Zalom et al., 2018), further exacerbates potential economic losses.

The use of introduced biological control agents to suppress lygus bug populations was justified given (1) the negative economic impact caused by these native insects, (2) the wide geographical and host plant ranges of this pest, (3) the lack of effective pre-existing native biocontrol agents of lygus bugs, (4) the unlikely risk of lygus bug extinction, and (5) the potential environmental improvements brought about through reduced insecticide use.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

In the 1960s, due to increasing economic losses from lygus bugs, the USDA-ARS initiated a project to investigate the role of natural enemies affecting lygus bug populations in the United States (Hedlund and Graham, 1987). These surveys found little to no parasitism of lygus bug nymphs. Day (2005) later reported average parasitism values of 10% or less for *L. lineolaris* in the eastern United States. Clancy and Pierce (1966) similarly found that <2% of *L. hesperus* nymphs in southern California were parasitized, and in central California no nymphs swept from alfalfa were found to be parasitized (Clancy, 1968). Van Steenwyk and Stern (1977) failed to find any native parasitoids of *Lygus* before their attempt to establish *Peristenus relictus* (= *stygicus*) in the 1970s in central California. Pickett et al. (2007) repeated these surveys in the late 1990s to look for parasitoids of lygus bugs in alfalfa in central California, and they also found no parasitism.

The lack of effective biocontrol of lygus bugs in the United States provided the incentive to begin explorations for novel parasitoids from abroad. In Central Europe, the endemic *Lygus rugulipennis* is widespread but is not commonly considered to be an important pest (Haye, 2004), suggesting natural enemies play an important role in controlling the bug's populations. In the late 1960s, the USDA-ARS initiated a survey of Europe, in cooperation with the Institute of Ecology at the Polish Academy of Sciences in Warsaw for braconids attacking *Lygus* spp. (Hedlund and Graham, 1987). These studies identified parasitoids associated with lygus bugs in Europe and developed rearing procedures suitable for the parasitoids (Bilewicz-Pawinska, 1975).

From this early work, two species emerged as potential candidates for release into North America: *Peristenus digoneutis* and *P. relictus* (then called *P. stygicus*). These nymphal parasitoids oviposit single eggs into young lygus nymphs (**Fig. 2**). The parasitoid larva consumes its host internally, killing it in the final nymphal developmental stage (**Fig. 3**). The discovery of these parasitoid species, which exhibited parasitism levels two to three times greater than

Figure 2. *Peristenus relictus* parasitizing a lygus bug nymph. (D. Nieto, Driscoll's Inc.)

Figure 3. *Peristenus relictus* larvae (instars 1–3) next to a 3rd-instar lygus bug nymph. (D. Nieto, Driscoll's Inc.)

that caused by the native parasitoid complex in the United States, led to a decades-long effort to import and permanently establish new nymphal parasitoids of lygus bugs in the United States (Hedlund and Graham, 1987; Pickett et al., 2017) and Canada (Broadbent et al., 2002, 2006).

Peristenus spp. were collected in Poland in 1970 and were subsequently released in Arizona after transiting through the USDA-ARS quarantine facility in Moorestown, New Jersey (Hedlund and Graham, 1987). These releases did not result in permanently established populations. Thereafter, collections and shipments of *P. digoneutis* and *P. relictus* came through either the USDA-ARS European Biological Control Laboratory (previously known as the ARS European Parasite Laboratory) in France or CABI Bioscience in Switzerland (Pickett et al., 2007). Beginning in the 1970s, Van Steenwyk and Stern (1977) received *P. relictus* (ex. France and Turkey) and made releases into a 2.8 ha (6.9 acre) pesticide-free plot of alfalfa in the southern San Joaquin Valley in California. While *P. relictus* successfully overwintered up to two years after release, parasitoid recoveries dwindled and ultimately did not lead to a permanently established population (Van Steenwyk and Stern, 1977). During the same period, releases of *Peristenus* spp. in Texas, Mississippi, and Canada (both *P. relictus* and *P. digoneutis*) similarly failed to result in permanent establishment (Hedlund and Graham, 1981; Broadbent et al., 2002). Pickett et al. (2007, 2009) in California resurrected this effort in 1998 after learning of the first successful permanent establishment of *P. digoneutis* in the United States, which occurred in northern New Jersey in the late 1980s (Day, 1996).

Releases in Central California

Both *P. relictus* and *P. digoneutis* were released in central California beginning in 1998 (Pickett et al., 2007). Releases were initially made for a minimum of three years at each of six sites. Release locations were free of pesticides and relied on alfalfa as an insectary plant because lygus bugs thrive on this forage crop, which is itself Eurasian in origin. At one such site, releases were made in a 0.2 ha (0.5 acre) plot of alfalfa in Sacramento managed by the California Department of Food and Agriculture (CDFA). The plot was periodically strip cut, leaving alternating 3-m (10-ft) strips of cut and uncut alfalfa. This practice provided continuous alfalfa foliage for lygus feeding and a buildup of thatch for overwintering of parasitized lygus bugs. To simulate rainfall, alfalfa was watered with overhead sprinklers and never flood-irrigated.

Of the six locations where *P. relictus* was released in central California by Pickett et al. (2007), initial recoveries were made at the CDFA-managed site in Sacramento and at the University of California (UC) Kearney Agricultural Center near Fresno. However, only at the CDFA site did the population of *P. relictus* continue to increase after releases, and that site is the presumed origin of the *P. relictus* population now established in central California. The successful establishment of *P. relictus* in Sacramento may be attributable to high lygus bug densities in the plot, the exclusion of insecticide applications, and the use of alfalfa cultivation practices favorable to parasitoid development (e.g., use of overhead sprinkler irrigation and leaving of alfalfa cuttings on soil as thatch).

Peristenus digoneutis was also recovered from the CDFA site in Sacramento during initial post-release collections, albeit less frequently than *P. relictus* (Pickett et al., 2007). However, by 2010, *P. digoneutis* was no longer recovered in central California (Pickett et al., 2013). The failure of this species to permanently establish in central California is likely due to climatic factors (Pickett et al., 2007). Furthermore, climatic changes since these releases may have now made summer temperatures in central California too high for persistence of populations of *P. digoneutis*, given that the fecundity and longevity of this species decline at or above 30°C (86°F) (Whistlecraft et al., 2010). Similarly, Day et al. (2000) found that the southern expansion of *P. digoneutis* in the eastern United States stopped in areas where summer high temperatures reached 30°C (86°F) for 14–30 days.

Releases in the Central California Coast

Given the successful establishment of *P. relictus* in the Central Valley, a release program was initiated on the Central Coast of California, which possesses a large strawberry production area where lygus bugs are key pests that trigger frequent insecticide application (Pickett et al., 2009). We were invited by Dr. Sean Swezey of UC Santa Cruz to make releases into organically certified strawberries at the Eagle Tree site, grown by Pacific Gold Farms, Inc. in Monterey County. In addition to producing pesticide-free strawberries, the owner Larry Eddings, with Dr. Swezey's guidance, was experimenting with alfalfa trap crops—one row of managed alfalfa for every 50 rows of strawberries (Swezey et al., 2007) (**Fig. 4**). The alfalfa was highly attractive to lygus bugs and was routinely vacuumed for mechanical control of this mirid pest (**Fig. 5**).

Figure 4. Alfalfa trap crop adjacent to organic strawberries: (a) closeup of alfalfa strip to right of strawberries; (b) strips of alfalfa trap crops as they appear at the organic commercial strawberry farm in Monterey County. (a: D. Nieto, Driscoll's Inc.; b: C. Pickett, CDFA)

Surveys conducted before releases on the Central Coast scarcely detected the native parasitoid *Peristenus* nr. *howardi* (≤6%) in either lygus bugs or an exotic European mirid, *Closterotomus norvegicus*, collected from wild vegetation. Furthermore, when lygus nymphs were collected from alfalfa or strawberry, no parasitism was detected (Pickett et al., 2009). *Peristenus relictus* and *P. digoneutis* were subsequently released into wild vegetation at two sites on the Central Coast, near Castroville in Monterey County and near Watsonville in Santa Cruz County in 2002 and 2003.

Figure 5. Tractor-mounted vacuum used to remove lygus bugs from strawberry rows. (C. Pickett, CDFA)

In 2004, the first releases of *P. relictus* and *P. digoneutis* were made into single rows of alfalfa (interplanted among rows of strawberry), which were left intact over several consecutive years (i.e., the alfalfa remained, even after adjacent strawberry rows were rotated out of production). This intercrop allowed for continuity in the host population, which was critical for sustaining initial populations of *Peristenus* species. Recoveries of *P. relictus* were made during the following years of 2003 to 2007 at all three release sites (i.e., in alfalfa rows, strawberries, and wild vegetation). These releases led to permanently established populations in Santa Cruz and Monterey counties (Pickett et al., 2009).

Peristenus digoneutis, on the other hand, was not recovered during the initial post-release collections from 2003 to 2007 on the Central Coast. Unlike central California, summers along the Central Coast are mild and thereby not likely to negatively affect *P. digoneutis* development (Pickett et al., 2007). However, insufficiently cold winters along the coast may not meet the 'chilling' requirement of *P. digoneutis* needed to break diapause after overwintering. The population of *P. digoneutis* released on the coast was particularly ill-suited in this respect. The region of Catalonia in Spain, where *P. digoneutis* was collected, has only a moderate compatibility rating of 0.54 (on a scale of 0–1) with the release sites near Salinas, California (Pickett et al., 2007).

In response, renewed efforts were made to collect *P. digoneutis* from a European location that was climatically more like the Central Coast of California. To that end, lygus parasitoids were collected from Brittany, France, which also experiences mild winters. Work was conducted with help from the USDA-ARS European Biological Control Laboratory, and the new population of parasitoids was released across six sites in Monterey County from 2013 through 2019. Subsequent recoveries of *P. digoneutis* on the Central Coast, however, have been infrequent and limited. While the prospects for this parasitoid's establishment have dimmed, we remain hopeful. Day et al. (1990) reported that several years (post-release) passed before notable recoveries of *P. digoneutis* were made in the eastern United States.

Releases in the Southern California Coast

In coastal Santa Barbara and Ventura Counties, lygus bugs are pests of strawberry and, increasingly, of raspberry and blackberry. Releases of *P. relictus* were made by the senior author (CHP) along the southern California coast in the town of Oxnard in 2009 (Pickett et al., 2010). As *P. relictus* was not recovered in post-release sampling, additional releases were made in Oxnard from 2016 through 2017. Subsequent recoveries of *P. relictus* collected from weeds, cover crops, and alfalfa were made from 2016 through 2018 (Nieto et al., 2020). In September 2021, four 200-sweep samples were taken from a row of organic alfalfa in Oxnard with 51.6 ± 8.1% parasitism by *P. relictus*, showing that *P. relictus* has successfully established on the southern California coast.

HOW WELL DID BIOLOGICAL CONTROL WORK?

Impact in the Northeastern United States

The establishment of *P. digoneutis* in the northeastern United States had a substantial impact on regional lygus bug population densities. By 1992, thirteen years after the first releases of *P. digoneutis* in Blairstown, New Jersey, *P. digoneutis* could be found northward as far as New York state. Nymphal parasitism of *L. lineolaris* increased several-fold, reaching 50% in alfalfa fields where the parasitoid was first released (Day, 1996). Lygus bug nymphal numbers in the same locations decreased by 75% from estimates made in the early 1980s. Day et al. (2003) reported that apple fruit damage from lygus bugs following parasitoid establishment in New Hampshire declined by 63% over a ten-year period, which was attributed to the impact of *P. digoneutis* on the pest population.

Impact in Central California

Peristenus relictus had a significant impact on the lygus bug population in the CDFA-managed plot of alfalfa located in an industrial area of Sacramento, California. In 2003, six years after the first trial plot was planted to alfalfa, lygus bugs that immigrated into the newly planted field reached a peak seasonal average of 11 nymphs per sweep (**Fig. 6**). The first release of a mixed population of ca. 1,100 *P. relictus* and *P. digoneutis* adults and developing larvae was made in September 1998. Subsequent monthly releases of ca. 2,200 adults and larvae (primarily *P. relictus*) were made at the same location during the summers of 1999 and 2000. Parasitism reached a seasonal average high of 54% in 2002; the monthly peak of 60% for that year occurred in August. Parasitism then declined as the host population correspondingly dropped to <2 nymphs per sweep during the final five years of sampling, 2007–2011 (**Fig. 6**). The biological control impact in this Sacramento trial plot demonstrates that the introduction of *P. relictus* can effectively suppress a lygus bug population, provided conditions are favorable (e.g., an initial high host density, lack of pesticide exposure, and stable overwintering sites).

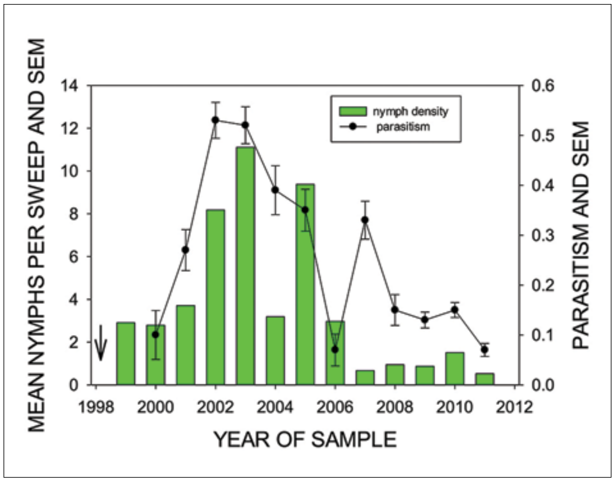

Figure 6. Parasitism of lygus bugs by *Peristenus* spp. in alfalfa in Sacramento, California. Arrow indicates date parasitoids were first released. (from Pickett et al., 2017; reprinted with permission from Elsevier)

After its initial establishment in central California, the *P. relictus* population spread to the surrounding areas in the San Joaquin Valley. By 2011, *P. relictus* was collected 213 km (134 mi) south, 35 km (22 mi) north, 35 km west and 30 (19 mi) km east of the original release site (Pickett et al., 2013). Parasitoid dispersal was most extensive to the south and included a recovery in Fresno County about 30 km away from the UC Kearney Agricultural Center where we had previously released *P. relictus* for several years without achieving establishment (Pickett et al., 2007; 2013). By 2018, *P. relictus* had also been collected from the southern-most counties in the San Joaquin Valley, about 500 km south of Sacramento, showing that this parasitoid had dispersed south throughout central California (Nieto et al., 2020). It should be noted, however, that biocontrol in these southern counties in central California, e.g. Kern Co., had only low rates (2%) of parasitism, and it may be that parasitism is hindered in that area by high summer temperatures.

Impact in the Central California Coast

Striking results were also recorded at two release sites comprised of wild vegetation in the Monterey Bay region along the Central Coast of California. From 2003 to 2014, lygus bugs were collected from unsprayed weedy vegetation—wild radish (*Raphanus raphanistrum*), mustards (*Brassica* spp.), and arrowleaf saltbush (*Atriplex triangularis*)—that grew near conventionally-grown strawberries. Average annual (for 2003–2014) parasitism peaked at 56% and 71% in 2011 for both of these sites, which caused lygus bug population density to decline by over 99% shortly thereafter (Pickett et al., 2017). These two Central Coast examples, along with the interior valley site in Sacramento, and those by Day (1996) in the northeastern United States, show the potential for biological control of lygus bug in undisturbed environments by the introduced European species of *Peristenus* parasitoids. Lygus bug numbers also declined in the organically produced strawberries at Eagle Tree (Prunedale, California) from a high of 2.6 nymphs per 50 suctions using a hand-held vacuum machine in 2003 to <1 nymph per 50 suctions, which is well below the economic threshold (Pickett et al., 2009, 2017). In habitats with higher levels of disturbance, such as conventionally managed strawberries, the impact of parasitism on lygus bug densities did not show a density-dependent response and did not meaningfully reduce pest populations.

The use of alfalfa trap crops in organic strawberry fields also proved useful for the biological control of lygus bugs. Unlike strawberries, which support extremely low lygus bug densities, trap crops provide spatially concentrated, host-rich habitats for *P. relictus*. Parasitism rates are consequently higher in the alfalfa strips than in the adjacent strawberry rows (Swezey et al., 2014). Trap-cropping thereby solves a conundrum that often afflicts biological control: high pest densities are needed to achieve optimal biocontrol from density-dependent parasitoids in what is typically a disturbed habitat. By interplanting alfalfa within strawberry fields, growers can benefit from *P. relictus* without suffering the excessive yield losses that are associated with high pest densities. Replicated field studies demonstrated that lygus bug parasitism in trap crops managed by using tractor-mounted vacuums was no different than non-vacuumed (control) trap crops at the organic strawberry ranch, Eagle Tree. Managing alfalfa with vacuum machines did not affect the percentage of lygus bug nymphs that were parasitized by *P. relictus* (**Fig. 7**). It should also be noted, however, that parasitism rates provided by *P. relictus* in alfalfa trap crops managed with conventional insecticides were reduced when compared to control trap crops.

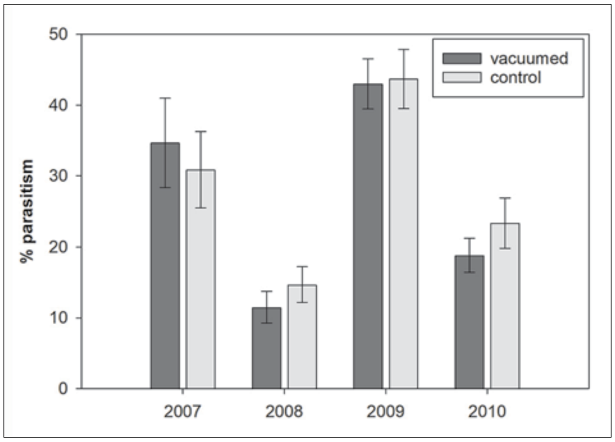

Figure 7. Mean percent parasitism of lygus bug nymphs in alfalfa trap crops with and without exposure to a tractor-mounted vacuum. (C. Pickett, CDFA)

Impact in the Southern California Coast

As in central California, *P. relictus* populations dispersed widely along the coast after establishment, particularly to the south. By 2011, populations of this parasitoid had extended into San Luis Obispo County, which is 150 km (93 miles) south from the original point of release (Pickett et al., 2013). By 2018, *P. relictus* was also collected along the southern California coast in Santa Barbara and Ventura counties, which meant that the parasitoid zone of establishment then covered all major areas of strawberry production in California (Nieto et al., 2020).

Side Effects of Lygus Biological Control on Native Insects

Classical biological control refers to the reunification of an exotic pest and a co-evolved natural enemy from its native range. The importation of *Peristenus* spp. for control of *Lygus* spp. in North America, however, falls into a category of biological control referred to as 'new associations' (Smith et al., 1993; Alleyne and Wiedenmann, 2001). *Lygus hesperus* and *L. lineolaris* are native to North America and likely became economically important to agriculture due to the ecological changes associated with modern, intensive farming practices, including use of exotic host plants. Ultimately, the rationale for biological control in both cases remains the same: the lack of specialized natural enemies that are capable of effectively regulating a pest population. Greathead (1995) has argued that new-association biocontrol makes sense if the target pest has close relatives from other parts of the world that are attacked by specialized natural enemies with limited or no impact on non-target species. Examples of new-association biological control can be found in Van Driesche et al. (2008).

Introductions of new-association biocontrol agents, including *Peristenus* species in North America (Day et al., 1990; Broadbent et al., 2002; Pickett et al., 2007) occurred before regulations requiring host range testing of new exotic insects for control of arthropods were implemented in the United States. At that point in time, the most important considerations pertaining to biocontrol candidates were their levels of parasitism, their likely impact on the target pest, and the freedom from contaminants such as hyperparasitoids in quarantine cultures. More limited host ranges indicate more specialized natural enemies (DeBach, 1964; Heimpel and Mills, 2017). Such natural enemies are considered safer to the environment; however, they may not be better biological control agents (see, Van Driesche et al., 2020). Demonstration of safety to non-target insects became a requirement to obtain a field release permit from USDA-APHIS beginning in the early 2000s.

Current evaluations of biocontrol agents are based largely on host specificity, which is estimated by a candidate's inability to parasitize non-target hosts in laboratory tests. Physiological host range tests, which often use no-choice exposures to non-target hosts in confined experimental settings, determine if a parasitoid is physiologically capable of using a given host for reproduction. A parasitoid's ecological host range, on the other hand, is limited to what is feasible given the physical, biological, and behavioral limitations that influence the parasitoid in its natural environment (Onstad and McManus, 1996). Female parasitoids searching for hosts must navigate their way through a visually and chemically complex plant habitat, guided by plant and animal volatiles, especially host kairomones. For parasitoids that are physiologically capable of being polyphagous, accounting for these real-world limitations is critical when formulating risk assessments. While *P. digoneutis* is more specialized towards lygus bug species than is *P. relictus*, both parasitoid species will nonetheless parasitize non-target hosts (i.e., non-*Lygus* mirids) in no-choice laboratory settings (Condit and Cate, 1982; Haye et al., 2005). However, field collections indicate that actual parasitism of non-*Lygus* hosts was <1% by *P. digoneutis* and generally <5% by *P. relictus* (Haye et al., 2004, 2005). Furthermore, field studies by Day (1999, 2005) demonstrated both the specificity displayed by *P. digoneutis* for lygus bug hosts and the corresponding small impact its establishment has had on non-target North American mirids, based on 19 years of post-release field collections. These studies collectively demonstrate the low probability of either *Peristenus* species suppressing populations of any non-target mirids in the United States.

The perception of impacts from the establishment of *Peristenus* species in the United States by regulatory agencies and the general public often focuses solely on potential risk. A more appropriate perspective on the consequences of this importation biocontrol project, however, would balance both the benefits and risks associated with such an endeavor. For instance, reductions to pest populations that are brought about by biological control should help to reduce insecticide use. Such reductions can be quite meaningful in California strawberries, which receive numerous, often calendar-based, broad-spectrum insecticide applications during each growing season (e.g., Pickett et al., 2009). This risk is made apparent to consumers by the routine inclusion of strawberries in the so-called 'Dirty Dozen' list, which names fruits and vegetables that contain the highest levels of pesticide residues based on USDA testing data (Environmental Working Group, 2021). Benefits of a diminished insecticide load include safer working conditions for farm laborers, less 'drift' into neighboring communities (Lombardi et al., 2021), less water pollution, and reduced pesticide residues in purchased fruit. Effective biocontrol also promotes a growing, robust organic farming sector, which currently constitutes nearly 13% of California's strawberry acreage (CSC, 2020). Consequently, future decisions regarding the appropriateness of releasing a biocontrol agent would be improved by adopting a benefit-risk analysis where the probability and magnitude of potential positive and negative outcomes are considered (Heimpel and Mills, 2017). Using *Peristenus* as a retroactive example, parasitism of non-target hosts is possible but quite minimal in its impact, whereas benefits are both realized and beneficial to society at large.

BENEFITS OF BIOLOGICAL CONTROL OF LYGUS BUG

To better understand the economic benefits provided by *P. relictus* in California strawberry fields, a simulation model of the type developed by Holst (2013) was used to approximate the impact of this parasitoid's introduction on lygus bug populations along the California Central Coast (Nieto et al., 2022). The theoretical lygus bug population used in this model was developed by incorporating 10 years of local weather data, a *L. hesperus* degree-day phenology model, and lygus bug immigration rates that were derived from local field studies. The extent to which parasitism affected this theoretical pest population was validated by 15 years of field-collected parasitism data from strawberry fields in Monterey and Santa Cruz counties from 2002 to 2018. When parasitism by *P. relictus* was applied and its population consequences compounded over a 10-year span, the theoretical lygus bug population was ultimately reduced by 70% when compared with a similar population that was never exposed to *P. relictus*. This diminished pest pressure equates to a reduction in yield losses due to lygus bug feeding that would save growers $4,697/ha ($1,900/acre) of organic strawberries. If applied to the 2021 acreage of organic strawberries in Monterey and Santa Cruz Counties, the annual savings due to the introduction of *P. relictus* is valued at over $3.75 million for the central California coast organic strawberry production alone.

Despite the observed and modeled reductions to lygus bug populations in California, *P. relictus* remains largely unrecognized as having made a positive contribution to the management of lygus bugs. This is partially because adult wasps are inconspicuous, and parasitized lygus bugs are not distinguishable from their non-parasitized counterparts by growers or field scouts. In addition, because most growers continue to perceive lygus bugs as a pest of economic importance, the establishment of *P. relictus* has not had a discernible effect on the frequency of lygus-directed conventional insecticide applications. A recent shift to conservation-based biocontrol strategies for *P. relictus*, such as by incorporating parasitoid-compatible insectaries, may improve both the performance of, and recognition for, this introduced biocontrol agent.

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CHAPTER 5

Better Control of Imported Cabbageworm, *Pieris rapae*, in Organic Brassicas in the Eastern United States

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NON-TECHNICAL SUMMARY

The cabbage butterfly (*Pieris rapae*, Lepidoptera: Pieridae) is the common white butterfly that we see in our gardens. The immature stage, referred to as the imported cabbageworm, feeds on broccoli, kale, and a variety of other vegetable plants in the genus *Brassica* (Brassicaceae). These larvae damage plants by chewing on the leaves, and severe infestations can cause complete defoliation. While *P. rapae* is one of the most common butterflies in the United States, it is a non-native invasive pest that reached North America in the 1860s. At that time, a very limited effort was made to use its European natural enemies against it. This effort resulted in the establishment of one parasitoid, *Cotesia glomerata* (Hymenoptera: Braconidae). This parasitoid, while becoming common, did little to reduce the damage caused by imported cabbageworm. A century later (from the 1960s through 1988), an attempt was made to introduce a different species, *Cotesia rubecula*, whose biology was more likely to provide some control of this pest. In 1988, introduction of *C. rubecula* from China succeeded. Work in New England showed that this new parasitoid reduced the damage done by *P. rapae* caterpillars in non-sprayed organic vegetable farms by 75%. The introduction of *C. rubecula* also reduced *C. glomerata* by more than 90%, which allowed a native, non-pest white butterfly, *Pieris oleracea*, to start expanding its range after a century of decline.

HISTORY OF INVASION AND NATURE OF PROBLEM

Pieris rapae (**Fig. 1**) (Lepidoptera: Pieridae) is a common pest of brassica crops (Brassicaceae or the mustard family) in Eurasia that was first found in North America in Quebec, Canada in 1860, possibly transported on stored cabbages on ships. By 1869, it was recorded in Boston, Massachusetts (Scudder, 1889). It soon became a common and important pest in the United States on cabbage and, over time, in all related brassica crops in both gardens and commercial fields (Chittenden, 1926). Biological control efforts in the 1880s

Van Driesche, R. G. 2022. Better control of imported cabbageworm, *Pieris rapae*, in organic brassicas in the eastern United States, pp. 49–58. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

Figure 1. The imported cabbageworm, *Pieris rapae.* (Ansel Oommen, Bugwood.org CC BY-NC 3.0 US)

Figure 2. The native veined white, *Pieris oleracea.* (Susan Elliott, iNaturalist.org CC BY-NC 4.0)

(Clausen, 1978) eventually led to displacement of a native butterfly, the veined white (*Pieris oleracea*) (**Fig. 2**), from southern New England due to the introduction of the parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) (Benson et al., 2003a). New biocontrol efforts were not made until the 1960s through the 1980s. In 1988, the successful establishment of a more specialized parasitoid (*Cotestia rubecula*) (Van Driesche and Nunn, 2002) provided better control of the pest and, by outcompeting *C. glomerata*, allowed the veined white to begin to re-establish and spread in southern New England (Herlihy et al., 2014).

WHY CONTROL THIS INVASIVE SPECIES?

High levels of feeding by *P. rapae* caterpillars reduce yield and quality of brassica plants (cabbage, broccoli, Brussels sprouts, kale, collards) in both commercial fields and gardens. Without controls of some type, losses can be significant (e.g., Bare, 1940; Wolfenbarger and Hibbs, 1958). In commercial conventional fields, a variety of syntethic pesticides are widely and commonly used. On organic vegetable farms, pesticides, including products containing the bacterium *Bacillus thuringiensis,* and others approved approved by organic producers' organizations are used in a similar way.

THE ECOLOGY OF THE PROBLEM

Pieris rapae is an important pest of brassica crops, but it co-occurs with other leaf-feeding pests such as diamondback moth (*Plutella xylostella*) and cabbage looper (*Trichoplusia ni*), which also must be controlled for good crop production (Anon., 2002). *Pieris rapae* also feeds on many species of native and invasive mustards, which can sustain *P. rapae* populations outside of crop fields (Wagner, 2005). Adult butterflies produced in these non-crop areas can rapidly disperse over large areas and infest crops. Consequently, control in production fields is not aimed at long-term or regional control but rather at protecting the immediate crop, usually through conventional or organic pesticides or possibly using protective row covers over plants in the field during the most vulnerable periods of each crop (Adams et al., 1990). Cabbage and broccoli become relatively less susceptible to this pest as they mature. In contrast, Brussels sprouts, collards, and kale are attacked throughout the growing season.

Classical biological control can contribute to the reduction of this *P. rapae* damage in two ways. First, specialized parasitoids can reduce the numbers of the pest produced on non-crop mustard weeds in non-cultivated areas. This lowers the regional pool of invading adults available to attack newly planted crop fields. Secondly, on organic farms, parasitism by *C. rubecula* parasitoids can avoid up to 70% of the crop damage by killing larvae while they are still small (4th instar) (Rahman, 1970) rather than allowing them to become mature (5th instar).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Three parasitoids were released in North America against *P. rapae*; *Cotesia glomerata* and *Cotesia rubecula* are both braconids that attack young larvae while *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae) is an egg parasitoid (Clausen, 1978; Van Driesche and Nunn, 2002). A fourth resident species, *Pteromalus puparum* (Hymenoptera: Pteromalidae), attacks the chrysalis (pupal stage) of *P. rapae*; *P. puparum* either was accidentally introduced into North America (Clausen, 1978) or is native (having been recorded in Canada in 1844 [Scudder, 1889]). *Pteromalus puparum* also attacks pupae of butterflies in several other families (Barron et al., 2003). Although *P. puparum* sometimes causes high rates of parasitism in *P. rapae* pupae collected from non-sprayed brassica crops (Lasota and Kok, 1986), by itself it does not reduce damage from *P. rapae*.

Use of the egg parasitoid *T. evanescens* was based on its seasonal mass release, rather than the action of established populations. Following mass releases, *T. evanescens* was recovered for a short time in release fields (Oatman et al., 1968). When used as a biological pesticide (whereby releases are made of thousands of wasps reared in commercial insectaries), it has shown promise for control at the field level (Parker, 1970; Oatman and Platner, 1972). However, it does not appear to either establish or spread. Therefore, it is likely not present in fields where it has not been released (e.g., Shelton et al., 2002) or in wild weedy mustard plants. Augmentative releases of insectary-reared *T. evanescens* are not presently used against *P. rapae* in the United States, likely due to the cost of such releases (relative to the cost of pesticides), limited availability of *T. evanescens* from suppliers, and the difficulty of integrating such releases with pesticides used for control of other pests in conventional brassica crops.

Cotesia glomerata (**Fig. 3**), a larval parasitoid of *P. rapae* in Europe, was chosen for importation by the USDA in the 1880s based on brief surveys in England and Germany. After some initial failures (Clausen, 1978), the parasitoid's cocoons were imported from England, and the adults that emerged were released in 1884, resulting in the establishment of *C. glomerata* in eastern North America (Clausen, 1978). In Europe, *C. glomerata* is principally a parasitoid of the large white (*Pieris brassicae*); however, in North America *C. glomerata* adapted well to the small white (= imported cabbageworm), *P. rapae*. Because *C. glomerata* produces many offspring (20–100) in each caterpillar it attacks, this parasitoid spread widely and became common. Two disadvantages of *C. glomerata* were that it has a wide host range (discussed in a later section) and that it does not kill imported cabbageworms until they are fully grown. It was subsequently shown that parasitized caterpillars eat more than healthy caterpillars (Clausen, 1978). Consequently, even high rates of *C. glomerata* parasitism do not reduce damage in individual fields.

Figure 3. The parasitoid *Cotesia glomerata*. (David Marquina Reyes, iNaturalist.org CC BY-NC_ND 4.0)

Figure 4. The parasitoid *Cotesia rubecula.* (David Cappaert, Bugwood.org CC BY-NC 3.0 US)

In contrast to *Cotesia glomerata*, *C. rubecula* (**Fig. 4**) produces only one offspring in each caterpillar that it attacks. However, *C. rubecula* kills caterpillars while they are still small, therefore avoiding more than 70% of the feeding damage that would otherwise occur (Rahman, 1970). Efforts to establish *C. rubecula* in the United States were not initially successful, most likely due to a poor match between the climate where the wasps were collected (in the former Yugoslavia) and where they were released in the central and eastern United States (Nealis, 1985). However, a population of this parasitoid was discovered in British Columbia in the 1960s, presumably from an unknown introduction. This strain was redistributed to Missouri, New Jersey, South Carolina, and Ontario (Puttler et al., 1970), but generally failed to establish, except perhaps in Ontario (Corrigan, 1982). A second attempt to establish the species in the eastern United States was made by collecting it in the former Yugoslavia, which was believed to be a better climate match than the previous attempt because its cold winters were more like the intended release areas. Wasps from Yugoslavia were released in Missouri, Virginia, and Ontario, and in 1988 it was recovered in Virginia, but that population did not persist. A third attempt was then made using wasps obtained from Beijing, China whose climate was a good match to New England. Wasps collected from China were released from 1988 to 1993 at 17 locations in Massachusetts, Connecticut, and Rhode Island, and the species established easily and spread rapidly (Van Driesche and Nunn, 2002). Further releases were subsequently made in additional states.

HOW WELL DID IT WORK

What Impacts Really Matter?

What matters to farmers faced with infestations of imported cabbageworms is the level of damage in their crops. If that can be measured, it is the best assessment of how well any control measures have worked. However, this is complicated because populations of imported cabbageworm exist both inside crop fields and in the much greater expanse of uncultivated meadows and river borders where sunny fields support native and introduced species of wild mustard plants suitable as food for *P. rapae* caterpillars.

Single samples of the pest and its parasitoids taken from a farmer's field can only partially show how important a biocontrol agent might be (a method commonly called "sample percent parasitism"). A better approach is to calculate how each life stage of the pest survives or dies over a whole generation of the pest, and what kills the individuals that die. This approach is called building life tables, which are similar to the actuarial tables used by life insurance companies to set insurance rates for human policy holders. Life tables sum up events over a whole generation of the pest and show which types of mortality are most important. An important feature that affects what farmers care most about—how much damage happens—is exactly how old caterpillars are when they die. There are five life stages of caterpillars (called instars) for imported cabbageworm, and nearly 70% of the total feeding by a caterpillar is due to feeding in the final 5th instar, which can be a thousand times more than a newly hatched caterpillar (Rayman, 1970). Control measures that kill larvae before they reach that stage are much more effective at reducing damage. In the following section we consider the effects of *C. rubecula* on *P. rapae* in view of these various ways to measure parasitoid impact on a pest.

Impact in Research Plots and Farm Surveys

In the mid-1980s in Massachusetts, before the introduction of *C. rubecula* from China, I measured rates of parasitism of *P. rapae* larvae due to *C. glomerata* and found them to be in the 60–80% range in a patch of non-sprayed collard plants (a brassica crop that remains suitable for *P. rapae* egg laying all season) (Van Driesche, 1988). Using information from this study, I constructed life tables that confirmed *C. glomerata* really was an important mortality factor affecting the changes in *P. rapae* numbers from one pest generation to the next in my collard field (Van Driesche and Bellows, 1988). However, due to the biology of *C. glomerata*, all the mortality caused by *C. glomerata* occurred in fully grown 5th-instar caterpillars. So, pest damage in the crop was not reduced.

In 2007, nine years after I had released *C. rubecula* from a Chinese population, that parasitoid was well established in southern New England. To find out how common and widespread it was, I surveyed organic vegetable farms in central and western Massachusetts in spring, toward the end of the first generation of *P. rapae* in various brassica crops. I found *C. rubecula* parasitizing young *P. rapae* caterpillars at every one of the 20 locations I sampled (based on 30 or more caterpillars from each site). In those samples, *C. rubecula* had attacked 75% of the caterpillars collected. Only 1% of these caterpillars had been attacked by *C. glomerata* (Van Driesche, 2008). In 2009, I repeated this survey in the same general area at 21 farms, but I sampled in the fall during the last generation of *P. rapae* to see how the levels of *C. rubecula* attack might compare to the spring in 2007 (**Fig. 5**). I found the same overall rate of parasitism, 75%, and a modestly higher proportion of that was from *C. glomerata* (12% of all parasitized hosts) (Herlihy and Van Driesche, 2013).

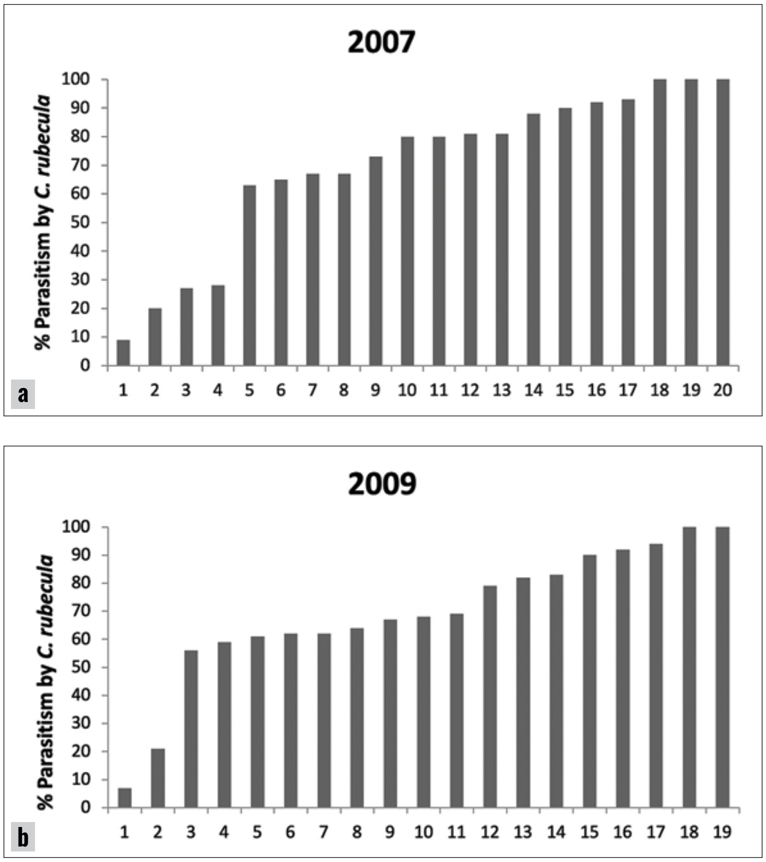

Figure 5. Rates of parasitism by *Cotesia rubecula* in western Massachusetts organic vegetable farms in (a) spring of 2007 and (b) fall of 2009, both surveys showing an average rate of parasitism of 75%. (modified from Herlihy and Van Driesche, 2013)

In a detailed field experiment at one organic collard farm in western Massachusetts in 2011, we measured the survival of imported cabbageworm caterpillar groups initiated on four different start dates throughout the growing season. Parasitism by *C. rubecula* averaged 62.4% among the measured caterpillar groups. No parasitism by *C. glomerata* was observed. Most importantly, parasitism by *C. rubecula* reduced crop damage because parasitized *P. rapae* larvae died when they were still small (4th instars) and did not become the large 5th-instar caterpillars that do most of the damage (Herlihy and Van Driesche, 2013).

The above findings in Massachusetts are supported by similar findings in New Zealand where *P. rapae* also invaded and where *C. rubecula* was also intentionally introduced. In the New Zealand study, *C. rubecula* caused 70% parasitism; cumulatively this level of parasitism lowered the density of the largest caterpillars (5th instars) at harvest by 85% (from 1.65 to 0.25 caterpillars/plant), greatly reducing plant damage (Cameron and Walker, 2002).

Regional Impact of *Cotesia rubecula*

To see how far the Chinese strain of *C. rubecula* had spread and whether the high level of parasitism we saw in Massachusetts was widespread, a survey was conducted in September of 2011 in 14 states and two Canadian provinces, from New England to North Dakota, southward to North Carolina and northward to New Brunswick and Quebec. We found *C. rubecula* all the way west to North Dakota (our furthest observation point). It had become the dominant parasitoid of imported cabbageworm in the northeastern and north central United States and adjacent parts of southeastern Canada and had displaced the previously common *C. glomerata* in that area. However, we found that *C. glomerata* remained dominant in more southern areas, from Virginia to North Carolina and westward to southern Illinois (below latitude N 38° 48') (Herlihy et al., 2012).

Side Effects of this Biocontrol Project

In addition to the main effect of controlling the pest *P. rapae*, there were three side effects caused by this biocontrol project: one negative (in the 1880s) and two positive (in the 1980s). The harmful effect was the attack on non-target native butterflies by *C. glomerata*, and the two positive effects were the subsequent suppression of *C. glomerata* by the second parasitoid, *C. rubecula*, and then the recovery of *P. oleracea*, the native butterfly that had nearly disappeared from Massachusetts due to *C. glomerata*. We investigated all three of these side events in Massachusetts, and the details are given in the following sections.

Harm to native butterflies by *Cotesia glomerata*

Cotesia glomerata attacks butterflies in three genera, including several species of *Pieris* in Europe and North America (Puttler et al., 1970; Lees and Archer, 1974; Laing and Levin, 1982; Sato and Ohsaki, 1987), several *Tatochila* in Chile (where *C. glomerata* was also introduced) (A. Sharpiro, pers. comm.), and one *Aporia* in Asia (Jiang, 2001). In laboratory tests in New England, we found that both of our native white butterflies—*P. oleracea* (the veined white) and *Pieris virginiensis* (the West Virginia white)—were suitable hosts for *C. glomerata* (Van Driesche et al., 2003). However, in the field we only found attacks on *P. oleracea* (Benson et al., 2003a) because *C. glomerata* did not fly in the forested areas where *P. virginensis* is found. Placement of *P. rapae* caterpillars in forests did not attract any attacks by *C. glomerata*, indicating *P. virginensis* is not at risk (Benson et al., 2003b).

Reduction of *Cotesia glomerata* by *C. rubecula*

Collections of *P. rapae* caterpillars at organic vegetable farms in western Massachusetts showed that within 20 years of its introduction, *C. rubecula* had replaced *C. glomerata* as the dominant parasitoid of *P. rapae* at sites that were widely separated in forested, hilly country (Van Driesche, 2008; Herlihy and Van Driesche, 2013). By 2007–2009, *C. glomerata* had been reduced to only 1% of total parasitism in the spring survey (2007) and 12% in the fall survey (2009). Similar results were reported from a study in New Zealand where in plots without *C. rubecula*, the parasitism by *C. glomerata* was 10–60%, but in plots with *C. rubecula* present, it was less than 10% (Cameron and Walker, 2002).

It is likely that the displacement of *C. glomerata* by *C. rubecula* in Massachusetts happened much sooner than the dates of our 2007–2009 surveys. In the research plot of collards where we made

Figure 6. The invasive form of cuckoo flower, *Cardamine pratensis* var. *pratensis* (a) has been a key reason for the population rebound of the veined white butterfly, *Pieris oleracea*, in Massachusetts, because its (b) ground-hugging rosette provides a suitable host from April to November. (a: Tomás Curtis; b: Graham Buck; a,b: iNaturalist.org CC BY-NC 4.0)

our first releases of *C. rubecula* in 1988, we observed that densities of *C. glomerata* cocoon masses had declined by 81% between 1988 and 1990, just two years after release of *C. rubecula*. We measured this decline as the percentage of the sampled plants on which we found *C. glomerata* cocoon piles. Specifically, in 1988 the number of sampled plants bearing *C. glomerata* cocoons was 16% (661 plants with cocoons/4,098 plants sampled over the growing season), but this had dropped to just 3% (82 plants with *C. glomerata* cocoons/2,706 plants sampled) by 1990, just two years after release of *C. rubecula* at this site (Van Driesche and Nunn, 2002).

Recovery of the native butterfly *Pieris oleracea*

Pieris oleracea (formerly *P. napi oleracea*) was historically the common white meadow butterfly of southern New England. In the mid-to-late nineteenth century, its range in Massachusetts declined (Scudder, 1889) to just remnant populations in western Massachusetts, and then was presumed to have disappeared. However, in 1986, a single large population of *P. oleracea* was discovered in western Massachusetts in a wet meadow where the caterpillars fed on an introduced variant of a wild mustard called cuckoo flower. This plant, *Cardamine pratensis* var. *pratensis* (**Fig. 6a**), is a spring-blooming biennial that is present throughout the year as a ground-hugging rosette (**Fig. 6b**).

We undertook studies to understand why this site supported a large population of the butterfly and to understand its likely future status. We observed that at this site, cuckoo flower's rosettes remained suitable for the butterfly's caterpillars from May through November, allowing for the development of four *P. oleracea*e generations each year (Herlihy et al., 2014). For most of the growing season, cuckoo flower's rosettes were over-topped by other species of plants, leading us to question if this provided any protection to caterpillars from parasitism. To find out, we introduced *C. glomerata* adults into open-bottomed field cages placed over either tall vegetation or similar areas where the tall vegetation had been removed by clipping. We placed *P. oleracea* caterpillars in these cages by putting them on either small collard or cuckoo flower plants in pots and placing the pots on the ground. In cages with over-topping vegetation, caterpillars suffered significantly lower *C. glomerata* parasitism (22.2%, 18/81 recovered larvae) compared to cages from which the tall plants had been removed by clipping (72.9%, 62/85 recovered larvae) (Herlihy et al., 2014). This suggested that even in the presence of *C. glomerata* populations in the habitat, this new host plant provided significant protection against parasitism because it was hidden most of the year by larger plants.

The second factor we found to have affected the native butterfly's improved survival was the displacement of *C. glomerata* (which attacks *P. oleracea*) by *C. rubecula* (which does not). Historically, the decline of *P. oleracea* began after the invasion of *P. rapae* (Scudder 1889), but there was no evidence of any strong competition between the butterflies' caterpillars for food. Research in Massachusetts showed that *C. glomerata*, which was the dominant parasitoid of *P. rapae* during this period of *P. oleracea* decline (Van Driesche and Bellows, 1988), could parasitize *P. oleracea* (Van Driesche et al., 2003) and did so in field tests (Benson et al., 2003a).

Chronologically, events were as follows. In 1986, a single large surviving population of *P. oleracea*e was discovered on cuckoo flower in western Massachusetts. We believe *C. glomerata* had long been long present at that site because in 2001 we found that 100% of trap host larvae of *P. oleracea*e were parasitized by *C. glomerata* (Van Driesche et al., 2004) in a cow pasture just 52 km (30 miles) away from the surviving population of *P. oleracea*. In 2007, we showed that *C. glomerata* was present at the cuckoo flower/*P. oleracea* site (Van Driesche, 2008), but at a much lower level that in our 2001 study (Van Driesche et al., 2004). From 2008 to 2010, we exposed *P. oleracea* larvae on potted plants with over-topping vegetation at the site with the *P. oleracea* population to try to detect parasitism. Of the 417 *P. oleracea* larvae recovered after field exposure in those experiments, only 3 (0.7 %) were parasitized by *C. glomerata* (and none by *C. rubecula*) (Herlihy et al., 2014). These studies demonstrated that by 2007 (the date we showed *C. glomerata* had been displaced by *C. rubecula* [Van Driesche, 2008]), pressure of *C. glomerata* on the native *P. oleracea* was gone at this site (Herlihy et al., 2014).

In summary, when *P. oleracea* adopted the invasive plant cuckoo flower as a new host, this conferred some degree of protection from *C. glomerata* because the plant's rosettes were obscured most of the year by taller vegetation that interfered with parasitoid foraging. Also, cuckoo flower provided an abundant resource that was available for the whole growing season, allowing four generations of the butterfly per year. These host plant benefits, combined with the removal of threats from *C. glomerata* (due to its displacement by *C. rubecula*), enhanced the population growth rate and hence density of the native butterfly. We expect that the butterfly's population will now re-expand across its historical range in Massachusetts.

BENEFITS OF BIOLOGICAL CONTROL OF IMPORTED CABBAGEWORM

There were two clear benefits from the introduction of *C. rubecula* for biological control of imported cabbageworm. The first of these was a 75% reduction in the number of large caterpillars in organic brassica crops, with a corresponding reduction in damage. This benefit is potentially offset to some degree because other pests also attack brassica crops, and their control is still necessary. However, it does provide benefits where pesticides are not applied, as in organic farms and many home gardens. The other benefit has been the suppression of another parasitoid, *C. glomerata*, which has largely eliminated the harm it caused to a native butterfly, the veined white (*P. oleracea*).

WORK STILL TO BE DONE

This story has been developing for over three decades, and while the broad outlines are now clear, there are several points that remain to be investigated. These include the degree of impact of *C. rubecula* and *C. glomerata* on *P. rapae* in non-cultivated habitats (meadows and along the margins of larger rivers) where imported cabbageworm feeds on invasive species of mustards. The rate of parasitism by these two *Cotesia* species on *P. rapae* outside of farm fields is worth exploring because this habitat is quite extensive, and these patches of wild mustards are potentially a large source of the butterfly and its associated parasitoids. Secondly, I predict that the range of the veined white, *P. oleracea*, will increase over time and will follow increases in the range of the introduced cuckoo flower. The veined white is also likely to increasingly be able to feed on weedy mustards in meadows and along rivers as the pressure of *C. glomerata* declines. Surveys of the veined white in southern New England are needed to track the recovery of this butterfly over more of its historical range.

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Chapter 6

Biological Control of Silverleaf Whitefly in the United States

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NON-TECHNICAL SUMMARY

Bemisia tabaci was described in 1889 as a tobacco pest in Greece and named *Aleyrodes tabaci*, the tobacco whitefly. Many name changes (Brown et al., 1995) have occurred since its first description. Perring (2001) and later DeBarro et al. (2011) and Boykin and De Barro (2014) determined that the different biotypes were a species complex, each with different biological attributes, cross-mating success, and differences in virus transmission. In the 1990s, improved transportation technology and increased frequency of international transport of plant material contributed to the extension of the geographical range of the *B. tabaci* complex. At present, it is globally distributed and occurs on all continents except Antarctica (Martin et al., 2000). Losses due to this species complex in agricultural worldwide have been extensive. Damage includes leaf silvering in cucurbit crops such as squash, transmission of viruses that reduce quality and yield of tomatoes, sticky fiber in cotton from honeydew production, and direct damage from feeding on horticultural/floricultural crops such as poinsettias (Gerling and Henneberry, 2001).

In the early 1990s, a biological control program was initiated by the U.S. Department of Agriculture (USDA) in response to widespread outbreaks of the particularly damaging biotype B, which at that time had been described as the silverleaf whitefly, *Bemisia argentifolii* (Bellows et al., 1994). This involved worldwide exploration for natural enemies in the tropics and subtropics where *B. tabaci* was known to be endemic. Many unique populations and species of parasitic wasps in the genera *Eretmocerus* and *Encarsia* (both Hymenoptera: Aphelinidae) were imported, reared, and released in the United States to reduce the impacts of this pest. For several years, the *Eretmocerus* species were the dominant parasitoids in the field, post-release (1999–2012) (Goolsby et al., 2004; Pickett et al., 2013). However, now (2012–2022) *Encarsia sophia* (formerly *E. transvena*) is most responsible for parasitism of *B. tabaci* in North America (Goolsby et al., 2009a; Xiao et al., 2011; Naranjo, 2018; Davis et al., 2020). *Encarsia sophia* has likely emerged as the dominant species because it can exist at lower silverleaf whitefly population levels. *Encarsia sophia* is an autoparasitoid, meaning it can parasitize its own female progeny (if necessary) to produce males. Along with resident species of predators, *E. sophia* has driven whitefly populations to even lower levels in the mid-2000s, compared to 1999 when the *Eretmocerus*

Goolsby, J. A., K. A. Hoelmer, and J. R. Gould. 2022. Biological control of silverleaf whitefly in the United States, pp. 59–72. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

spp. became the dominant parasitoids (Goolsby et al., 2009a,b; Naranjo et al., 2018). Overall, the imported parasitoids, in combination with the local whitefly predators, have dramatically lowered pest populations an estimated 90%, allowing for the development of integrated pest management programs that further reduce the damage from the pest and allow for sustainable production of field/greenhouse crops and ornamental plantings. In the early 2000s, the silverleaf whitefly biological control program was estimated to be saving $300 million annually (Robinson and Taylor, 1996). For a complete review of the program, see Gould et al. (2008).

HISTORY OF INVASION AND NATURE OF PROBLEM

The first specimens of *Bemisia tabaci* (Hemiptera: Aleyrodidae) (**Fig. 1**) collected in the Western Hemisphere were found in 1894 in the United States on sweet potato. They were initially described as *Aleyrodes inconspicua* and given the name sweetpotato whitefly, but were later recognized as *B. tabaci* biotype A. Except for its role as a vector of cotton leaf crumple in the late 1950s and early 1960s, *B. tabaci* was not recognized as an economic pest in the United States. However, by the 1980s, *B. tabaci* became a serious problem of agricultural communities in the United States and northern Mexico. Outbreaks occurred in California and Arizona in 1981 and were initially presumed to be the long-present *B. tabaci* biotype A. Field crops such as cotton and melons in the Imperial Valley of California and Lower Rio Grande Valley of Texas were significantly affected (Birdsall et al., 1995; Riley and Ciomperlik, 1997). These outbreaks were followed by heavy infestations on poinsettia crops and by the appearance of silverleaf symptoms on squash (Price et al., 1986; Maynard and Cantliffe, 1989). The source of these problems was soon recognized as a new biotype of *B. tabaci*, first formally recognized from Florida. Based on several attributes—high reproductive capacity, resistance to pesticides, alternative host plant utilization, and other differences—the new pest was designated as *B. tabaci* biotype 'B' (Costa and Brown, 1990) and subsequently as a new species, the silverleaf whitefly, *Bemisia argentifolii* (Bellows et al., 1994). Subsequent studies placed *B. argentifolii* as a member of the Middle East-Asia Minor clade (De Barro et al., 2011), and recently it has been grouped within the North Africa-Mediterranean-Middle East species complex (de Moya et al., 2019).

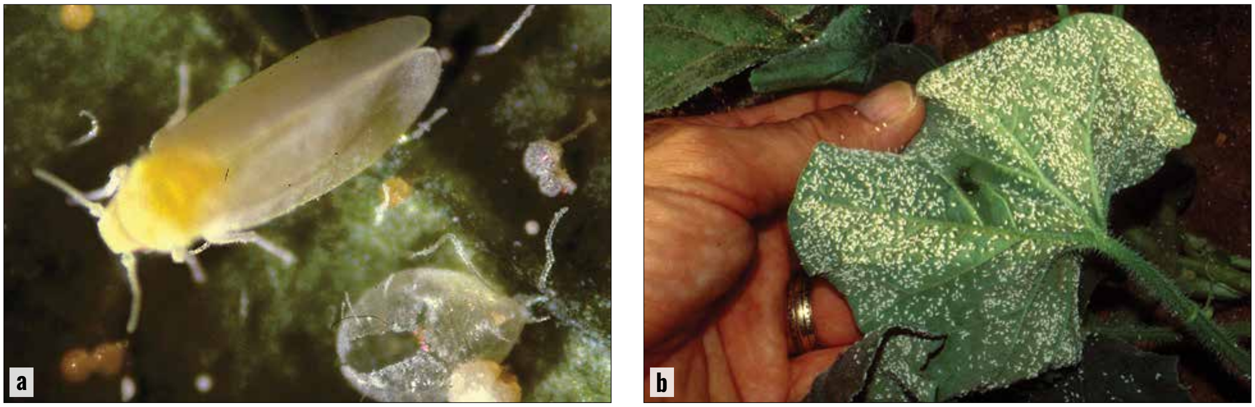

Figure 1. *Bemisia tabaci* on underside of a melon leaf. (a: J. Goolsby, USDA-ARS; b: K. Hoelmer, USDA-ARS)

WHY CONTROL THIS INVASIVE SPECIES?

In Arizona, California, Texas, and Florida, losses in 1991 and 1992 were estimated to range from $200 to $500 million (Perring, 1996). In the Imperial Valley of California between 1991 and 1995, over $100 million were lost annually (Birdsall et al., 1995). In Arizona, California, and Texas, cotton growers spent $154 million from 1994 through 1998 to control silverleaf whitefly and prevent cotton lint stickiness (Ellsworth et al., 1999). Gonzalez et al. (1992) estimated that for every $1 million of primary silverleaf whitefly-induced crop loss in a multi-commodity-growing agricultural community, there was an estimated $1.2 million loss of farm income. Infestations in U.S. greenhouse and ornamental production also caused losses estimated in the millions (Barr and Drees, 1992). Losses to the tomato industry in Florida in 1991 were reported to exceed $125 million (Schuster, 1992). Similar crop and financial losses occurred in adjacent agricultural areas in northern Mexico (Silva-Sanchez, 1997).

These unacceptable whitefly-caused financial, social, and environmental losses highlighted the need for a nationally coordinated effort to provide long- and short-term solutions to the problem. The reasons for the outbreaks were unknown but clearly suggested biological and host plant preference differences between the outbreak populations of whitefly and the previously known *B. tabaci* populations. Actions to address the issues arising from these unprecedented outbreaks of the silverleaf whitefly led to the development of a classical biological control program against this new form of *B. tabaci*.

THE ECOLOGY OF THE PROBLEM

The impact of released classical biological control agents on invasive whitefly pests has been well documented (e.g., Quezada, 1974; DeBach and Rose, 1976; Bellows et al., 1992). Designing methods to evaluate the impact of parasitoids released against silverleaf whitefly, however, was not straightforward. Evaluating the success of classical biological control programs for silverleaf whitefly was complicated due to its broad host plant range, coupled with the influence of farming practices, variations in cropping patterns across states, and differences in climate among locations. To further complicate matters, these factors operated at different spatial and temporal scales and were likely interrelated. In addition, landscape pests with broad host ranges like *B. tabaci* are also widespread in unmanaged non-crop habitats, and it is in these landscape populations that natural enemies are often most valuable. To address this complexity, data on parasitoid releases in the Imperial Valley of California were collected and analyzed using multivariate statistics to determine the impact of introduced whitefly parasitoids.

Silverleaf whitefly became a significant agricultural pest in states from North Carolina to California in the early and mid-1990s. The whitefly attacked a wide range of plants, many of them important agricultural crops (Davis et al., 2020). It was able to complete up to 20+ generations per year by moving from spring cucurbit crops like cantaloupe and watermelons into cotton in the summer months. Whiteflies overwintered on cole crops and winter weed species, as well as on ornamental plants around houses. Whiteflies reached their peak in cotton, often creating massive migrations from that host crop when it was defoliated. In peak years, clouds of whiteflies leaving cotton fields were so thick that fall melons became impossible to grow because of the overwhelming numbers of the whitefly and the plant viruses that they carried.

In the Imperial Valley of California, as well as other growing areas of the southern United States, a succession of host crops are available to silverleaf whitefly throughout the year. Under favorable weather conditions, gravid females can cause a population explosion if suppressive measures are not in place. Alfalfa (*Medicago sativa*) is the major crop in the Imperial Valley, with alfalfa planting varying from 71,000 to 91,000 ha (175,000–225,000 acres) over a 20-year period. Although present year-round, *B. tabaci* populations are generally low in alfalfa compared to cantaloupe (*Cucumis melo reticulus*) or cotton (*Gossypium hirsustum*). Alfalfa therefore provided a more stable habitat for *B. tabaci* parasitoids. Landscaping plants at residences or businesses scattered throughout the Imperial Valley also provided perennially stable habitats and refuges for both whiteflies and parasitoids. Seasonal crops are exceptionally good hosts for *B. tabaci*, but generally for short periods of time, making it difficult for parasitoids to discover, build up, and suppress whitefly populations on such short-cycle crops before the crops are harvested and plowed under.

If overwintering whitefly populations found on fall and winter cole crops (*Brassica* spp.) are not controlled, they may quickly colonize spring crops. Shortly after the cole-crop season ends and the fields are plowed under, spring cantaloupes and cotton emerge, providing highly favorable *B. tabaci* hosts. Cantaloupe fields planted for spring harvest are present into June, and cotton fields are hosts through September or October. A monitoring program using traps, conducted jointly by the Imperial County Agricultural Commissioner's office and USDA-APHIS (Animal and Plant Health Inspection Service), showed that peak *B. tabaci* populations historically occurred in August or September. Under crop production patterns used during the 1990s, cotton was the dominant host crop during these months, supporting the highest populations of *B. tabaci*. During the height of the of silverleaf whitefly outbreaks in the late 1980s and early 1990s, cantaloupe fields planted for fall harvest were available as whitefly habitat from July through November, but growers were forced to significantly reduce fall cantaloupe acreage because of pressure from *B. tabaci* populations and associated control costs (Legaspi et al., 1997; Gould et al., 2008).

Many control strategies were developed and implemented in the United States to control *B. tabaci* in the 1990s (Gould et al., 2008). New chemical tools became available that proved especially useful in controlling silverleaf whitefly in cantaloupe and cole crops, reducing extremely high populations observed in these crops during the peak outbreak years. Imidacloprid, applied to the soil, was widely used in cole crops and cantaloupe (in California 1990–2003); tank mixes of acephate and fenpropathrin were used on cotton (in California 1990–2003). Other factors that influenced *B. tabaci* populations were undergoing changes as well. Control costs for pink bollworm (*Pectinophora gossypiella*), boll weevil (*Anthonomus grandis*), and silverleaf whitefly, combined with increasing competition in world markets, led to reductions in cotton acreage from over 40,000 ha (99,000 acres) in the 1970s to less than 8,000 ha (20,000 acres) annually from 1989 through 2002. Acreage of cantaloupe peaked in the 1980s then plummeted in 1992, largely due to *B. tabaci* damage, and many fields were left unharvested. Between 1990 and 1991, fall cantaloupe acreage dropped by approximately 30%, and the gross value of the crop decreased by over $15 million. This reduction in cotton and cantaloupe (especially fall cantaloupe) acreage available to *B. tabaci* probably reduced regional whitefly populations. Alfalfa has remained the most stable whitefly host plant and parasitoid refuge, with the 2010–2020 area ranging from 65,000 to 89,000 harvestable ha (161,000–220,000 acres) annually. This increase in alfalfa acreage and reduction of more *B. tabaci*-susceptible crops most likely contributed to the overall reduction in silverleaf whitefly population levels and damage in the Imperial Valley. Similar changes occurred in the Lower Rio Grande Valley of Texas with reduced agreage of melons and cotton and subsequent increases in grain sorghum production. Shifts in cropping patterns can greatly affect the ecology of silverleaf whitefly.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The classical biological control program directed against silverleaf whitefly in the 1990s was one the largest and most comprehensive programs in the history of biological control in the United States (Legaspi et al., 1996; Kirk et al., 2000, Gould et al., 2008). A team of scientists from USDA-APHIS, USDA-ARS (Agricultural Research Service), CDFA (California Department of Food and Agriculture) and several universities contributed to the discovery, importation, evaluation, release, and colonization of a suite of natural enemies. Field entomologists with USDA-APHIS were stationed in Arizona, California, and Texas to carry out the research where the infestations were the most damaging. To support the foreign exploration for natural enemies for *B. tabaci* and other biological control projects, a new APHIS quarantine facility was built at Moore Airbase near Edinburg, Texas, which eventually coordinated with mass-rearing facilities at the field locations to maximize the release efforts for the biological control program. The substantial level of commitment by USDA matched the level of damage caused to a wide range of crops by this 'super pest.' The national program leadership of ARS and APHIS produced a remarkable achievement in redirecting research programs, obtaining additional research and implementation funding from Congress, and developing a

5-Year National Research and Action Plan for Development of Management and Control Technology for the Silverleaf Whitefly (1992–1996), which was followed by the Silverleaf Whitefly 5-Year National Research, Action, and Technology Transfer Plan (1997–2001) to help organize research and track progress.

The foreign exploration program for natural enemies of *B. tabaci* resulted in more than 130 shipments of natural enemies from 30 countries being sent to quarantine facilities in the United States between 1991 and 1998 (**Table 1**). Climate matching software (CLIMEX) was used to match the affected areas in the United States with locations within the native distribution of *B. tabaci* and to rank areas for possible foreign exploration (Goolsby et al., 2004). The USDA-ARS European Biological Control Laboratory in Montpellier, France, contributed to the biological control program by having its staff engage in nearly year-round exploration, which led to the discovery of many parasitoids, predators, and pathogens for evaluation by U.S. researchers.

At the USDA-APHIS Mission, Texas, Biological Control Quarantine Laboratory, 50 populations of natural enemies (parasitoid wasps and predatory insects) were held in culture, including 16 new species of *Eretmocerus* and *Encarsia* parasitoids (Legaspi et al., 1996; Legaspi et al., 1997; Goolsby et al., 1998; Kirk et al., 2000; Gould et al., 2008). Only parasitoids that had been reared from *B. tabaci* (any biotype, as the biotypes could not be determined at the time of collection) and were either primary or autoparasitic species were considered for release. Predictive, pre-release studies were conducted in quarantine and in field cages to determine which species showed the most potential to control the whitefly populations (Goolsby et al., 1996, 1998). This information was used to rank species for large-scale releases based on mass rearing. Low-performing parasitoid species were also released at selected locations in substantial numbers to validate the predictions.

The silverleaf whitefly program was the first large-scale biological control program to use molecular genetic methods to characterize the imported natural enemies (Vacek et al., 2008). RAPD-PCR was used on the natural enemies reared in quarantine to identify potential cryptic species and maximize the release of the genetic diversity available from the exploration efforts. This was critical because many of the most valuable *Eretmocerus* species were extremely similar morphologically but had unique biological traits such as specific host-plant preferences and climatic adaptations. Molecular methods were also used to assure colony purity and to identify potentially exotic specimens recovered from the field. Taxonomic keys were developed to identify and describe the imported *Eretmocerus* parasitoids of *B. tabaci* in North America (Zolnerowich and Rose, 1998).

Mass-rearing facilities were established in Tucson, Arizona; the cities of Imperial and Sacramento, California; and Mission, Texas. At these locations, hundreds of millions of *Eretmocerus* and *Encarsia* species were mass reared (**Fig. 2**) over several years for release and evaluation in the areas affected by silverleaf whitefly (Roltsch et al., 2008a; Gould et al., 2008, Goolsby et al., 2009b), which included the subtropical agricultural areas of the United States and Mexico. Mass-rearing techniques improved dramatically over the course of the program, beginning with laboratory rearing in environmental chambers on whitefly-infested hibiscus plants, to heated outdoor field cages with large pots of kale and eggplant, to highly managed greenhouses that used large-leaf eggplants and mechanical removal of parasitoid pupae (Simmons et al., 2008a; Goolsby et al., 2009b). The large number of parasitoids available for release enabled a large-scale field evaluation of biological control as an integrated component of management programs.

Field evaluation programs were conducted in Phoenix, Arizona; Brawley and Sacramento, California; and Mission, Texas. Candidate natural enemies were tested in field cages on multiple crops, including alfalfa, broccoli, cotton, and melons (Hoelmer, 2007; Hoelmer and Roltsch, 2008). The results showed strong tri-trophic interactions and verified the importance of adequate climatic adaptation. The four species of Palearctic *Eretmocerus* that established in the western United States were morphologically similar, representing a group of closely related taxa that appear to be specialist parasitoids of the *B. tabaci* complex of biotypes. Their ability to readily attack whiteflies in the *B. tabaci* complex may have given them an advantage in the field versus the native North American *Eretmocerus tejanus* (**Fig. 3a**) and *Eretmocerus eremicus*, which have broader host ranges that include *Trialeurodes* species.

Table 1. Parasitic Hymenoptera imported into the United States and evaluated for biological control of *Bemisia tabaci* (biotype "B"), 1992 to 1998. (All specimens were collected from *Bemisia tabaci* complex unless otherwise noted.)

Species	MBCL Asseccion Code	MBCL DNA Pattern	Collection Locality	Collector[1]	Date	Identifier	Host Plant	Biology
***Encarsia* species**								
Enc. bimaculata	M92018	EN-1	India, Parbhani	G. Butler	Jan-92	Woolley & Schauff		
Enc. bimaculata[2]	M93010	EN-1	India, Parbhani	G. Butler	Jan-92	Woolley & Schauff		Autoparasitoid
Enc. formosa[3]	M92017	EN-2	Greece, Angelohori	J. Kashefi	Jan-92	Woolley & Schauff	Bean	Uniparental
Enc. formosa	M92030	EN-2	Egypt, Nile Delta	Kirk & Lacey	Jan-92	Schauff	Lantana	Uniparental
Enc. lutea	M93064	EN-10	Cyprus, Mazotos	Kirk & Lacey	Jan-93	Woolley & Johnson	Lantana	
Enc. nr. *hispida*	M94056	EN-16	Brazil, Sete Lagoas	Rose	Feb-94	Rose & Woolley	Poinsettia	Uniparental
Enc. lutea	M94107	EN-10	Israel, Givat Haim	Kirk & Lacey	Oct-94	Woolley & Johnson	Cotton	Autoparasitoid
Enc. lutea	M94115	EN-10	Israel, Ein Gedi	Kirk & Lacey	Oct-94	Woolley & Johnson	Lantana	Autoparasitoid
Enc. lutea	M94129	EN-10	Spain, Mazarron Casas Nuevas	Kirk & Lacey	Nov-94	Woolley & Johnson	*Ipomea* sp.	Autoparasitoid
Enc. lutea	M96044	EN-10	Sicily, Ragusa	Kirk & Campobasso	Sep-96	Johnson	Solanaceous weed	Autoparasitoid
Enc. pergandiella	M94055	EN-15	Brazil, Sete Lagoas	Rose	Feb-94	Rose & Woolley	Poinsettia, Soybean	Uniparental
Enc. sophia	M93003	EN-7	Spain, Murcia	Kirk & Lacey	Jan-93	Woolley & Schauff	Lantana	Autoparasitoid
Enc. sophia	M94017	EN-3	Taiwan, Shan-Hua	Legaspi, Carruthers, Poprawski	Mar-94	Woolley & Johnson	Soybean, Tomato	Autoparasitoid
Enc. sophia	M94019	EN-4	Taiwan, Shan-Hua	Legaspi, Carruthers, Poprawski	Mar-94	Woolley & Johnson	Soybean, Tomato	Autoparasitoid
Enc. sophia	M94041	EN-5	Thailand, Chiang Mai	Kirk & Lacey	Mar-94	Woolley & Johnson	Poinsettia	Autoparasitoid
Enc. sophia	M94047	EN-5	Malaysia, Kuala Lumpur	Kirk & Lacey	Mar-94	Woolley & Johnson	*Mussaenda* sp.	Autoparasitoid
Enc. sophia	M95107	EN-5	Pakistan, Multan	Kirk & Lacey	Nov-95	Goolsby	Cotton	Autoparasitoid
Enc. sophia	M96065	EN-5	Pakistan, Jalari	Kirk	Oct-96	Goolsby	Cotton	Autoparasitoid
Enc. sophia[3]	M94014	EN-11	Philippines, Benguet	Legaspi, Carruthers, Poprawski	Mar-94	Woolley & Johnson	White potato	Autoparasitoid
Enc. sophia	M94016	EN-11	Taiwan, Shan-Hua	Legaspi, Carruthers, Poprawski	Mar-94	Woolley &Johnson	Poinsettia	Autoparasitoid
Encarsia sp.[2]	M95023	EN-5	Thailand, Doi Suthep	Carruthers & Legaspi	May-95		unknown woody plant	Autoparasitoid
Encarsia sp.	M94024	EN-6	Thailand, Kampang Saen	Kirk & Lacey	Mar-94	Woolley & Johnson	Snakeweed	Autoparasitoid
Enc. sp. (*parvella* group)	M95001	EN-18	Dominican Republic, Azua	Ciomperlik	Jan-95	Schauff	Tomato	Autoparasitoid

Table 1. (continued)

Species	MBCL Asseccion Code	MBCL DNA Pattern	Collection Locality	Collector[1]	Date	Identifier	Host Plant	Biology
***Eretmocerus* species**								
Eret. emiratus	M95104	ERET-12	United Arab Emirates	Porter, Romadon	Nov-95	Rose & Zolnerowich	Okra	Biparental
Eret. sp. nr. *furuhashii*[2]	M95026	ERET-11	Taiwan, Chiuju	Kirk	May-94	Goolsby	Cabbage	Biparental
Eret. sp. nr. *furuhashii*	M95098	ERET-11	Taiwan, Tainan	Talekar & Jones	Oct-95	Rose & Zolnerowich	Tomato	Biparental
Eret. hayati	M93005	ERET-2	India, Thirumala	Kirk & Lacey	Jan-93	Rose & Zolnerowich		Biparental
Eret. hayati	M95012	ERET-10	Pakistan, Multan	Kirk, Lacey & Akey	Apr-95	Rose & Zolnerowich	Mulberry	Biparental
Eret. hayati	M95105	ERET-10	Pakistan, Multan	Kirk & Lacey	Sep-95	Rose & Zolnerowich	Eggplant	
Eret. hayati[2]	M96064	ERET-10	Pakistan, Jalari	Kirk	Oct-96	Goolsby	Cotton	Biparental
Eret. melanoscutus	M94036	ERET-3	Thailand, Chiang Mai	Kirk & Lacey	Mar-94	Rose & Zolnerowich	*Chromolaena*	Biparental
Eret. melanoscutus	M94040	ERET-3	Thailand, Kampang Saen	Kirk & Lacey	Mar-94	Rose & Zolnerowich	Cotton	Biparental
Eret. melanoscutus[2]	M94023	ERET-8	Thailand, Sai Noi	Kirk & Lacey	Mar-94	Rose & Zolnerowich	Eggplant, Melon	Biparental
Eret. melanoscutus	M95097	ERET-3	Taiwan, Tainan	Talekar & Jones	Oct-95	Rose & Zolnerowich	Tomato	Biparental
Eret. mundus	M92014	ERET-1	Spain, Murcia	Kirk, Chen, Sobhian	Jan-92	Schauff	Cotton	Biparental
Eret. mundus	M92019	ERET-1	India, Padappai	Kirk & Lacey	Jan-92	Rose & Zolnerowich	Eggplant	Biparental
Eret. mundus	M92027	ERET-1	Egypt, Cairo	Kirk & Lacey	Jan-92	Rose & Zolnerowich	Lantana	Biparental
Eret. mundus[2]	M93004	ERET-1	Spain, Murcia	Kirk & Lacey	Jan-93	Woolley & Schauff	*Sonchus*	Biparental
Eret. mundus	M93058	ERET-1	Taiwan, Tainan	Moomaw	Dec-93	Rose & Zolnerowich	Tomato	Biparental
Eret. mundus[2]	M94085	ERET-1	Italy, Frascati	Kirk & Campobasso	Sep-94	Rose & Zolnerowich	Hibiscus	Biparental
Eret. mundus	M94092	ERET-1	Italy, Castel Gondolfo	Kirk & Campobasso	Sep-94	Rose & Zolnerowich	*Ipomea* sp.	Biparental
Eret. mundus	M94097	ERET-1	Italy, Testa Di Lespe	Kirk & Campobasso	Sep-94	Rose & Zolnerowich	Eggplant	Biparental
Eret. mundus	M94103	ERET-1	Israel, Gat	Kirk & Lacey	Oct-94	Rose & Zolnerowich	Kohlrabi	Biparental
Eret. mundus	M94105	ERET-1	Israel, Gat	Kirk & Lacey	Oct-94	Rose & Zolnerowich	*Sonchus* sp.	Biparental
Eret. mundus	M94120	ERET-1	Israel, Golan Ma'Aleh Gamla	Kirk & Lacey	Oct-94	Rose & Zolnerowich	Melons	
Eret. mundus	M94124	ERET-1	Israel, Negev Desert	Kirk & Lacey	Oct-94	Rose & Zolnerowich	Cucumber	Biparental
Eret. mundus	M94125	ERET-1	Israel, Golan Kibutz	Kirk & Lacey	Oct-94	Rose & Zolnerowich	*Euphorbia*	Biparental
Eret. mundus	M96028	ERET-1	Sicily, Santa Groce	Kirk & Campobasso	Sep-96	Goolsby	Eggplant	Biparental
Eret. mundus	M97046	ERET-1	Cyprus, Nicosia	Kirk	Jul-97	Goolsby	Lantana	Biparental
Eretmocerus sp.	M96076	ERET-13	Ethiopia, Melka Werer	Gerling, Terefe	Nov-96	Goolsby	Cotton	Biparental

[1] Affiliations of collectors: A. Kirk, L. Lacey, R. Sobhian (USDA-ARS- EBCL, Montpellier, France), D. Akey and G. Butler (USDA-ARS Phoenix, Arizona),G. Campobosso (USDA-ARS, Rome, Italy), W. Jones (USDA-ARS, Weslaco, Texas), J. Kashefi (USDA-ARS, Thessaloniki, Greece), J. & B. Legaspi (USDA-ARS, Weslaco, Texas), M. Rose & C. Moomaw (Texas A&M Univ.), T. Poprawski, R. Carruthers (USDA-ARS, Weslaco, Texas), N. Talekar (AVRDC, Shanhua, Taiwan), D. Gerling (Israel), E. Porter & L. Romadon (USDA-APHIS-FAS, United Arab Emirates), A. Terefe (Melka Werer, Ethiopia)

[2] Not evaluated; all other species evaluated at MBCL quarantine and/or in field

[3] Host *Trialeurodes vaporarorum* or *Trialeurodes* sp.

Figure 2. Field cage production system (a–i) and greenhouse production system (j–o) for mass rearing *Bemisia* parasitoids (explanation of images given on following page).

Figure 2 Explanation. (a) *Eretmocerus hayati* male anntenating emerging female in advance of mating; (b) *Eretmocerus mundus* pupae inside exuviae of whitefly, note empty areas on either side of pupae which allows the parasitized whiteflies to float as compared to unparasitized whiteflies that sink. This feature is used for separation of parasitoid pupae; (c) *Encarsia sophia* pupae inside exuviae, note presence of dark meconia that are characteristic of this parasitoid genus; (d) vacuum collection of whitefly adults from the mother colony to use for infesting field cages; (e) release of parasitoid adults onto infested plants in field cage; (f) field cage full of infested eggplants that have been inoculated with parasitoids. Plants are mature and ready for harvest of leaves with parasitized whitefly; (g) harvested leaves drying on racks for one day; (h) harvested leaves inside Plexiglass emergence cages that are used to collect adult parasitoids; (i) emergence cage with black shroud to force adult parasitoids towards light and into petri dishes used for collection; (j) large eggplants infested with *B. tabaci* shrouded to contain adult parasitoids that have just been released; (k) eggplants held in greenhouse for maturation of parasitoid pupae; (l) funnel showing bulk unparasitized *B. tabaci* and parasitoid pupae floating in water, which have just been removed from the eggplant leaves using a high-pressure flat fan sprayer; (m) funnel showing separation of unparasitized whitefly that sink to bottom and top layer of floating parasitoid pupae; (n) parasitoid pupae drying on nylon mesh cloth; (o) parasitoid pupae being weighed to determine approximate numbers. (a,b: Mike Rose, TAMU; c: Walter T. Nagamine, d–q: J. Goolsby, USDA-ARS)

The climate in the native range of each of the four imported *Eretmocerus* species closely matched the climate in the areas of the United States where they established: (1) *E. mundus* (from Mediterranean Europe and the Mediterranean climate of the San Joaquin Valley of California), (2) *Eret. emiratus* (from the dry, hot desert of the Arabian Peninsula and the Imperial Valley of California), (3) *Eret.* nr. *emiratus* (from the subtropical desert of Ethiopia and the areas around Yuma and Phoenix, Arizona), and (4) *Eret. hayati* (from the subtropical desert of the Indus River Valley and the Rio Grande Valley of Texas) (Goolsby et al., 2004; Pickett et al., 2013). The exotic autoparasitoid (i.e., a parasitoid that produces male progeny by parasitizing its own developing female progeny) *Enc. sophia* (**Fig. 3b**) also established at the same sites in California, Arizona, Florida, South Carolina, and Texas. As noted earlier, *Enc. sophia* did not become a dominant parasitoid until 2010 (to present), many years after its release in the 1990s.

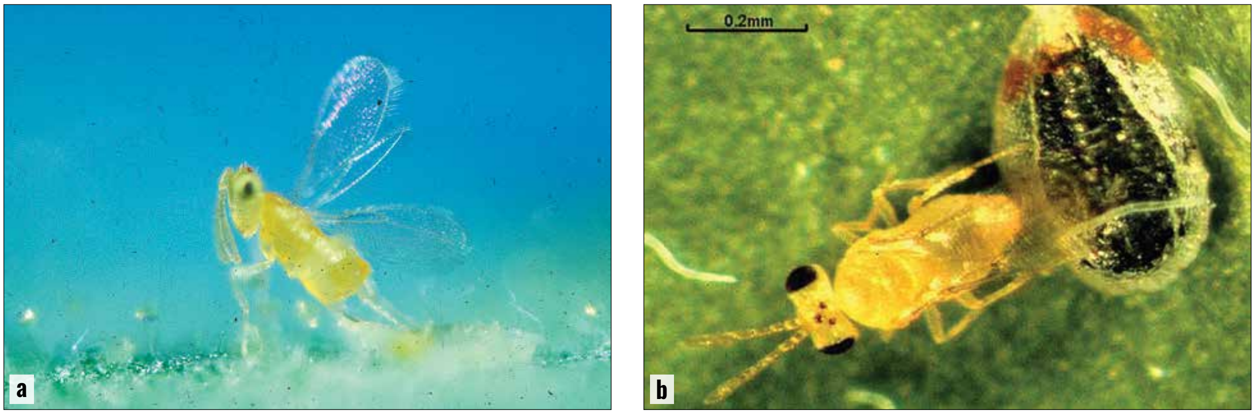

Figure 3. (a) *Eretmocerus tejanus* ovipositing into 2nd-instar silverleaf whitefly nymph; (b) *Encarsia sophia* autoparasitizing its own female pupa. (a: Mike Rose, TAMU; b: Walter T. Nagamine)

Several release methods were developed to enhance the likelihood of establishment of the released parasitoids in annual cropping systems. It was important that whiteflies and their habitat be available for parasitoids so that they could persist in the environment after the annual crops were plowed under. Refuge strips, home gardens, and commercial landscape nurseries were used as release sites because they had stable year-round populations of *B. tabaci* and were free of the use of broad-spectrum pesticides (Roltsch et al., 2008b). In addition to inoculative release methods, a more efficient method for augmentation of parasitoids was developed for use in cucurbit crops by using seedling transplants (bearing parasitized whiteflies) known as 'banker plants' (**Fig. 4**) (Goolsby and Ciomperlik, 1999; Pickett et al., 2004.). More recently, banker plants were modified for use in vegetable crops in Florida (Yinfang et al., 2011). Papaya plants infested with the less damaging greenhouse whitefly (*Trialeurodes vaporariorum*) that were parasitized by *Enc. sophia* were used for early-season releases of parasitoids into vegetable and greenhouse crops. This technique avoided the use or release of *B. tabaci* into the cropping system.

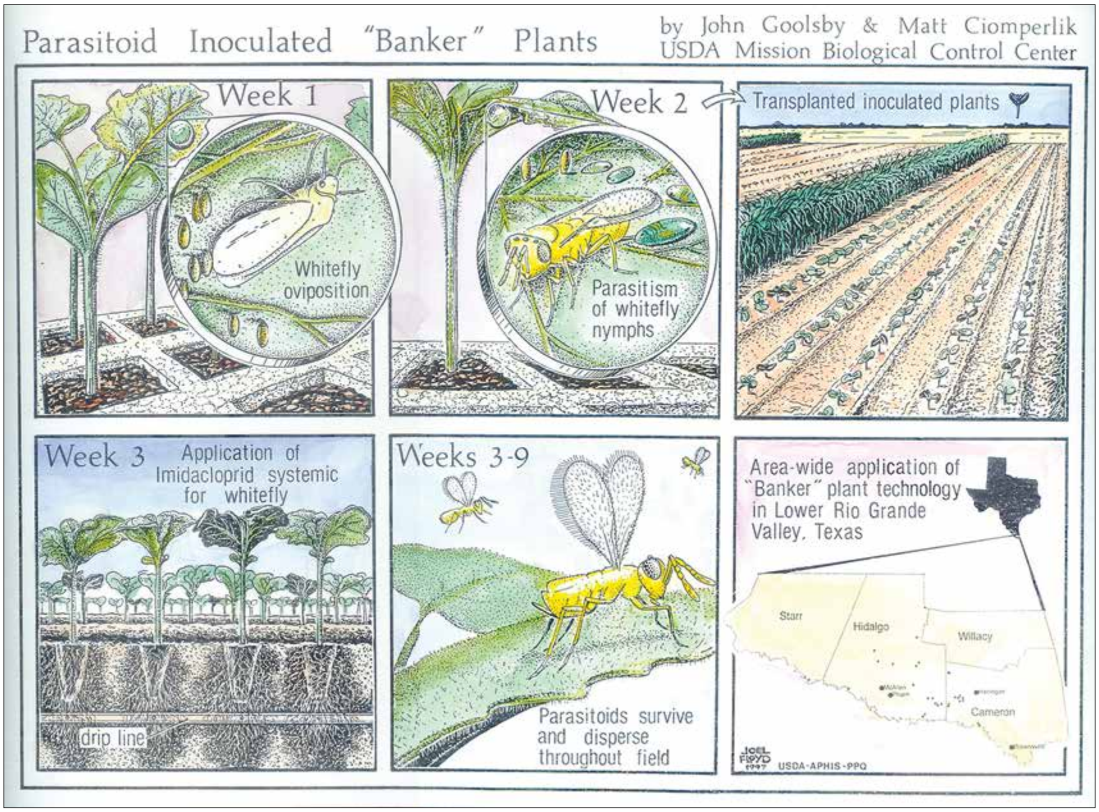

Figure 4. Parasitoid-inoculated 'Banker Plant' graphic used to show growers the technology. (J. Floyd, USDA-APHIS)

Field efforts to control *B. tabaci* were aided by the development of several narrow-spectrum insecticides that were effective against *B. tabaci* while still allowing substantial parasitoid activity. Biological control intensive-Integrated Pest Management (IPM) strategies were developed to take advantage of the new selective insecticides. Banker plants were transplanted into imidacloprid-treated fields, which reduced the cost of release and demonstrated how biological control could be incorporated in the IPM and local farming practices used during the release programs of the 1990s (Goolsby and Ciomperlik, 1999). A field-scale demonstration of mass-reared augmentation releases of *Eret. emiratus* in crops in the Imperial Valley in California showed that it was possible to increase parasitism in field crops through augmentative releases of parasitoids (Simmons et al., 2008b).

While more studies are needed to get a full assessment of the efficacy of the whole biological control program, some detailed quantitative studies have been conducted that indicate impacts of the introduced parasitoids were substantial in the years immediately following establishment in areas such as the Lower Rio Grande Valley. During these studies, regional differences in efficacy were apparent: life table studies conducted for survivorship of *B. tabaci* on cotton in Maricopa, Arizona documented the regional dominance in parasitism of *E. sophia*. The same studies also showed that in cotton, the most effective natural enemies were predatory insects (Naranjo, 2018). This contrasted with cotton in Turkey where life tables showed that parasitism by *Eret. mundus* was a significant mortality factor (Karut and Naranjo, 2009). Similar research is needed for other regions and habitats where the exotic parasitoids have become established. Only after evaluations of many crops, associated weeds, and other landscape hosts over time will we truly be able to accurately measure the impact and significance of benefits derived from the interagency silverleaf whitefly biological control program.

HOW WELL DID IT WORK?

What Impacts Really Matter?

Establishment of silverleaf whitefly parasitoids reduced the reservoir populations of whiteflies and helped to stabilize agricultural production in the affected farming areas in Arizona, California, Florida, Puerto Rico, South Carolina, and Texas within a few years, and populations of silverleaf whitefly continued to drop over time (**Table 2**). Production of crops affected by *B. tabaci*, such as squash, melons, and cucumbers again became economical, especially with the integration of new insecticides that were not as toxic to the silverleaf whitefly parasitoids and predators. Integrated pest management programs for crops such as cotton and alfalfa were able to lower and even eliminate insecticide use once the late-spring migration of silverleaf whitefly from melons into cotton was reduced. Insecticide use for whiteflies on ornamentals in urban landscapes was largely eliminated once the biological control agents became well established. In commercial greenhouse crops, methods for early-season release of commercially produced whitefly parasitoids stabilized production of crops such as tomatoes, cucumbers, peppers, and poinsettias.

Table 2. Timeline of the silverleaf whitefly, *Bemisia tabaci*, biological control program.

1990	Invasion of silverleaf whitefly in USA
1991	Initiation of multi-agency biological control program
1992	Foreign exploration for biocontrol agents
1995	Establishment of *Eretmocerus* and *Encarsia* species
1996	Biocontrol agents shared with Australia and Mexico
1997	Biocontrol program shows early benefits, *Eretmocerus* species dominant
2001	Silverleaf whitefly outbreaks eliminated
2001	IPM programs reduce direct and virus-related damage
2010	*Encarisa sophia* becomes dominant
2011	Biocontrol agents shared with China, Uganda
2022	Silverleaf whitefly field populations remain low

BENEFITS OF BIOLOGICAL CONTROL OF SILVERLEAF WHITEFLY

Reduction in pesticide use against outbreak populations of silverleaf whitefly produced significant but uncalculated monetary benefits for the agricultural sector. Benefits to the environment and safety of agricultural workers were also significant, but not measured. The silverleaf whitefly biological control program clearly demonstrated the potential benefits of classical biological control in annual row-crop agriculture. The program also confirmed the utility of predictive evaluations, which showed that a multiple-species release strategy was needed due to the varied climates involved and the many different crops that were damaged by *B. tabaci*. This strategy should be considered for future biological control programs directed at multi-crop, multi-host plant invasive pests that become widely distributed in the United States.

Parasitoids imported for this project have also been shared globally with Mexico, the Dominican Republic, China, Australia, Tanzania, and Uganda. This international cooperation has allowed for greater benefit sharing of biological control agents, especially from the countries that received these silverleaf whitefly agents from the United States.

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CHAPTER
7

Classical Biological Control of Codling Moth in the Western United States

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NON-TECHNICAL SUMMARY

Codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae) is a notorious global pest of pome fruit and walnuts, causing severe economic losses due to the direct damage caused by larval feeding within the flesh and core of pome fruit and the kernels of walnuts. As an invasive species, it has become a key pest for pome fruit and walnut production in almost all regions of the world, and management has generally required extensive use of insecticides to reduce damage of harvestable produce to an acceptable level. Early attempts at classical biological control led to the introduction of the egg-larval parasitoid *Ascogaster quadridentata* (Hymenoptera: Braconidae) from Europe and its establishment in the western United States in the 1920s. As this failed to provide a sufficient degree of control, a more detailed approach to the selection of candidate parasitoids for introduction from Central Asia was made in the 1990s, which led to the establishment of the gregarious cocoon parasitoid *Mastrus ridens* (Hymenoptera: Ichneumonidae). As a cocoon parasitoid, *M. ridens* exhibits several traits that may enhance the impact of parasitism on codling moth abundance. Levels of parasitism of codling moth in individual orchards during the introduction of this parasitoid to the western United States varied, but in some cases were as high as 70% in California and 52% in Washington State. The direct impact of parasitism by *M. ridens* on codling moth in pesticide-treated orchards may be limited as only very low levels of this pest can be tolerated in commercial crops. In contrast, however, such parasitism can contribute effectively to codling moth management in non-commercial habitats, such as backyard and wild trees, by greatly reducing the opportunity for mated female moths to disperse from such areas with higher density populations into commercial, pesticide-treated orchards.

HISTORY OF INVASION AND NATURE OF PROBLEM

The codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae, **Fig. 1**), is presumed to have spread from Central Asia along with the cultivation of its host plants, particularly apple. The history of the cultivated

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Figure 1. Adult female codling moth (*Cydia pomonella*) on the surface of an apple. (N. Mills, UC Berkeley)

apple is complex and began with the domestication of the wild apple (*Malus sieversii*) in the mountains of Central Asia between 4,000 and 10,000 years ago (Cornille et al., 2014). Cultivated apples subsequently travelled westward along the Silk Route to Europe and underwent extensive hybridization with Caucasian (*Malus orientalis*), Siberian (*Malus baccata*), and European (*Malus sylvestris*) crab apples. Domesticated apples, now known as *Malus domestica*, eventually arrived in North America with colonists in the 17th century. Consequently, codling moth is thought to have been present in North America since the mid-1700s and to have spread as far as California by 1872, representing a very early example of an insect invasion that was almost certainly aided by human transportation.

Codling moth has subsequently spread to almost all parts of the globe where pome fruit and walnuts are cultivated, with the exception of South Korea and Japan. One of the more recent examples of spread has been in China, which has almost 50% of the world production of apples (Zhu et al., 2017). First recorded in China in Xinjiang Province (in the northwest) in 1953, codling moth then spread eastward to other apple-producing provinces, reaching Gansu by 1989 and Liaoning by 2009.

WHY CONTROL THIS INVASIVE SPECIES?

As codling moth is a direct fruit-boring pest, if populations are not effectively controlled, they can build up over successive years and have severe effects on farmers' ability to harvest marketable fruit and nuts. At even relatively low levels of abundance, the extent of damage can be sufficient to cause rejection or downgrading of harvested produce, causing significant economic impact on growers. Control is not an option, but a necessity for this invasive pest. The question, however, is how best to control it over the longer-term productivity of perennial tree crops?

While control has been based on the use of insecticides, codling moth does have specialized natural enemies in its region of origin in Central Asia, and recent surveys suggested that parasitism levels there were higher than in the western United States (Unruh 1998; Lacey and Unruh, 2005; Mills, 2005). Together with an increasing demand for reduction of insecticide residues in exported fruit, these survey data refocused attention on opportunities for classical biological control of codling moth.

THE ECOLOGY OF THE PROBLEM

Codling moth is a fruit-boring pest that feeds primarily on apples, pears, and walnuts, but also occasionally on quince and stone fruit such as apricots, plums, nectarines, and peaches. It has from one to four generations a year, depending on

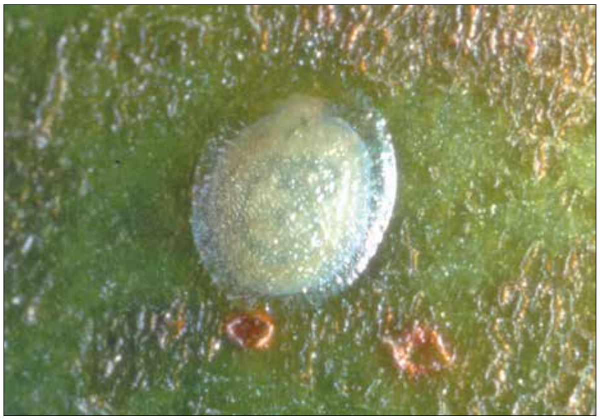

Figure 2. Codling moth (*Cydia pomonella*) egg on the husk of a walnut. (N. Mills, UC Berkeley)

temperature, and overwinters as mature larvae in cocoons under the bark or in debris on the ground beneath its host trees (Welter, 2009). Eggs are laid singly on fruit or leaves (**Fig. 2**), and larvae, after feeding briefly on the fruit surface, bore into the fruit and feed on the flesh and seeds (or nut kernels, **Fig. 3**). There are five larval instars, and the mature larvae exit the fruit and pupate under bark or on the ground.

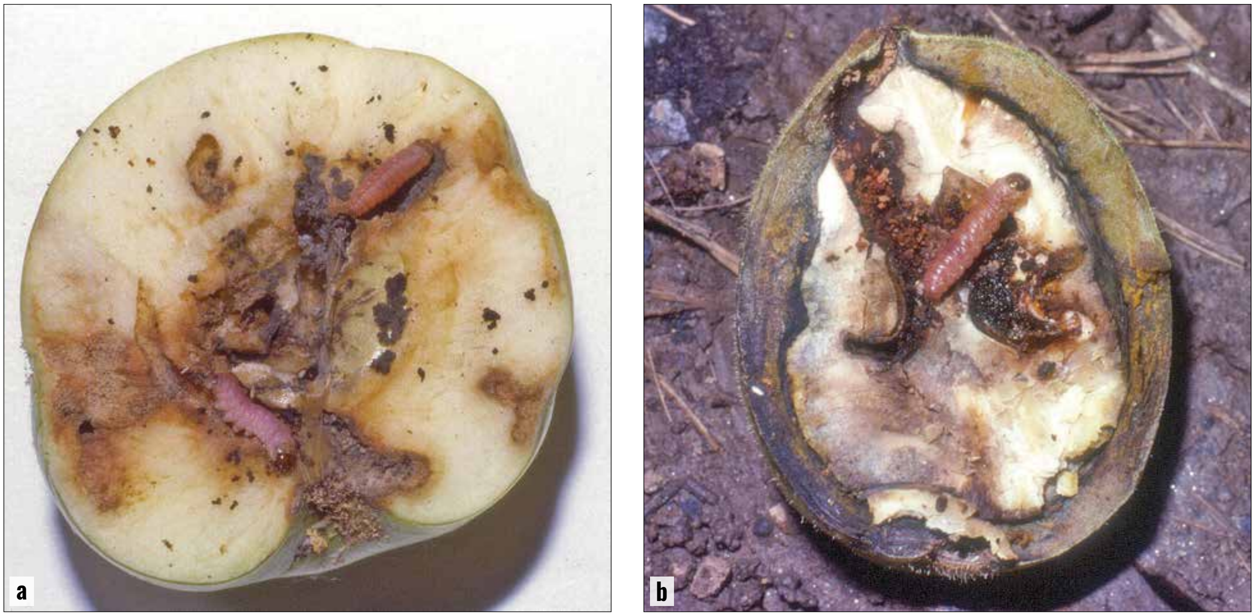

Figure 3. Codling moth (*Cydia pomonella*) larvae in (a) the flesh and core of an apple and (b) the kernel of a walnut. (N. Mills, UC Berkeley)

As a direct fruit pest, codling moth can cause extensive crop damage. The potential for damage increases with the number of moth generations per year (which varies by region) and is greater in apples than walnuts or pears. In regions with two or more generations per year, damage in apples can exceed 80%, whereas damage in walnuts can vary from 55 to 72% and more typically averages 42% (Barnes, 1991). By 2002, apple and walnut production in the western United States had each exceeded 80,000 ha (198,000 acres), with annual revenues of $1.15 billion and $0.3 billion, respectively. Pear production covered 28,000 ha (69,000 acres), with an annual revenue of $0.25 billion (Mills, 2005).

Management of codling moth has relied heavily on the use of insecticides, beginning with lead arsenate in the early 1900s, switching to DDT in the 1940s, organophosphates in the 1950s, carbamates and pyrethroids in the 1970s, and to newer classes of insecticides (insect growth regulators, neonicotinyls, diamides) in the 1990s (Croft and Riedl, 1991; Doerr et al., 2012). As a consequence, insecticide resistance has consistently occurred throughout the history of codling moth management, starting with resistance to lead arsenate in 1929 (Croft and Riedl, 1991). The introduction of pheromone-based mating disruption in the 1990s provided an opportunity to reduce reliance on insecticide use and at the same time to both slow the development of resistance and increase the potential for control by natural enemies (Jones et al., 2009, 2016).

In addition to conventional fruit and nut orchards, pome fruit and walnuts are often planted in residential gardens and even as roadside trees. These non-commercial settings are often unmanaged and serve as a reservoir for codling moth populations that can colonize surrounding commercial orchards. While insecticide use has been the standard approach for coddling moth control in conventional orchards, classical biological control has the advantage that it can also contribute to management of populations in organic settings and consequently can reduce the pressure from this pest on neighboring commercial, pesticide-treated orchards.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Figure 4. The established European egg-larval parasitoid *Ascogaster quadridentata* on the surface of an apple. (N. Mills, UC Berkeley)

Figure 5. The released solitary cocoon parasitoid *Liotryphon caudatus* on the bark of a walnut tree. (N. Mills, UC Berkeley)

Most early studies of parasitoid complexes of codling moth were conducted in Europe (Rosenberg, 1934; Lloyd, 1960). Several unsuccessful attempts to introduce parasitoids from Europe to North America were made in 1904–1905, 1921, and 1935–1936 (Clausen, 1978). From these attempts, one species, the specialized solitary egg-larval parasitoid *Ascogaster quadridentata* (**Fig. 4**), was established in Washington State in the 1920s and has since become widely distributed (Johansen, 1957). Despite being widespread, however, levels of parasitism are typically <5%, and this species has contributed little to the suppression of codling moth populations in the United States (Mills, 2005).

Among the species that failed to establish in the western United States during this early phase of parasitoid importations from Europe was *Liotryphon caudatus* (= *Calliephialtes messor*) (Hymenoptera: Ichneumonidae), a solitary cocoon parasitoid (**Fig. 5**). Its successful establishment in New Zealand (Cole and Walker, 2011), however, prompted further collections from the Black Sea (Unruh, 1998) and Europe (N. Mills, pers. obs.) from 1990 to 1994. The collections in Europe were concentrated in Austria and Switzerland, where a lesser-known solitary larval parasitoid—*Microdus rufipes* (= *Bassus rufipes*) (Hymenoptera: Braconidae)—had also been documented. These collections provided evidence that *M. rufipes* was present at low abundance in the Rhone Valley region of southern Switzerland, but that the related species *Microdus conspicuus* (Hymenoptera: Braconidae) was present in eastern Austria. In addition, these collections provided a colony of *L. caudatus*, which was reared on codling moth cocoons at the University of California, Berkeley. A total of 46,000 individuals of this parasitoid were released in apple, pear, and walnut orchards in both coastal and inland regions of California from 1991 to 1997. Additional releases of 6,500 individuals were made at various locations in Washington State (Unruh, 1998; Lacey and Unruh, 2005). Although successful parasitism of codling moth cocoons was evident at some release sites during the year of parasitoid release, there was no evidence of establishment at any of the field sites in subsequent years.

Also, during the 1990s, renewed interest in biological control of codling moth was stimulated by studies of its natural enemies and their significance in Kazakhstan (Makarov, 1982; Zlatanova and Tarabaev, 1985). These studies found that not only were levels of parasitism higher in the foothills of the Tien Shan mountains in southern Kazakhstan, but that the dominant parasitoid species were different from those that had previously been found in Europe. This prompted additional surveys for parasitism of codling moth in the regions around Almaty, Kazakhstan from 1993 to 1998 and the Ili Valley, in the Xinjiang province of China, in 1993 and again from 1999 to 2002 (Unruh, 1998; Mills, 2005). These surveys confirmed that the dominant parasitoid species were the gregarious cocoon parasitoid *Mastrus ridens* (**Fig. 6**), initially misidentified as *M. ridibundus*, and *Microdus rufipes* (**Fig. 7**), which had also been found at low abundance in Switzerland. Other parasitoids recovered from these surveys included *A. quadridentata* and *L. caudatus*, the larval endoparasitoid *Pristomerus vulnerator* (Hymenoptera: Ichneumonidae), the two pupal parasitoids *Apechthis* sp. and *Dibrachys* sp., and some unidentified hyperparasitoids. It was also notable that *A. quadridentata*, though present in Kazakhstan, was not found in northwestern China.

Figure 6. The established gregarious cocoon parasitoid *Mastrus ridens*, standing over a codling moth cocoon. (N. Mills, UC Berkeley)

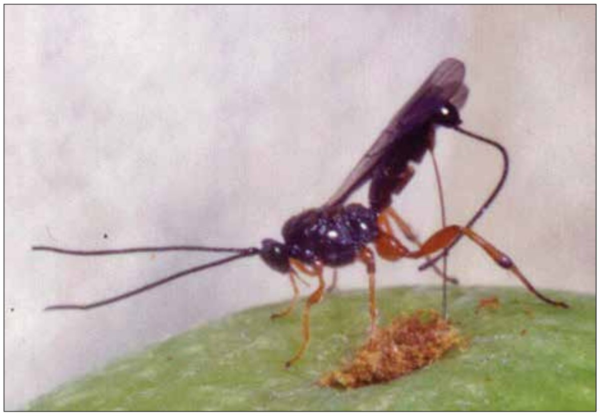

Figure 7. The released larval parasitoid *Microdus rufipes* probing into a codling moth larval entrance hole in an apple. (N. Mills, UC Berkeley)

To maximize the potential for successful suppression of codling moth populations in the western United States, efforts were made to predict the potential impact of candidate parasitoids before their introduction to the region and to select the most promising species from Kazakhstan. Stage structured matrix models were constructed to describe the demography of codling moth populations and to identify the life cycle stages that are most vulnerable to additional mortality from introduced parasitoids (Mills, 2005). Using this approach, 2nd-instar larvae and the cocoon stage were identified as the two points in the life cycle in which parasitism would have the greatest potential to suppress codling moth populations.

Of the two dominant parasitoids in Kazakhstan, *M. rufipes* attacks early-instar larvae and *M. ridens* attacks the cocoon stage. Consequently, colonies of these two species were established on codling moth in quarantine at the University of California, Berkeley. A total of 317,000 individuals of *M. ridens* and 196 individuals of *M. rufipes* were subsequently released in California from 1995 to 2000. The low numbers of *M. rufipes* released resulted from difficulty in rearing this species. Additional releases of 7,600 individuals of *M. ridens* were also made in 1996–97 in central Washington State (Unruh, 1998; Lacey and Unruh, 2005). There were no recoveries of *M. rufipes* from release sites, but *M. ridens* became established with levels of parasitism in some cases reaching 70% in California (Mills, 2005) and 52% in Washington State (Unruh, 1998; Lacey and Unruh, 2005).

HOW WELL DID IT WORK?

Since the initial establishment of *M. ridens* was determined, no follow-up studies have taken place to evaluate the impact of the parasitoid in the western United States. As has often been the case for classical biological control programs, funding support is available during the foreign exploration and introduction phases of a program, but often runs out before adequate evaluations can be made of the success and economic benefits of the program. Nonetheless, anecdotal observations made at the time of the parasitoid releases clearly indicated that local populations of codling moth in non-sprayed orchards were reduced substantially the year following release of *M. ridens*. The establishment of *M. ridens* in the western United States prompted interest from other countries, and this species has been successfully established in Argentina (Tortosa et al., 2014), Chile (Devotto et al., 2009), and New Zealand (Charles et al., 2019). It has also been introduced into Australia (Williams, 2018) and France (Borowiec et al., 2020). In addition, through simulation modeling, Wearing and Charles (2022) found that parasitism by *M. ridens* should be sufficient to suppress codling moth populations on non-sprayed, non-commercial trees in New Zealand, which would contribute to a reduction in adult moths colonizing neighboring commercial orchards.

The potential impact of *M. ridens* on codling moth populations is enhanced by the fact that (1) it attacks the most vulnerable stage in the life cycle of its host (Mills, 2005), (2) it exploits the larval-aggregation pheromone of codling moth to locate cocooned hosts (Jumean et al., 2005), which may account for its ability to show a positive response to patches of higher cocoon density (Bezemer and Mills, 2001), and (3) it has a numerical advantage over its host due to a shorter generation time and gregarious development (Bezemer and Mills, 2003). A shorter generation time than its host is of particular importance; in California, this trait allows *M. ridens* to complete two generations on overwintering cocooned larvae in the fall with adults emerging early enough in spring to start a third generation before the cocooned larvae of codling moth pupate. Similarly, the completion of two generations on overwintering cocoons, one in the fall and a second in the spring, has been observed in New Zealand (Charles et al., 2019).

BENEFITS OF BIOLOGICAL CONTROL OF CODLING MOTH

As a direct pest with larvae that bore into harvestable fruit and nuts, the codling moth is a difficult target for effective classical biological control in pesticide-treated orchards in the western United States. Damage thresholds for fruit and nut production tend to be lower than can be consistently achieved by parasitism from introduced parasitoids, and the widespread use of insecticides in conventional orchards is not conducive to parasitoid persistence in such environments. Consequently, the greatest benefits of biological control to the management of codling moth should occur at a broader landscape or regional scale through a reduction in its abundance in non-managed environments such as abandoned orchards and roadside or backyard trees. Unmanaged trees support much higher levels of codling moth abundance than those in commercial orchards and are a constant source of dispersing mated female moths that pose a significant threat to commercial orchards, particularly those using pheromone-based mating disruption to reduce reliance on insecticides (Wearing, 1979). The simulation model of Wearing and Charles (2022) confirms that in commercial orchards *M. ridens* would only contribute minimally to codling moth control, but that observed levels of parasitism would be sufficient to reduce mean adult population abundance by 74% in habitats where trees are not treated with pesticides.

The benefits of effective biological control of codling moth also extend to other potential insect pests in commercial fruit and nut orchards, as reduced insecticide usage leads to increased activity of the natural enemies of secondary pests that include leafrollers, leafhoppers, sap-sucking insects, and spider mites. Such benefits have been particularly notable in New Zealand where there has been a 90% reduction in the intensity of insecticide use in apple orchards and, consequently, a significant increase in export revenue (Walker et al., 2017).

REMAINING WORK

In the absence of a post-release monitoring phase in the classical biological control program for codling moth in the western United States, there remains an ongoing need to verify both the longer-term establishment and the spread of *M. ridens* throughout the western region. As it is unlikely to be sufficiently abundant in commercial orchards, monitoring surveys of trees in unmanaged environments to assess presence and levels of parasitism would be particularly informative. In addition, given that California implemented a system for reporting applications of all pesticides in 1990 (Wilhoit, 2018), it may also be possible to document the extent to which biological control and other IPM practices, such as pheromone-based mating disruption, have contributed to a reduction in pesticide use in pome fruit and walnut orchards in this state.

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CHAPTER 8

Biological Control of Invasive Citrus Leafminer, *Phyllocnistis citrella*, in Florida and Implications for Citrus Pest Management

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NON-TECHNICAL SUMMARY

The citrus leaf miner (CLM), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), is a significant threat to citrus crops in Florida. Its larvae damage leaves, shoots, and sometimes fruit by making serpentine mines in the cuticle, which can also affect the appearance and health of the trees. It also exacerbates the spread of the devastating citrus canker disease by providing opportunities for the pathogen to infect surfaces damaged by the larvae. Classical biological control to reconstruct the natural enemy complexes of CLM was initiated soon after it invaded Florida in 1993. Among the three exotic species of parasitoids introduced from Asia, successful establishment and impact are documented for one species, *Ageniaspis citricola* (Hymenoptera: Encyrtidae), with parasitism rates of 70–100% being reported at different times and locations. Native parasitoids and predators also contribute to CLM suppression. However, the efficacy of these beneficial organisms has been significantly reduced over the last 17 years by the increased use of insecticides needed to suppress Asian citrus psyllid (*Diaphorina citri*), the vector of the devastating disease known as huanglongbing or citrus greening disease, in commercial citrus. Effective natural enemies of CLM cause mortality in both commercial orchards and urban citrus planting, reducing the need for chemical control. Assessments of the impacts of the introduced parasitoids on CLM and their interactions with other natural enemies, the environment, and chemical control, can help in making integrated pest management decisions in Florida citrus.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

The citrus leaf miner (CLM; **Fig. 1**), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), is a serious pest of citrus and other plants in the Rutaceae. It is a major pest of citrus in Eastern Hemisphere countries, where it is native to southern Asia. The CLM has spread to all citrus-growing regions of the world (Meyrick, 1909; Janse,

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Figure 1. Adult of the citrus leafminer, *Phyllocnistis citrella.* (Lyle J. Buss, University of Florida)

1917; Clausen, 1931, 1933; CIE, 1970, 1986; Vari and Kroon, 1986; Beattie, 1989; Hoy and Nguyen, 1997) and has been recorded in several countries from Asia, Africa, and the Pacific region (Heppner, 1993). In the United States, CLM was intercepted in 1914 on citrus and other horticulture imports of the Atalantia company from the Philippines (Sasscer, 1915). However, the first record of CLM establishment in the continental United States, or elsewhere in the New World, was in southern Florida in May 1993 (Heppner, 1993). An infestation level of 90% mining of leaves was reported in Homestead, Florida on about 200 acres of Persian limes, and in a single growing season, CLM had spread throughout peninsular Florida, infesting nurseries, groves, and dooryard citrus. The CLM is now well established in Florida and has spread to the other Gulf Coast states. Considered an important pest in citrus nurseries and on either young or top-grafted trees, CLM causes feeding damage in both young and mature trees, which promotes the spread of citrus canker disease. By 1994, CLM was present in Alabama, Louisiana, and Texas (Nagamine and Heu, 2003), and by 1995, it was detected in Central America, western Mexico, and several Caribbean islands (Jones, 2001). By 2000, CLM had reached southern California, likely from Mexico (Grafton-Cardwell, 2009). By 2002, CLM had been detected in the five main Hawaiian Islands (Nagamine and Heu, 2003). In the Mediterranean basin, CLM was found in 1994 and has since spread rapidly. It is also spreading in Central and South America and has been reported from southern Africa and West Africa (CABI, 2021).

Nature of the Problem

Citrus leafminer adults are very small moths (**Fig. 1**) (2 mm long, with 4 mm wingspans) that are active during the early morning and early evening hours. Females lay individual eggs, usually next to the midvein on underside of young leaves; eggs hatch in 2–10 days. Females may lay more than one egg per leaf when flush growth is scarce or when leafminer numbers are high. Larvae (**Fig. 2a**) make serpentine mines under the leaf cuticle, and there can be one to several larvae and mines per leaf, depending upon the pest level (**Fig. 2b**). Gottwald et al. (2002) reported that a single larva can consume 1–7 cm^2 (0.2–1 in^2) leaf area, and 2–3 larvae per leaf can consume half the leaf area. Four or more mines per leaf causes leaf distortion and drop (Peña and Duncan, 1994). Damaged leaves lose water, followed by curling, necrosis, chlorosis, leaf deformation, and reduction of photosynthetic activity (Peña et al., 2000). Pupation generally takes place inside the mine in a pupal chamber at the leaf margin (**Fig. 2c**). Succulent leaves with thin cuticles are most favorable for larval mining (Latif and Yunus, 1951), which is why infestations on young plants are more intense than those on mature trees (**Fig. 3a,b**) (Uygun et al., 2000; Garcia-Marí et al., 2002). Heppner (1995) also reported heavy infestations and severe damage to the growth of young plants and possible damage to the fruit rind, particularly for grapefruit. The extent of damage in terms of length of the mines varies greatly among different host plants, with the longest mines on elephant lemon (*Citrus medica*) and the shortest mines on lime (*Citrus aurantifolia*) (Pandey and Pandey, 1964). The life cycle of CLM varies with different citrus species and temperatures (Shevale and Pokharkar, 1992; Patel and Patel, 2001). In laboratory and field tests in Egypt, young flush leaves (1–5 days old) of several varieties of grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis*), Baladi orange, navel orange and acid lime (*Citrus medica* var. *limonum*) were susceptible to CLM infestation (Mogahed, 1999). *Citrus jambhiri*, *Citrus karna*, and *Citrus limonia* were also susceptible to CLM damage, while the trifoliate orange, *Poncirus trifoliata,* and its

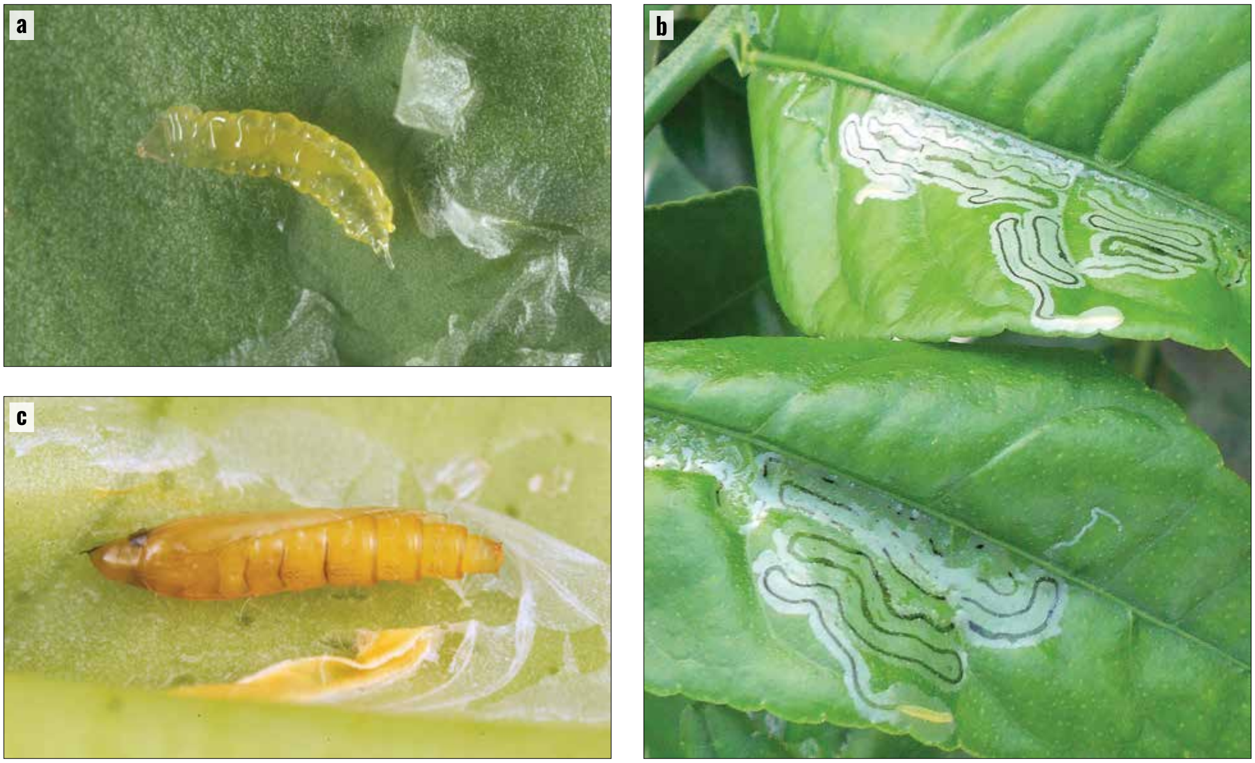

Figure 2. Citrus leafminer, *Phyllocnistis citrella* (a) larva; (b) larvae and serpentine mines in citrus leaves; (c) pupa. (a,c: Lyle J. Buss, University of Florida; b: Mongi Zekri, University of Florida)

hybrids were least susceptible. Grapefruit, lime, and oranges were also reported susceptible in Argentina (Goane et al., 2008). Negative effects on yield from CLM infestations are uncommon in mature trees, but reductions have been reported for heavily infested limes. The most serious effect of CLM infestation is indirect, with CLM making plants more susceptible to plant pathogens, such as *Xanthomonas axonopodis* pv *citri*, the bacterium responsible for Asiatic citrus canker (Sohi and Sandhu, 1968; Cook, 1988; Gottwald et al., 2002; Junior et al., 2006; Canteros et al., 2017). Leaf tissues damaged by larval feeding become more susceptible to *Xac*. Citrus canker was first found in Florida around 1912, and despite multiple eradication attempts it remains established in the state, causing significant losses in citrus crops.

Figure 3. Citrus leafminer, *Phyllocnistis citrella*, infestation on young plant growth. (a,b: Mongi Zekri, University of Florida)

WHY CONTROL THIS INVASIVE SPECIES?

Citrus leafminer larvae cause feeding damage to almost all types of citrus, including oranges, grapefruit, mandarins, lemons, limes, and their close relatives such as kumquat and calamondin. Mature trees generally tolerate CLM feeding damage; however, young plants in nurseries and orchards are more seriously affected. The larval feeding tunnels, called mines, are generally in the lower surface of the leaves but may include the upper surface in heavy infestations. The damaged leaves are curled and distorted, which slows growth of young trees. In heavily infested young trees, new shoots are visibly distorted, and damage reduces photosynthesis. Citrus leafminer damage to fruits may make them less marketable (Heppner and Fasulo, 2010). Controlling CLM is also critical to prevent the pest from increasing the rates of citrus canker disease. The larvae, through their feeding and movement, can move the bacterium of citrus canker throughout the mine and intensify infestation (Gottwald et al., 2002; Belasque et al., 2009). Citrus canker initially is visible as lesions on leaves, stems, and fruits, followed by defoliation, blemished fruits, premature fruit drop, twig dieback, and tree decline (Diez et al., 2006), which affects both the quality and quantity of fruit (**Fig. 4**). Following the 1912 detection of citrus canker in Florida, it was declared eradicated, including in adjacent states, by 1933 (Loucks, 1934; Dopson, 1964). In 1986, citrus canker was discovered a second time in Florida, and despite multiple eradication attempts it is still present in the state (Schoulties et al., 1987; Stall and Civerolo 1991; Schubert et al., 1996; Gottwald et al., 1997). Costs of those eradication efforts included the removal or cutting back of over 1.56 million commercial citrus trees and nearly 600,000 infected and exposed dooryard trees (Gottwald et al., 2002). These regulatory actions received considerable press attention and faced legal challenges, which had a far-reaching political and socioeconomic impact in Florida, with implications for national and international trade (CABI and EPPO, 1997; APHIS USDA, 1999). Most citrus varieties grown in Florida are moderate to highly susceptible to citrus canker disease. Several factors such as wind, rain, actions of people, and feeding by CLM contribute to the spread of the disease. The spring and summer rains, when combined with wind speeds of more than 8 m/s (18 mph), can greatly increase damage from the disease (Serizawa and Inoue, 1974). After the invasion of CLM in Florida in 1993, the incidence of citrus canker increased due to feeding damage by CLM larvae (Gottwald et al., 1997). Feeding by CLM larvae on the epidermal cell layer forms galleries beneath the foliar cuticle, and any splits in the cuticle can lead to the formation of large lesions due to the direct exposure of mesophyll tissues to the pathogen of citrus canker. The combination of canker pathogens and the CLM can lead to significant field infection and spread of the disease, even on highly resistant cultivars and species related to citrus such as calamondin and kumquat (Gottwald et al., 2002).

Figure 4. Citrus canker symptoms on leaves and fruit. (Mongi Zekri, University of Florida)

THE ECOLOGY OF THE PROBLEM

The degree and severity of CLM damage vary with the availability of new shoots on citrus trees. In Florida, most mature trees do not produce new growth during the winter months from November to February, and CLM populations are low to negligible even on young citrus during this period. Because of the strong impact of biological control by the predators and parasitoids on CLM, and the use of insecticides in the conventional citrus during the year, particularly for suppression of the Asian citrus psyllid (the vector of the huanglongbing [HLB] or citrus greening disease), populations of CLM decline in density toward the end of the year. The scarcity of new growth and the lower temperatures in winter impede reproduction and population increase by CLM. Citrus trees have their major flush of new growth each year in spring, followed by smaller, less predictable flushes of growth in summer and early fall. However, CLM infestation is low to negligible in the spring flush, and levels do not start to increase until April or May, with peak populations in summer and early fall. Similar patterns of lower damage levels during winter have been reported in Argentina (Diez et al., 2006) and during spring in China and Australia (Binglin and Mingdu, 1996). One generation is produced about every three weeks in summer and fall in Florida. Citrus leafminer attacks all types of citrus and its close relatives (Rutaceae), whose flushing patterns vary with citrus varieties and tree age (Knapp et al., 1993). Other factors may also influence the timing and magnitude of flush production. Climatic changes may result in warm weather or rain events in winter that promote the production of new growth, stimulating increases in winter CLM populations. Positive effects of temperature, humidity, and rainfall on CLM density have been reported by Patel and Patel (2001). Also, trees that are infected with the causal pathogen of HLB can experience changes in citrus plant physiology that include irregular production of flushes, which may also affect levels of CLM infestations. Practices intended to reduce the unwanted flushes of new growth, such as managing irrigation and fertilization, may also be useful to reduce the CLM feeding and spread of citrus canker.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Following the discovery of CLM in Florida in 1993, its population expanded and high density populations developed in most of the citrus-producing areas of the state within six months (Knapp et al., 1993). Initial efforts were based on repeated applications of various insecticides and had little effect, and concern developed that CLM would greatly harm citrus groves and nurseries. A classical biological control program against CLM, intended to establish natural enemies from its native region or other regions where effective parasitoids of CLM were known, was initiated soon after CLM's invasion. Successful management of CLM through use of native or introduced parasitoids has been reported from Australia, India, Japan, and Israel (Ishii, 1953; Batra and Sandhu, 1981; Argov and Rossler, 1996; Peña et al., 1996).

In 1994, the parasitic wasp *Ageniaspis citricola* (Hymenoptera: Encyrtidae; **Fig. 5**), originally from Thailand but obtained from Australia where it had been released previously, was released at 52 sites in southwest Florida as part of a statewide program of biological control against CLM (Hoy and Nguyen, 1997; Pomerinke and Stansly, 1998; Hoy and Nguyen, 2003). Before its introduction to Florida, *A. citricola* was imported from Thailand into Australia, where it underwent risk assessment (Neale et al., 1995). Based on those host range data and an assessment of the climatic similarity between Queensland (where the parasitoid had established successfully) and Florida, permission was obtained to import *A. citricola* into quarantine in Florida. Marjorie Hoy hand-carried large numbers of *A. citricola* adults and pupae from Australia into Florida on April 25, 1994. In view of the lack of space and hosts in the quarantine facilities in

Figure 5. Adult of the parasitoid *Ageniaspis citricola*. (Salman Alshami, University of Florida)

Figure 6. Pupae of the parasitoid *Ageniaspis citricola.* (Salman Alshami, University of Florida)

Florida, and the risk analysis information provided by Australian scientists, a request for direct release of *A. citricola* in Florida was submitted to the Division of Plant Industry and approved, which was followed by immediate submission to the USDA-APHIS. John LaSalle at the British National Museum confirmed the identity of the parasitoids from Australia, which facilitated the permission to release *A. citricola* from that source into Florida. First releases of adults *A. citricola* against CLM were made in April 1994 (Hoy and Nguyen, 1997). Females of *A. citricola* lay their eggs into either the eggs or first-instar larvae of CLM, and pupae are produced within the pupal chamber made by the CLM host (**Fig. 6**) (Edwards and Hoy, 1998). *Ageniaspis citricola* is polyembryonic, and females typically deposit two eggs per oviposition event, one of which develops into a male. The second egg splits, producing two daughters (Zappalà and Hoy, 2004); this reproductive strategy may contribute to the success of this parasitoid when host populations are low as each brood can mate with its own siblings. *Ageniaspis citricola* was able to overwinter in Florida despite frost events, and its recovery in spring of 1995 and again in 1996 confirmed its establishment. Parasitism levels by *A. citricola* at monitored groves increased from 2% in May 1994 to 86% in October 1995, apparently unhindered by competition from native parasitoids. In contrast, apparent parasitism of CLM from local parasitoids fell from 30% to 2% during the same period (Pomerinke and Stansly, 1998). Dispersal of *A. citricola* from release sites was reported to occur as far as 48 km (30 mi) from the nearest release point, most likely aided by wind (Pomerinke and Stansly, 1998). Climatic conditions in Florida proved suitable for *A. citricola*. This parasitoid soon became a permanent component of the biological control of CLM in all major citrus-producing areas of the state. Survivorship of *A. citricola* was greatest at 80–95% RH in studies in southwest Florida in 1995 and 1996 (Edwards and Hoy, 1998). Parasitism of CLM by *A. citricola* steadily increased at most sites following the initial release, whereas the proportion of host pupal chambers with local parasitoids declined. A second population of what was thought to be *A. citricola* was imported from Taiwan and released in Florida. This was later determined to be a distinct but cryptic species. However, no evidence of its establishment was found (Hoy et al., 2000; Alvarez and Hoy, 2002).

Cirrospilus (=*quadristriatus*) *ingenuus* (Hymenoptera: Eulophidae), another Asian parasitoid of CLM, was introduced from Australia and released in Florida in 1994 (Hoy and Nguyen 1994, 1997; Smith and Hoy, 1995). The natural range of *C. ingenuus* includes Australia, China, India, Indonesia, Japan, Malaysia, Oman, Taiwan, and Thailand (Schauff et al., 1998; Zhu et al., 2002). This species was known previously as *Scotolinx quadristriatus* (Waterhouse, 1998) and *Cirrospilus quadristriatus* (Subba Rao and Ramamani, 1966). The introductions of this species into Australia, Cyprus, Israel, Morocco, Oman, Syria, Tunisia, and Turkey were under the name of *C. quadristriatus* (Schauff et al., 1998). Evans (1999) synonymized *C. quadristriatus* with *Cirrospilus ingenuus*, and the latter was the name used in Schauff et al. (1998) and LaSalle et al. (1999). *Cirrospilus ingenuus* is a solitary ectoparasitoid of late-instar larvae or prepupae of the CLM, and it generally is restricted to this host. Females may deposit more than one egg in a mine, and its larvae feed on the larvae or prepupae of CLM, but only one adult parasitoid emerges per mine. Ujiye and Adachi (1995) reported a female-biased sex ratio, with approximately 60% female progeny. Adult longevity is about two weeks, and a generation requires two to three weeks, depending upon temperature (Smith and Hoy, 1995).

Initial monitoring in 1994–1996 of CLM populations at locations across Florida failed to detect *C. ingenuus* (Hoy and Nguyen, unpub. data), although it was reported established later in November 1997 and January 1998 around Homestead, Florida (LaSalle et al., 1999). *Cirrospilus* sp. (unidentified specimens) was detected at low levels (<5% parasitism) during the fall of 2017 in Fort Pierce, Florida (Khalid and Qureshi, unpub. data). This parasitoid also caused mortality through probing of the host with the ovipositor (without oviposition) and then feeding on host hemolymph (termed "host feeding") (Neale et al., 1995).

Cirrospilus is a large genus, with approximately 130 species of parasitoids of various lepidopteran or dipteran leafminers (Zhu et al., 2002). Besides *C. ingenuus*, *Cirrospilus* species that have been reported attacking CLM include *C. cinctiventris, C. diallus, C. jiangxiensis, C. longifasciatus, C. lyncus, C. nigriscutellaris, C. nigrivariegatus, C. phyllocnistis, C. pictus, C. variegatus, C. vittatus* sp. nr. *lyncus*, as well as other undescribed species from Honduras, Argentina, Japan, and Colombia (Schauff et al., 1998).

Semielacher petiolatus (Hymenoptera: Eulophidae) is another larval parasitoid of CLM, and it also is capable of host feeding (Argov and Rossler, 1998; Mineo and Mineo, 1999a,b). It has been found attacking the CLM in Australia (Boucek, 1988; Smith et al., 1997) and in the Solomon Islands (Schauff et al., 1998), and it is considered endemic in those regions. It has also been introduced into other areas where CLM invaded, including Cyprus, Israel, Morocco, Oman, Syria, Tunisia, Turkey, Egypt, Greece, and Spain (Schauff et al., 1998). In 1998, *S. petiolatus* was found in Italy for the first time, but the path of its introduction there is unknown (Mineo et al., 1998). By 2001, it appeared to be the most efficient parasitoid of CLM in Italy, with parasitism rates up to 80%. In 2002, *S. petiolatus* was recovered in all the citrus-growing areas of Sicily, with its greatest abundance on CLM in early summer (June–August). In contrast, parasitism rates by an introduced eulophid, *Citrostichus phyllocnistoides*, were higher in the latter part of the growing season (September–October) (Siscaro et al., 2002). *Semielacher petiolatus* was imported and evaluated in quarantine in Florida, but not released because the potential risk of disrupting biological control by *A. citricola* was considered higher than the potential benefit of establishing *S. petiolatus* in Florida (Lim and Hoy, 2005; Lim et al., 2006). *Semielacher petiolatus* appears to be able to attack leafminer species other than the CLM although at low rates.

HOW WELL DID IT WORK?

Ageniaspis citricola has many of the attributes of an effective natural enemy (Rosen and Huffaker, 1983). These include relatively high host specificity (Neale et al., 1995), the ability to locate low-density host populations, and to discriminate between previously healthy and parasitized hosts (Edwards and Hoy, 1998; Zappalà and Hoy, 2004. However, it is not effective in regions with low relative humidity (Yoder and Hoy, 1998), and its population growth lags behind that of CLM populations in the spring in Florida (Villanueva-Jimenez et al., 2000). *Ageniaspis citricola* populations can increase from very low densities to detectable levels by the second flush cycle in Florida and, if not disrupted by drought or pesticide applications, can cause up to 100% parasitism of CLM by the fall, which significantly reduces the overwintering population of CLM (Villanueva-Jimenez et al., 2000; Zappalà et al., unpub. data). However, the biological control of CLM is significantly reduced by the frequent the use of foliar sprays, as occurred in Florida in an effort to suppress Asian citrus psyllid (the vector of HLB disease) after HLB was detected in Florida in 2005.

Although *C. ingenuus* was not detected in initial surveys conducted after its release, it did establish and was recovered from the CLM in November 1997 and January 1998 near Homestead, Florida (Knapp et al., 1999). *Cirrospilus* sp. parasitoids were also detected at low levels (<5% parasitism) in the fall of 2017 in Fort Pierce, Florida. However, establishment has not been confirmed for *C. phyllocnistoides*, another parasitoid that was imported and released against CLM in Florida (J. Qureshi, unpub. data). Both *C. ingenuus* and *C. phyllocnistoides* are also known to probe the host with the ovipositor and engage in host feeding, an additional source of mortality besides parasitism. Additional work is therefore needed in both commercial orchards and urban areas to measure the impacts of the parasitoids introduced for control of CLM populations in Florida.

BENEFITS OF BIOLOGICAL CONTROL OF CITRUS LEAFMINER

The biological control program against CLM provided significant benefits to the Florida citrus industry by establishing a sustainable, inexpensive form of management for CLM, a pest that not only caused direct feeding damage to citrus but also increased the spread of citrus canker. The natural enemies of CLM introduced by this program also help reduce the spread of both the pest and pathogen between commercial and urban areas. Suppression of CLM also reduces citrus production costs by reducing the need for pesticide applications, thus also supporting pesticide-resistance management in citrus. Resistance management is becoming increasingly important for citrus production in Florida due to the widespread occurrence of Asian citrus psyllid and HLB disease. The increased use of insecticidal sprays against Asian citrus psyllid also affects other pests, increasing the risk of pest resistance in many pests found in citrus. In areas without canker disease, natural enemies can probably provide sufficient control of CLM in both tree nurseries and mature groves. However, in situations where canker or the vector-HLB complex exists, extensive use of insecticides is needed. The release of host specific-natural enemies from climatically similar regions is a successful pest control tactic as seen with the introduction of *A. citricola* into Florida. Despite the increased use of insecticides in commercial production systems during the past two decades to suppress the vector of HLB, biological control still provides effective control of CLM in commercial citrus orchards and urban environments.

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Chapter 9

Successful Biological Control of Glassy-Winged Sharpshooter, *Homalodisca vitripennis*, in Invaded Regions

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NON-TECHNICAL SUMMARY

Glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae), is an example of a U.S.-native insect that became a serious pest by invading new parts of the United States and other countries that lie beyond its native range. This pest has been accidentally moved into new areas through trade in live plants, which can carry its egg masses on the plants' leaves. In the absence of specialized egg parasitoids, *H. vitripennis* populations can reach extremely high densities. *Homalodisca vitripennis* is also problematic, in part, in native areas and parts of the invaded range, because of its ability to transmit subspecies of a xylem-limited bacterium, *Xylella fastidiosa*, which causes several lethal, scorch-like diseases in plants. The most infamous of these is Pierce's disease of grapes. In all invaded regions (i.e., California, French Polynesia, Hawaii, Cook Islands, and Easter Island), *H. vitripennis* has been successfully controlled through intentional or accidental introductions of the egg parasitoid *Cosmocomoidea ashmeadi*.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae: Proconiini) (**Fig. 1**), formerly known as *H. coagulata*, is native to the southeastern United States and northeastern Mexico (Triapitsyn and Phillips, 2000). Sometime in the 1980s, *H. vitripennis* was accidentally introduced into southern California, most likely through trade in live ornamental plants from the southeast United States (i.e., intracontinental movement) (Sorensen and Gill, 1996; Blua et al., 1999). Following its introduction into California, this pest had high population growth, and these high-density populations potentially enabled further invasions into new areas. In 1999, *H. vitripennis* established in Tahiti and rapidly spread through French Polynesia (Grandgirard et al., 2000). It is likely *H. vitripennis* was introduced into Tahiti on nursery

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Hoddle, M. S. and D. J. W. Morgan. 2022. Successful biological control of glassy-winged sharpshooter, *Homalodisca vitripennis*, in invaded regions, pp. 92–103. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

plants that originated from California. In 2004, *H. vitripennis* was recorded from Hawaii, and then from Easter Island and the Cook Islands in 2005 and 2007, respectively (Petit et al., 2008a; Rathe et al., 2015). In all instances, movement of live plants was the most likely conduit for long distance spread into new areas of the South Pacific. *Homalodisca vitripennis* eggs (**Fig. 2**) on the undersides of leaves were probably the life stage that was moved undetected into new areas (Petit et al., 2009). Long distance movement of adult *H. vitripennis* in the absence of host plant material via aircraft may also be possible, but the fitness of surviving females may be reduced by a lack of food and exposure to low temperatures in cargo storage areas (Rathe et al., 2015).

Figure 1. Adult glassy-winged sharpshooters, *Homalodisca vitripennis*, feeding on a citrus plant. (Mike Lewis, Center for Invasive Species Research, UC Riverside)

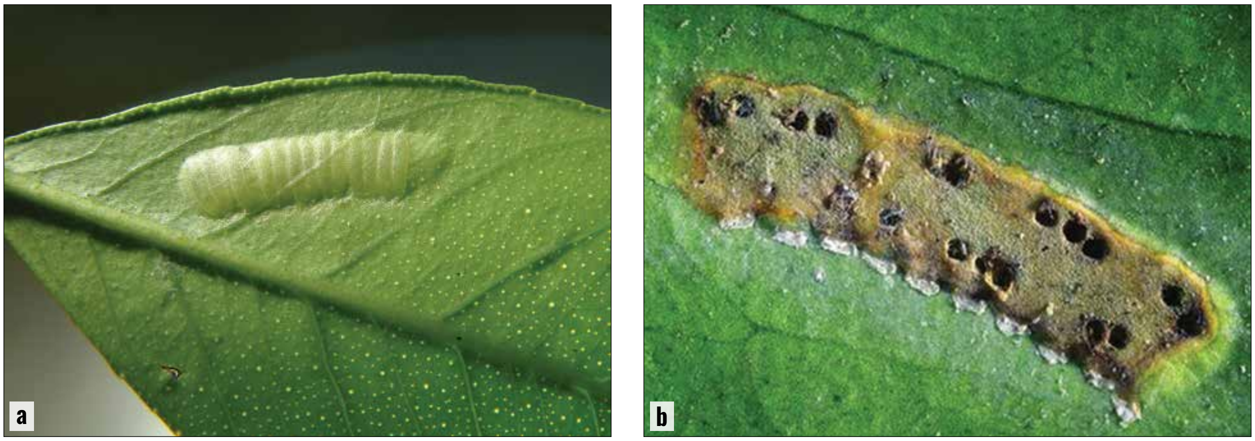

Figure 2. (a) *Homalodisca vitripennis* egg mass laid underneath the epidermis on the underside of a citrus leaf. An egg mass is comprised of individual eggs laid side by side. (b) *H. vitripennis* egg mass with emergence holes made by adult parasitoids that emerged from eggs. (a: Mike Lewis, Center for Invasive Species Research, UC Riverside; b: Regents, University of California)

Nature of the Problem

The damage *H. vitripennis* does, in both its native region and parts of its invaded range, is due primarily to its ability to transmit a xylem-limited bacterium, *Xylella fastidiosa*, of which at least five different subspecies have been identified (Baldi and La Porta, 2017). These subspecies may cause lethal diseases to some crops, urban ornamental plants, and native trees. Some well-known plant diseases that result from *X. fastidiosa* infection following *H. vitripennis* feeding include Pierce's disease of grapes, oleander leaf scorch, and similar maladies in liquidambar (**Fig. 3**), almonds, peaches, plums, and olives. Several of these diseases (e.g., olive leaf scorch) had not been observed prior to the establishment of *H. vitripennis* in California. This pest also increased the severity of existing *X. fastidiosa* problems in California, especially Pierce's disease in grapes. These outcomes were most likely the result of high *H. vitripennis* population densities and broad host range (Sicard et al., 2018). Fortunately, these diseases have not been observed following extra-continental invasion of *H. vitripennis* into the South Pacific as it appears that there were no sources of *X. fastidiosa*, despite the potential of imported plant species being present that could act as asymptomatic reservoirs of *X. fastidiosa*.

Figure 3. (a) Liquidambar tree in an urban area of southern California showing decline due to infection with *Xylella fastidiosa;* (b) a healthy liquidambar devoid of symptoms. (a,b: Mark Hoddle, UC Riverside)

It should also be noted that *X. fastidiosa* has invaded new regions (e.g., Italy [Sicard et al., 2018; Lindow, 2019]) independently of *H. vitripenni*s.

Another problem with high densities of *H. vitripenni*s nymphs and adults is that they are a significant public nuisance due to their production of copious amounts of watery waste that “rains” down from host trees such as crepe myrtle (*Lagerstroemia* spp.). Tree fruit productivity (e.g., of mangoes in French Polynesia) may decline under such heavy feeding pressure. Furthermore, adult insects are attracted to lights at night and readily fly in through open windows and doors, especially in tropical areas. Each morning there is a need to clean up and remove hundreds of dead insects from light fixtures, floors, tabletops, porches, etc. Another problem arises when adult *H. vitripenni*s “buzz” people’s heads when they are attracted to lights at night as the wing beat frequency can be very irritating to some people’s hearing. Rarely, adult *H. vitripenni*s may land on people and attempt to probe exposed skin, and “bites” of this nature are painful.

In French Polynesia, *H. vitripenni*s may have had a significant negative impact on populations of native spiders. Laboratory and field studies indicated that *H. vitripenni*s adults and nymphs were toxic to some species of native spiders that consumed them. The mechanism underlying this putative toxic effect is unknown but could have increased invasion severity in French Polynesia through the creation of natural enemy free space (Suttle and Hoddle, 2006).

WHY CONTROL THIS INVASIVE SPECIES?

*Homalodisca vitripenni*s is a highly polyphagous species (Hoddle et al., 2003) that presents significant threats to perennial trees and vines growing in agricultural areas, urban landscapes, and native forests because of its ability to inoculate host plants with a pathogen, *X. fastidiosa*, which is potentially lethal. The economic losses resulting from *H. vitripenni*s-vectored *X. fastidiosa* is estimated to be in the tens to hundreds of millions of dollars per year (Pilkington et al., 2005; Tumber et al., 2014). Reducing losses and minimizing disease incidence relies, in part, on reducing population densities of vectors such as *H. vitripenni*s that spread the pathogen (Haviland et al., 2021). Vector control is necessary as negative impacts stem primarily when *H. vitripenni*s reaches high densities on a diversity of host plants. Fortunately. *H. vitripenni*s is not an efficient vector of *X. fastidiosa* and this provides opportunities for using biological control and other sustainable Integrated Pest Management (IPM) tools to reduce vector densities with concomitant decreases in incidence of *X. fastidiosa*-related diseases (Daugherty and Almeida, 2019).

During the initial phases of the *H. vitripenni*s invasion in California, large populations of this pest lived in urban areas, from where they could invade neighboring agricultural production zones. Also, *H. vitripenni*s adults can overwinter in organic perennial tree crops (e.g., organic citrus is an excellent overwintering host for *H. vitripenni*s) or minimally managed farms (i.e., low-input IPM citrus), which can also be close to commercial vineyards. In spring, *H. vitripenni*s can move from these overwintering areas into crops like grapes that are susceptible to Pierce’s disease (Blua et al., 2005). Programs to protect grapes focus on vector-reduction through use of insecticides to deter *H. vitripennis* feeding (i.e., sub-lethal doses of systemic neonicotinoid insecticides) or to kill nymphs and adults. Area-wide management programs using insecticides can be effective in reducing the vector's density (Haviland et al., 2021). However, widespread insecticide applications may not be acceptable in urban areas, organic orchards or farms, or wilderness areas, and resistance development is also a significant concern (Byrne and Redak, 2021).

In urban areas in California, *H. vitripennis* can reach high densities because of high levels of host plant diversity, limited pest management, and year-round irrigation. As a result of feeding by *H. vitripennis* in urban areas, scorch-like mortality of oleanders, liquidambar trees, and olives —a phenomenon that used to be uncommon in California—increased significantly because of *X. fastidiosa* infections from *H. vitripennis* feeding. This cycle of infestation and urban tree mortality has significant economic costs, mainly due to the value of these large trees (i.e., tree appraisal values made by professional arborists for property valuations) and subsequent expenses for removing large dying or dead trees and replacing them. Area-wide insecticide treatments in urban areas for *H. vitripennis* control, however, are costly and unpopular with the public.

Similarly, massive spray programs are not feasible in wilderness areas where lower densities of *H. vitripennis* occur, as for example, in natural areas of French Polynesia (Petit et al., 2008a). This is particularly true for invaded islands of the South Pacific, especially French Polynesia, where natural areas are not only large but also extremely mountainous (Petit et al., 2008a). These obstacles to large-scale control can be overcome by using biological control instead of chemical control if a safe and effective natural enemy can be identified. For *H. vitripennis*, the egg parasitoid *Cosmocomoidea ashmeadi* (formerly known as *Gonatocerus ashmeadi*) (Hymenoptera: Mymaridae) (**Fig. 4**) is such a natural enemy. Its successful introduction has been very effective at reducing *H. vitripennis* populations in urban, agricultural, and wilderness areas in invaded regions (Grandgirard et al., 2008, 2009). Similar positive impacts in California were also attributable to *C. ashmeadi* (Gutierrez et al., 2011).

Figure 4. *Cosmocomoidea ashmeadi*, an egg parasitoid that parasitizes eggs of *Homalodisca vitripennis*. (Mike Lewis, Center for Invasive Species Research, UC Riverside)

THE ECOLOGY OF THE PROBLEM

Homalodisca vitripennis is much larger than the sharpshooters native to California (e.g., *Graphocephala atropunctata* [Cicadellini]), which can also transmit resident strains of *X. fastidiosa*, such as *X. fastidiosa* subsp. *fastidiosa* that causes Pierce's disease in grapes. The larger size of *H. vitripennis* allows it to feed on a wide range of woody hosts such as oleanders that were previously unsuitable for feeding by the native sharpshooters, thereby potentially inoculating new plant species with various subspecies of *X. fastidiosa*. Also, in comparison to native Californian sharpshooters, *H. vitripennis* can disperse further from point sources into crops (Haviland et al., 2021). The enhanced capabilities of this new vector have provided opportunities for subspecies of *X. fastidiosa* to exist together in the same host plant, a phenomenon that was likely uncommon in the absence of *H. vitripennis*. This co-occurrence, in turn, sets up opportunities for bacterial recombination events. Genetic changes resulting from recombination may result in bacterial subspecies that can cause infection and disease in new plant species (Nunney et al., 2012). Therefore, plant pathologists have recommended that steps be taken to minimize the likelihood of different *X. fastidiosa* subspecies being inoculated into the same host plants, to limit opportunities for new subspecies to arise through recombination events (Nunney et al., 2010).

Reducing vector densities is one way to reduce the rate and likelihood of such recombination events. In the absence of persistent spray programs, which have significant inherent problems such as non-target impacts and resistance development (Byrne and Redak, 2021), biological control provides an alternative approach to suppressing vector densities. When successful, biological control can help reduce not only vector densities but also the rates at which new *X. fastidiosa* subspecies arise because numbers of feeding *H. vitripenni*s are reduced, which subsequently may also reduce grower reliance on insecticides for pest control as fewer plant disease-causing strains of *X. fastidiosa* are evolving.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Biological Control Efforts in California

Egg parasitoids of *H. vitripenni*s occur in two hymenopteran families: Mymaridae (e.g., *Cosmocomoidea* [formerly *Gonatocerus*]) spp. and Trichogrammatidae (e.g., *Ufens* spp. [Al-Wahaibi et al., 2005]). *Cosmocomoidea ashmeadi* was accidentally introduced into California, likely through the unintentional movement of parasitized *H. vitripenni*s egg masses on plants that originated from the home range of this pest (Vickerman et al., 2004). It is possible that *C. ashmeadi* established during the early phases of the *H. vitripenni*s invasion, or it may have established before the *H. vitripenni*s invasion by using the eggs of a native California Proconiini sharpshooter, *Homalodisca liturata* (formerly known as *H. lacerta*]) (Burks and Redak, 2003; Vickerman et al., 2004). Retroactive host specificity testing indicated that *C. ashmeadi* cannot parasitize native California sharpshooter species outside of the tribe Proconiini (Boyd and Hoddle, 2007).

As part of an importation biological control program targeting *H. vitripenni*s, four species of egg parasitoids were introduced into California. Three of these parasitoids, *Cosmocomoidea fasciata* (a gregarious egg parasitoid formerly known as *Gonatocerus fasciatus*), *Cosmocomoidea triguttata* (a solitary egg parasitoid formerly known as *Gonatocerus triguttatus*), and *Cosmocomoidea morrilli* were sourced from the native range of *H. vitripenni*s in the southeast United States (Triapitsyn and Phillips, 2000; Triapitsyn et al., 2003; Pilkington et al., 2005; Boyd and Hoddle, 2007). The fourth species, *Anagrus epos* (a gregarious, new association egg parasitoid) was imported from Minnesota (USA) where it attacks eggs of *Cuerna fenestella* (Hemiptera: Cicadellidae), a native, univoltine proconiine sharpshooter (Krugner et al., 2008). New association natural enemies (or neoclassical biological control agents) used in classical (or introduction) biological control are species that have no evolutionary association with the target pest but which have the ability to attack a novel host for food (e.g., a predator species) or as a reproductive host (e.g., a parasitoid species) (Hokkanen and Pimentel, 1989; Stenberg et al., 2021).

Table 1 summarizes releases of seven different parasitoid species into California for the biological control of *H. vitripenni*s. Of these seven parasitoids, *C. triguttata* and *C. fasciata* failed to establish permanent populations in California, despite extensive releases and follow-up monitoring efforts (**Table 2**). For example, a total of 1,154,169 *C. triguttata* were released in California across 4,249 release sites from 2000 to 2013 (**Table 2**). A total of 617 field-collected *H. vitripenni*s egg masses resulted in the rearing of 2,028 *C. triguttata* over the period 2000–2015. No field recoveries of this parasitoid have been made since 2015, and it is now assumed that this parasitoid has failed to establish in California (**Table 2**).

Similarly, *A. epos* likely did not establish in California. This may have been due, in part, to rearing difficulties because only 7,485 parasitoids were released over the period 2005–2009 across six counties: Fresno Co. (82 released), Kern Co. (3,538 released), Riverside Co. (2,050 released), Santa Clara Co. (390 released), Tulare Co. (658 released), and Ventura Co. (767 released).

Cosmocomoidea morrilli is native to the southeast United States and northeast Mexico, and the first record of *C. morrilli* in California was of specimens reared from an unidentified Prociniini egg mass (possibly *H. liturata*) in 1981 (Triapitsyn, 2006) before the establishment of *H. vitripenni*s. It is uncertain if *C. morrilli* is native to California or whether it was accidentally introduced. This species was originally

Table 1. Total number of parasitoids released by species by year in California for biological control of *Homalodisca vitripennis*. Parasitoid releases were made primarily by the California Department of Food and Agriculture (CDFA).

PARASITOID SPECIES AND NUMBERS RELEASED								
Year	***C.* ashmeadi***	***C. morrilli***	***C. walkerjonesi***	***C. morgani***	***C. triguttata***	***C. fasciata***	***A.** epos***	***Ufens* sp.**
2000	0	0	0	0	1,440	0	0	0
2001	26,800	0	755	0	107,625	0	0	0
2002	36,598	0	27,195	0	215,442	3,498	0	0
2003	23,901	0	19,902	0	271,404	176,866	0	0
2004	23,666	0	8,836	0	78,536	88,540	0	0
2005	10,737	3,788	2,894	0	42,988	50,540	600	0
2006	12,437	45,122	0	0	42,833	30,850	3,882	0
2007	7,540	79,917	0	2,140	58,325	0	1,926	0
2008	79,865	97,171	0	16,114	89,162	0	1,077	0
2009	14,616	77,445	0	18,051	64,376	0	0	0
2010	8,438	86,490	0	29,941	75,876	0	0	0
2011	2,268	90,644	0	35,654	68,444	0	0	0
2012	3,811	26,635	0	19,374	19,093	0	0	0
2013	699	25,069	0	22,689	18,225	0	0	0
2014	586	17,946	0	29,222	0	0	0	0
2015	2,634	20,075	0	20,407	0	0	0	0
2016	4,570	24,157	0	17,049	0	0	0	0
2017	1,084	16,115	45	7,405	0	0	0	54
2018	12,673	12,517	14	15,998	0	0	0	855
2019	24,956	9,438	0	8,187	0	0	0	58
2020	18,016	8,612	0	6,191	0	0	0	0
2021***	10,939	9,800	0	4,659	0	0	0	0
Total	**269,643**	**650,941**	**59,641**	**253,081**	**1,153,769**	**350,294**	**7,485**	**967**

* *Cosmocomoidea*
** *Anagarus*
*** data available for *C. ashmeadi* through to the end of August

confused with the morphologically similar *Cosmocomoidea walkerjonesi* (see below), a parasitoid native to California but not recognized as a new species at the time. Molecular data and morphological traits have since been identified that separate *C. morrilli* and *C. walkerjonesi* (Triapitsyn, 2006). As part of the classical biological control program targeting *H. vitripennis*, *C. morrilli* was intentionally introduced into California from Texas and Tamaulipas (Mexico) in 2001 (Morgan et al., 2002; Pilkington et al., 2005).

A second new association biological control agent, *Cosmocomoidea deleoni* (formerly known as *Gonatocerus deleoni*), was also considered for potential release and establishment in California. This natural enemy is a solitary egg parasitoid native to Argentina that was originally reared from eggs of *Tapajosa rubromarginata* (Cicadellidae: Proconiini). It was subjected to host range testing in quarantine (Lytle et al., 2012a), but it was found to be competitively inferior to *C. ashmeadi* (Irvin and Hoddle, 2011) and was not released. A third potential new association species, the trichogrammatid *Pseudoligosita plebeian*, was reared from *H. liturata* eggs collected in Mexico. It was studied but not released (Lytle et al., 2012b).

Table 2. Release and recovery data for two species of egg parasitoids—*Cosmocomoidea triguttata* (white) and *C. fasciata* (gray)—released in California for classical biological control of *Homalodisca vitripennis*. Releases and recovery surveys were made primarily by the California Department of Food and Agriculture (CDFA).

Year	No. of release sites		No. of releases at site		Total no. parasitoids released		No. survey visits to release sites		No. GWSS egg masses recovered		No. parasitoids reared from GWSS eggs		No. of survey sites with parasitoid recoveries	
	C. tri	*C. fasc*	*C. tri*	*C. fasc*	*C. tri*	*C. fasc*	*C. tri*	*C. fasc*	*C. tri*	*C. fasc*	*C. tri*	*C. fasc*	*C. tri*	*C. fasc*
2000	4	0	4	0	1,440	-	-	-	0	0	0	0	0	0
2001	42	0	137	0	107,625	0	-	-	1	0	10	0	1	0
2002	34	8	197	13	215,442	0	162	162	42	2	140	2	13	1
2003	46	38	527	332	271,404	3,498	312	317	34	2	182	10	15	1
2004	44	47	286	340	78,536	176,866	438	441	55	1	468	1	18	1
2005	67	57	200	244	42,988	88,540	428	430	13	0	36	0	5	0
2006	61	41	300	162	42,833	50,540	808	819	3	0	13	0	3	0
2007	65	0	270	0	58,325	30,850	982	985	121	0	425	0	21	0
2008	66	0	513	0	89,162	0	1,225	1,236	42	0	121	0	14	0
2009	65	0	380	0	64,376	0	1,246	1,247	20	0	82	0	9	0
2010	66	0	539	0	75,876	0	1,295	1,295	63	0	371	0	17	0
2011	65	0	546	0	68,444	0	1,268	1,268	28	0	157	0	9	0
2012	44	0	204	0	19,093	0	1,316	1,316	8	0	9	0	7	0
2013	41	0	146	0	18,225	0	1,043	1,043	3	0	3	0	3	0
2014	0	0	0	0	0	0	637	665	0	0	0	0	0	0
2015	0	0	0	0	0	0	570	661	1	0	11	0	0	0
2016	0	0	0	0	0	0	479	611	0	0	0	0	0	0
2017	0	0	0	0	0	0	0	528	0	0	0	0	0	0
2018	0	0	0	0	0	0	0	517	0	0	0	0	0	0
2019	0	0	0	0	0	0	0	494	0	0	0	0	0	0
2020	0	0	0	0	0	0	0	470	0	0	0	0	0	0
2021	0	0	0	0	0	0	0	346	0	0	0	0	0	0
Total	**710**	**191**	**4,249**	**1,091**	**1,153,769**	**350,294**	**12,209**	**14,851**	**434**	**5**	**2,028**	**13**	**135**	**3**

Two other species of parasitoids reared from *H. vitripennis* eggs collected in California that were subsequently mass reared and released for biological control of *H. vitripennis* included *Cosmocomoidea morgani* (possibly native to California [Triapitsyn, 2006]), and *C. walkerjonesi* (native to California [Triapitsyn, 2006]) (**Table 1**). Morphologically, *C. morgani* is more similar in appearance to some undescribed *Cosmocomoidea* species from Central America. Triapitsyn (2006) has speculated that *C. morgani* may not be native to California but may be an introduced species that established when *H. vitripennis* eggs masses were highly abundant. It is possible that *C. morgani* is native to California but simply was not detected before 2003 when it was reared for the first time. However, this seems unlikely (S. Triapitsyn, pers. comm.). Molecular studies may help determine the area of origin of *C. morgani*.

Biological Control Efforts in French Polynesia

Following the invasion and establishment in 1999 of *H. vitripennis* in Tahiti and Moorea (French Polynesia), surveys found very low levels of egg parasitism (<3%) by three local species of parasitoids (Grandgirard et al., 2007). These survey data strongly suggested that *H. vitripennis* was benefiting from natural enemy-free space and that the pest's high rate of population growth and rapid spread were due to natural enemy escape combined with a highly suitable climate that favored year-round reproduction. Consequently, the decision was made in 2004 to initiate an importation (= classical) biological control program with *C. ashmeadi* in French Polynesia, and a large-scale project was initiated with parasitoids from California (Grandgirard et al., 2008, 2009). Following the completion of studies that indicated an acceptably low level of risk to non-target parasitism, a total of 13,786 parasitoids were released at 27 sites on Tahiti between May and October of 2005 (Grandgirard et al., 2008). These releases resulted in rapid establishment, spread and impact, as described in the next section.

HOW WELL DID BIOLOGICAL CONTROL WORK?

In southern California, monitoring of *H. vitripennis* populations in non-sprayed citrus indicated that over the course of a 10-year period (2002–2012), *H. vitripennis* densities declined by >95%, and parasitism by *C. ashmeadi* was a likely cause (Banks et al., 2019) (**Fig. 5**). Densities of *H. vitripennis* in urban areas in California in 2022 now tend to be significantly lower compared to earlier levels. However, in some agricultural areas where insecticides have been consistently used to protect grapes from *H. vitripennis*-*X. fastidiosa*, pest populations remain unacceptably high (Byrne and Redak, 2021). This problem may be due, in part, to the development of insecticide resistance by *H. vitripennis* (Byrne and Redak, 2021), the damaging effects of the insecticides on natural enemies, including *C. ashmeadi*, and a very low tolerance threshold for the presence of this pest by grape growers.

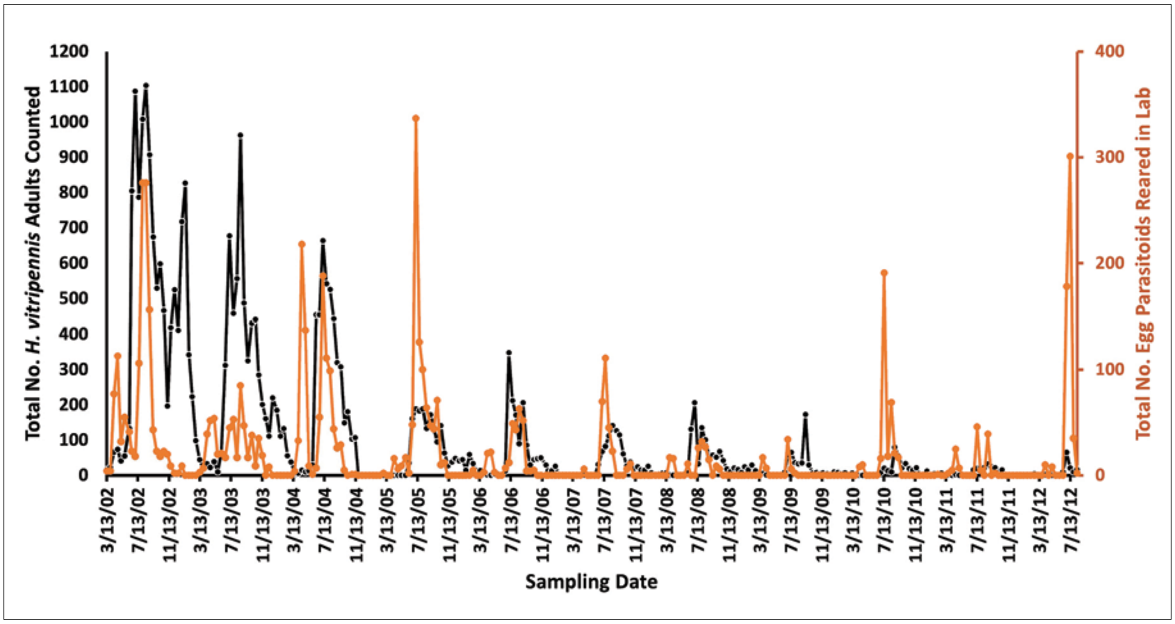

Figure 5. This chart shows an ~95% decline in *H. vitripennis* densities in non-treated lemons over the period 13 March 2002 to 8 August 2012 at Ag. Ops. UC Riverside, and the corresponding numbers of parasitoids reared from collected *H. vitripennis* egg masses that were found during timed visual searches of survey trees. (See Banks et al., 2019 for more details on field surveys and parasitoid rearing.)

In French Polynesia, the impact of *C. ashmeadi* on *H. vitripennis* population densities was extremely high and rapid. By December 2005, ~7 months after parasitoid releases began, parasitism of *H. vitripennis* egg masses averaged 80–100% in Tahiti and the neighboring island of Moorea. As a result of these high levels of egg parasitism, populations of *H. vitripennis* nymphs and adults decreased by more than 90% (Grandgirard et al., 2008, 2009).

This strong suppressive effect was later recorded across three additional archipelagos (Society, Austral, and Marquesas) in French Polynesia (Grandgirard et al., 2009). Interestingly, *C. ashmeadi* was not released into the Austral or Marquesas archipelagos; establishment of this parasitoid on these islands was likely due to the movement of parasitized *H. vitripennis* egg masses on plants being transported between islands (Petit et al., 2009). Similarly, for Hawaii, the Cook Islands, and Easter Island, *C. ashmeadi* was also moved inadvertently by people on plants, likely as parasitized *H. vitripennis* egg masses. Following the establishment (intentional or accidental) of *C. ashmeadi* in these new areas, *H. vitripennis* populations quickly declined to very low levels. Unfortunately, the impacts of *C. ashmeadi* across the Austral or Marquesas archipelagos in French Polynesia, Hawaii, and the Cook Islands have not been studied and deserve documentation. Similarly, for the islands of Tahiti and Moorea in French Polynesia, follow-up studies evaluating the long-term suppressive effect of *C. ashmeadi* on *H. vitripennis* populations are warranted.

BENEFITS OF BIOLOGICAL CONTROL OF *HOMALODISCA VITRIPENNIS*

Another significant but underappreciated consequence of elimination of *H. vitripennis* spread in the South Pacific region is that countries with significant agricultural enterprises (e.g., New Zealand, Australia, and Chile [Easter Island is a special territory of Chile]) were protected from invasion by this pest. The mechanism behind this protection was likely the substantial reductions in *H. vitripennis* densities in many areas due to biological control. There is also a high likelihood that when egg masses are accidentally transported into new areas, some eggs are parasitized by *C. ashmeadi*. In areas that receive parasitized *H. vitripennis* egg masses, the invader is less likely to establish because too few viable sharpshooters will be introduced to overcome the problem of failure to mate that arises as a small group of invaders spreads out randomly over a large area (Allee effect) (Petit et al., 2008b). Importantly, from a risk management perspective, the *C. ashmeadi* adults that emerge from *H. vitripennis* egg masses in areas where *H. vitripennis* invades but fails to establish are themselves unable to establish if suitable eggs for parasitism (i.e., Proconiini sharpshooter eggs) in receiving areas are not present.

Both New Zealand and Australia were sufficiently concerned about the invasion threat posed by *H. vitripennis* originating from infested islands in the South Pacific that proactive studies were undertaken to assess risks to native plants from *H. vitripennis*-*Xylella* (Rathe et al., 2014; Groenteman et al., 2015). A strong argument has been made that non-target impact assessments (i.e., host range and host specificity testing) should not be required for *C. ashmeadi* releases in New Zealand should *H. vitripennis* establish there because this natural enemy would pose little risk to New Zealand's native cicadellids, none of which are in the tribe Proconiini—the only group that is suitable for parasitism (Charles, 2012).

In southern California, especially urban areas, *H. vitripennis* populations are now very low, and it is rare to experience heavy levels *H. vitripennis* 'rain' (i.e., *H. vitripennis* excreta) falling from infested trees, even from highly preferred species like crepe myrtle. In agricultural areas, especially where insecticides tend to be applied frequently to protect grape cultivation, *H. vitripennis* is still considered a potential problem because of the risks it poses through the transmission of *X. fastidiosa*. The persistence of *H. vitripennis* in agricultural areas may also be due to reductions of natural enemy populations, including *C. ashmeadi*, because insecticides are accidentally killing these beneficial insects. Long-term, regular insecticide use is also driving resistance development in *H. vitripennis* in some major grape-growing areas (Byrne and Redak, 2021), and this phenomenon may exacerbate the risk this vector poses to commercial grape producers.

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CHAPTER 10

Biological Control of Cereal Leaf Beetle in the United States

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NON-TECHNICAL SUMMARY

Cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae), is one of the most serious pests of small grain crops worldwide. Without specialized natural enemies, this beetle can significantly reduce yields of wheat and oats, which are food crops important to large parts of the world. Native to Eurasia, cereal leaf beetle invaded North America in the early 1960s when it was first recorded in the United States in Michigan. The beetle quickly began to spread through wheat-growing regions and was soon the target of a regional eradication program by the USDA-APHIS. Within a few years, it was obvious that pesticides weren't going to slow the spread of cereal leaf beetle, and pesticides were a major cost to producers of various grain crops. In 1964, efforts were initiated to develop grain varieties resistant to feeding by this pest and to find parasitoids associated with cereal leaf beetle in its native range. The USDA-ARS European Parasite Laboratory, now called the European Biological Control Laboratory, spent six years (1964–1969) searching for parasitoids closely associated with cereal leaf beetle throughout Europe. Four species with promise for use in biological control were shipped to the United States, and eventually two of these became permanently established in the eastern United States. The larval parasitoid *Tetrastichus julis* (Hymenoptera: Eulophidae) was the most successful and quickly established new populations. *Anaphes flavipes* (Hymenoptera: Mymaridae), which attacks the egg stage of the beetle, also established permanent populations in the eastern United States in the 1960s and 1970s. By 1999, the cereal leaf beetle had spread across Canada and the Pacific Northwest grain-growing regions. Initially, growers in midwestern, eastern, and northwestern U.S. states applied pesticides to control the pest, but without achieving sustainable control. Following some success of the biological control program against cereal leaf beetle in the eastern United States, the two most successful parasitoids were obtained and shipped to Washington, Oregon, and nearby states. Recoveries of *A. flavipes* were never recorded in this region or Utah. However, parasitism of larvae by *T. julis* reached high levels in field insectaries designed to aid establishment of these parasitoids. Farmer pesticide-use records, required in Oregon, showed that as the larval parasitoid spread, the amount of pesticide used for cereal leaf beetle control in Oregon declined (Roberts and Rao, 2012).

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The beetle was first reported in northern California in 2013. Within one year of that observation, *T. julis* was collected in central Oregon for release just across the border in northern California. A field insectary was established at the University of California (UC) Research and Extension Center in Tulelake, California to produce *T. julis* for release throughout the Klamath River Basin grain production area of northern California. Between the natural dispersal of parasitoids from this insectary, redistribution of the parasitoid, and its natural spread from Oregon south into California, high levels of parasitism were soon recorded from nearby commercial small grain fields. Within five years (2018), numbers of cereal leaf beetle in grain fields declined significantly. Control of this pest with pesticides in northern California and the Pacific Northwest cost several million dollars per year, and these savings, following the success of this biological control program, were directly passed on to regional grain growers. Furthermore, the non-target effects of routine insecticide treatments to such common crops as wheat and oats was avoided.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Cereal leaf beetle (CLB), *Oulema melanopus* (Coleoptera: Chrysomelidae), is a serious pest of small grains and grass forage (**Fig. 1**). Native to Eurasia, the beetle was first reported in the United States in Michigan in 1959 (Dysart et al., 1973), and was formally identified as a new pest in North America in 1962. The beetle rapidly spread eastward but was slower in its westward movement (Haynes and Gage, 1981). By mid 1980s, CLB could be found in Montana, Idaho, and Utah, and it was first reported in Washington State in 1999 (Rao et al., 2003; Evans et al., 2006; Roberts, 2016). Populations can now be found throughout much of the continental United States except for the drier, warmer regions of the Southwest (southern California, Arizona, New Mexico, Texas, Oklahoma) where it has yet to be reported. The beetle has also spread north and can be found throughout the cereal-growing regions of Canada (Kher et al., 2013). Cereal leaf beetle was first reported in northern California along the Oregon border in 2013 (Dowell and Pickett, 2016).

The CLB was first found in the grain belt of the United States, and because it is such a serious pest of wheat and oats, the first response to CLB by the USDA was to limit its spread and achieve eradication (Haynes and Gage, 1981). In 1962, townships in Michigan and Indiana were placed in quarantine, and all small grains had to be treated before leaving these areas. Millions of bushels of hay, straw, and small grains were treated with insecticides. Repeated spraying of several hundred thousand acres of these same commodities

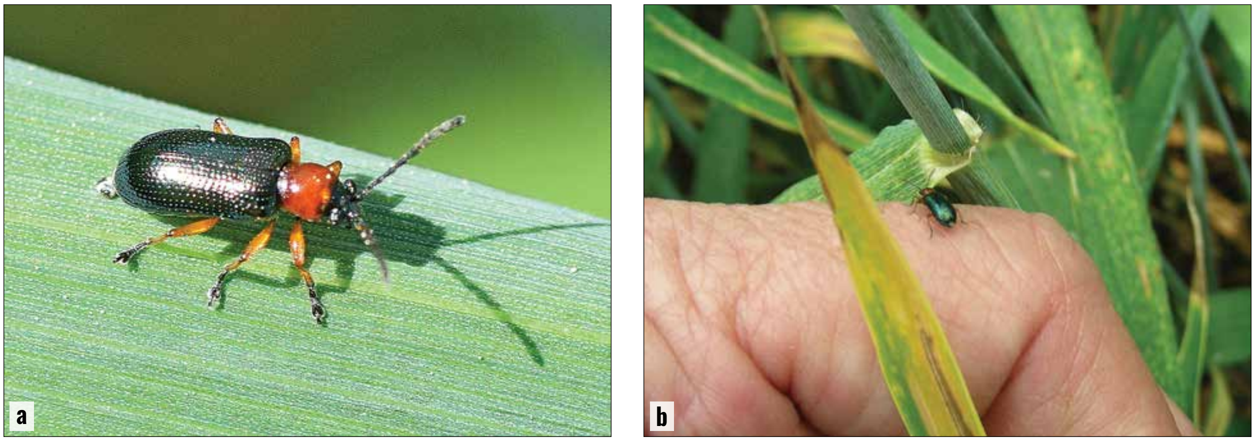

Figure 1. Adult cereal leaf beetle, *Oulema melanopus*, in wheat field (a) close-up; (b) further back for scale. (a: Kostas Zontanos, iNaturalist.org CC BY-NC 4.0; b: C. Pickett, CDFA)

took place in Indiana. Over whole regions, fields of grains and nearby suburban areas were treated with malathion and carbaryl. In 1967, after more than a million acres (>400,000 ha) of land in the Midwest had been treated with insecticides, the federal and state spray programs were abandoned; containment was deemed impossible. The California Department of Food and Agriculture imposed a quarantine against CLB during this early phase of its spread to include all states east and including Minnesota, Iowa, Missouri, Arkansas, and Louisiana (Anon., 2007).

Nature of Problem

The diet of CLB is limited to the leaves of small grains and forage grasses (Poaceae = Gramineae). It prefers oats (*Avena sativa*) over wheat (*Triticum aestivum*), barley, (*Hordeum vulgare*), or rye (*Secale cereale*) (Gallun et al., 1966). Although adults can be found feeding on grasses, it is the larval stage that causes serious economic damage (**Fig. 2a**). Larvae skeletonize and strip leaves of green tissue, and when high numbers are present, they cause fields to appear bluish (**Fig. 2b**). From 13 to 75% yield losses were reported in barley in the midwestern United States (Webster and Smith, 1979). Reduction in yield is directly related to consumption of foliage, which reduces the plant's photosynthesis (Haynes and Gage, 1981). Controlled field studies used insecticides to create pest-free fields (Buntin et al., 2004) for comparisons between fields of wheat with naturally high damage levels (>90% damaged flag leaves) and treated fields with undamaged leaves (Gallun et al., 1967). These comparisons showed a direct relationship between the number of CLB larvae on grain leaves and the degree of yield loss. Although CLB attacks many species of grasses, it prefers those with a C_3 pathway over C_4 plants (i.e., wheat over corn). Shortly after CLB was found in California, the rice industry requested studies on the potential impact of this beetle on rice, *Oryza sativa*, a C_3 plant. However, like many grasses, the crop proved to be unsuitable for CLB reproduction (Godfrey et al., 2002), suggesting there were other factors more than just C_3 vs. C_4 status that affected a grass species' suitability for this beetle.

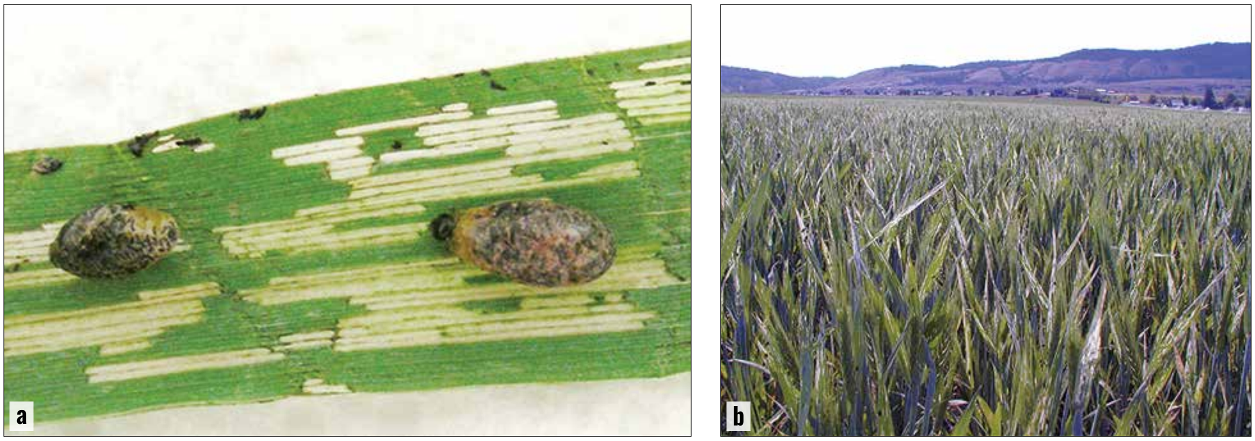

Figure 2. (a) Leaf skeletonization of oats caused by cereal leaf beetle, *Oulema melanopus* (CLB); (b) CLB-damaged wheat field. (a: Photo by C. Pickett, CDFA; b: D. Walenta, Oregon State University)

The invasion of the midwestern United States by CLB prompted the initiation of a classical biological control program against CLB. The rapid spread and increase of CLB's population demonstrated the inability of resident natural enemies to suppress its population growth, and this rapid spread triggered a regional quarantine and eradication program led by the USDA and local agencies (Haynes and Gage, 1981). As has happened with other invasions of exotic pests in the past, failed eradication efforts were followed by investment into host plant resistance, followed lastly by development of a classical biological control program. Later, when the Pacific Northwest was invaded, growers immediately started spraying on a calendar basis for CLB control. Once chemical treatment became too costly to sustain, natural enemies of CLB were imported from midwestern states where effective CLB parasitoids had become recognized following their release in that region.

WHY CONTROL THIS INVASIVE PEST

Wheat is the most important food staple in North America and other temperate regions of the world (Shewry and Hey, 2015). Similarly, oats are also a significant grain food staple with increasing demand due to its nutritional advantages: high fiber content important for digestion and low gluten content important to patients of celiac disease (Rasane et al., 2015). Both grains are heavily attacked by CLB. Thus, there was initially a major effort to eradicate this pest from the midwestern United States. At its peak, 1,616,807 acres (654,300 ha) were being treated with insecticides in Michigan, Illinois, and Indiana. The financial cost to growers in 1966 was $1.85 per acre ($0.75/ha), or in total $2,991,093 (Haynes and Gage, 1981). Adjusting for inflation, losses would be about $30 million today (2022). Because spraying included urban areas, lawsuits arose, adding another unanticipated cost. Delineating grain fields from houses and cars was considered too expensive to do when applying pesticides aerially, and this inability to target pesticides accurately brought the eradication effort to an end. Furthermore, pesticide treatments did not slow the spread of CLB into adjoining wheat-growing states. Growers of small grains in Utah and Washington reacted in the same way following the discovery of CLB in their states (Karren, 2008; Roberts and Rao, 2012). These states immediately started massive state-coordinated, county-wide spraying for this pest, greatly increasing the growers' production costs (Roberts and Rao, 2012). Not only did these eradication projects reduce grower profits, most likely spraying disrupted natural control of other pests. As the likely failure of eradication efforts became clear to state and federal agencies funding the efforts, CLB control efforts were widened to include other management strategies. In 1963 and 1964, considerable effort was directed at developing resistant varieties of the main affected grains (wheat, oats, and barley). While some lines of wheat resistant to CLB were developed, none were found for oats. Given that CLB would likely remain a serious problem unless resistant crop varieties could be developed for all major grains, funding was finally directed at classical biological control, beginning in the early 1960s. As has happened in many past invasions, classical biological control was the 'tool' of last resort.

The most important reason for pursuing classical biological control was the need for regional control that would make a serious pest of a major food staple more manageable for growers. Although pesticides could be used, they are costly and can be disruptive to the environment. But what made this approach realistic was the discovery of several primary, larval parasitoids attacking CLB in parts of its native range in Europe. Surveys of wheat, oats, and barley measuring parasitoid prevalence, species dominance, and percent parasitism found three potential candidates for importation (Dysart et al., 1973). During nine years of surveys by the European Parasite Laboratory, USDA-ARS (currently the European Biological Control Laboratory), no serious CLB damage was recorded in grain fields in Italy, France, Spain, Denmark, or Sweden. Records summarized by others in Europe since the 1850s until 1963 reported only a few outbreaks, described as 'sporadic' and 'localized' (Dysart et al., 1973).

PROJECT HISTORY THROUGH ESTABLISHEMENT

Midwestern United States

Midwestern releases

Beginning in 1965, large numbers of CLB larvae were field collected by the European Parasite Laboratory and held until parasitoid emergence. The adult parasitoids then were shipped to the

USDA-ARS laboratory in Moorestown, New Jersey. The USDA-ARS contracted with Michigan State University and Purdue University in Indiana to study the biology of these new parasitoids and to make initial releases (Maltby et al., 1971; Dysart et al., 1973; Haynes and Gage, 1981). Difficulty in rearing the larval parasitoids shifted the emphasis of the release effort towards using parasitoids reared from CLB collections in Europe for direct field releases in the United States (Dysart et al., 1973). Shipping parasitoids, primarily as diapausing larvae or pupae, provided the most vigorous parasitoids for release. The parasitoids were identified to species at the USDA-APHIS Laboratory in Niles, Michigan then shipped to cooperators. Delivery from Europe within 48 h through commercial air transportation aided rapid field release of healthy parasitoid adults. Three larval parasitoids (all Hymenoptera) were shipped for release to affected areas in eastern states from 1964 to 1971: 82,437 *Tetrastichus julis* (Eulophidae), 142,054 *Diaparsis* spp. (unknowingly a mix of two ichneumonids, *D. carinifer* and *D. temporalis*; Montgomery and De Witt, 1975; Haynes and Gage 1981), and 11,421 *Lemophagus curtus* (Ichneumonidae) (Dysart et al., 1973). During the same period, a fourth hymenopteran parasitoid, *Anaphes flavipes* (Mymaridae), was also imported from Europe. This species attacks CLB eggs (Maltby et al., 1971) and was easier to rear than the larval parasitoids. *Anaphes flavipes* was mass produced in a rearing facility built by USDA-APHIS in Niles, Michigan, starting in 1966. Nearly 300,000 egg parasitoids were reared and provided to cooperators for field releases, starting in Michigan and Indiana.

Midwestern recoveries

By 1972, all four parasitoids had been recovered from initial release locations in various states in the midwestern United States. *Tetrastichus julis*, *D. temporalis*, *L. curtus*, and *A. flavipes* were documented to have established in Michigan (Maltby et al., 1971; Dysart et al., 1973), but only *A. flavipes* was recovered in Ohio (Maltby et al., 1971). The most common larval parasitoids initially recovered in the midwestern states were *T. julis* followed by *D. temporalis*. The larval parasitoid *D. temporalis* was projected as most likely to control CLB based on its abundance in field collections in Europe, its biology, and its synchrony with CLB larvae (Dysart et al., 1973, Haynes and Gage, 1981). In 1975, *D. temporalis* was recovered from Michigan, Ohio, Indiana, West Virginia, Pennsylvania, and New York, but it hasn't been reported since then, while *L. curtus* continued to be collected, although only at low numbers. In contrast, *T. julis* was recovered with increasing frequency and at higher rates. *Tetrastichus julis* and *A. flavipes* continued to spread throughout Michigan and Ohio (Maltby et al., 1971; Dysart et al., 1973).

Western United States

Western releases

The first report of CLB in the western United States came from Utah in 1984. Despite county-wide spraying of malathion, CLB continued to spread throughout the state (Karren, 2008). In 1985, the two larval parasitoids *T. julis* and *D. temporalis*, and the egg parasitoid *A. flavipes* were imported and released into Utah (Karren, 1986) and shortly thereafter in Montana from the eastern United States, with help from USDA-APHIS (Blodgett et al., 2004). The first detections of CLB in Washington and Oregon were made in 1999. Cooperative Extension agents in these states led a coordinated survey for CLB and releases of their biocontrol agents (Rao et al., 2003; Roberts and Rao, 2012). The 'Western Cereal Leaf Beetle Team' of federal, state, and university personnel from seven states focused their efforts on establishing the parasitoids with potential to suppress CLB in the central and western United States and Canada (Logan et al., 1976; Harcourt and Guppy, 1977; Haynes and Gage, 1981; Ellis et al., 1988).

The initial releases coordinated by this western CLB team were of 13,928 *T. julis*, 25,631 *A. flavipes*, and 125 *L. curtus* from collections made in Pennsylvania and Montana (Roberts, 2016). Before being released, parasitoid shipments were examined at the Washington State University's quarantine facility. Releases were made, when possible, in field insectaries that were manipulated for high numbers of CLB in suitable stages. These insectaries were blocks of commercial or non-commercial grains grown in or near areas with high CLB density that were managed to maximize parasitoid production through high production of its host larvae, i.e., exposure to young pre-boot stage oat plants (Roberts, 2016). Sequential plantings of small grains from fall to the following spring ensured continuous presence of the optimal stage of plants for beetle development. The use of field insectaries was repeated in Oregon, but there are no published data on numbers of parasitoids released in that state. One project report (Bai et al., 2008) states that 383,626 *T. julis* and 114,160 *A. flavipes* were released from 2000 to 2008 in the northern half of Oregon. In 2013, CLB was reported for the first time in California. Most likely this was at the very early stage of the beetle's invasion into California given that 10 years of state-wide surveys by the California Department of Food and Agriculture (2001-2010) did not report any CLB adults (Dowell and Pickett, 2016). In 2014, one year after the 2013 CLB detection), a total of 630 *T. julis* parasitoids were collected from wheat fields near Portland, Oregon and released into northern California at an insectary following methods described in Roberts (2016) and managed by the UC Intermountain Research and Extension Center at Tulelake (Pickett et al., 2021). From 2015 to 2017, six releases of *T. julis* (from a low of 20 up to 530 individuals each) were made in commercial wheat fields using CLB larvae from this field station. Parasitoids were released as larvae inside their CLB hosts (**Fig. 3**); numbers of parasitoids were estimated for each release by dissecting 5–10 hosts to check for parasitism.

Figure 3. Parasitoid-filled cadaver of cereal leaf beetle, *Oulema melanopus*, showing emerging *Tetrastichus julis* larva, circled in red. (C. Pickett, CDFA)

Western recoveries

In Utah, only *T. julis* established permanent populations (Karren, 2008). In Washington and Oregon, *A. flavipes*, although released in large numbers, did not establish (Roberts, 2016). Similarly, *L. curtus* has not yet been reported as established in Washington, following releases of small numbers. In northern California, parasitism of CLB by *T. julis* reached 80% at the field insectary one year after initial releases (Pickett et al., 2021). Pre-release dissections at the same site showed *T. julis* was present but at low densities when parasitoids were first released. Interestingly, Roberts (2016) also reported 80% parasitism at one of their insectaries in Washington one year following releases, and they speculated, without data, that the parasitoid was already present at the time of initial releases. These results, and the discovery of *T. julis* outside of field insectaries in Washington and California (Roberts, 2016; Pickett et al., 2021) suggest this parasitoid was able to rapidly disperse and track populations of CLB invading new areas. Contrary to earlier studies on *T. julis* (Haynes and Gate, 1981), this species rapidly spread through the Pacific Northwest and dispersed into both crop and non-crop habitats (Evans et al., 2015).

> One caveat: the lack of recoveries of some parasitoid species that were released may not reflect their absence so much as the little effort expended in looking for them. They may be present on other non-target species or just present in very low numbers on CLB.

HOW WELL DID IT WORK?

Impact in the Midwestern United States

For the midwestern United States, little quantitative information on the impact of the project on CLB densities was recorded because few long-term post-release studies were carried out documenting parasitism and changes in CLB numbers. We do know that *T. julis* was repeatedly recovered from CLB larvae within just a few years of its release at the Kellogg Biological Station in Michigan, and parasitism there went from 20% in 1975 to 60% the following year. A parallel decline in the local CLB population also occurred in the same period at a site in Jackson County that was nearly 47 mi (75 km) away, where parasitoids had not been released. This decline led to the belief that causes other than parasitism were responsible (Haynes and Gage, 1981). However, because other releases of *T. julis* had been made in 1965 at sites near the USDA-APHIS rearing facility in Niles, Michigan (about 62 mi or 100 km from the Jackson County site), it is possible that the parasitoid had dispersed on its own to Jackson County by the mid-1970s. In 1975 there was little evidence that *T. julis* alone could have such a large impact on CLB, and expectations for its success at that time were low because *T. julis* was considered a poor disperser and possibly a poor competitor with *A. flavipes*, which at that time seemed more promising. Also, predictions of impact needed to consider an important difference between Europe and North America, which was that the introduced primary parasitoids were freed of any restraints imposed on them by their co-evolved, secondary parasitoids. Dysart et al. (1973) recorded three hyperparasitoids collected from *Oulema* spp. (CLB and its relatives) in Europe, any of which could have affected the impact of the primary parasitoids if present in the United States. To date, however, there have been no reports of hyperparasitism of the introduced CLB parasitoids in the United States.

Impact in the Pacific Northwest

More recently, in the Pacific Northwest the primary goal of the CLB team was to establish field insectaries for *T. julis* to support widespread releases. No effort was made to measure CLB numbers before and after releases, only their parasitism shortly after releases were made. In Washington State, Roberts (2016) provided detailed estimates of parasitism at several field insectaries. Where measurements were made over 2 to 4 years, the annual trends in parasitism were always upward, going from a low of 11% to a high of 100%. For seven locations, the average parasitism of CLB larvae in the final year of observations was 90%. Although a similar effort was not made in Oregon by Cooperative Extension, the USDA-APHIS did report on the change in acreage of cereals treated with pesticides for CLB, as well as the cost of pesticide treatment in that state (Roberts and Rao, 2012). In 2004, at CLB's peak density in Oregon, 64,200 acres (25,980 ha) were treated with insecticides at a total cost of $770,400. By 2007, three years after the start of releases of *T. julis*, the acreage treated had dropped by 70% to 19,141 ac (7,746 ha).

Impact in Northern California

In northern California, the impact of *T. julis* on CLB was measured at the initial field insectary located at the UC Intermountain Research and Extension Center in Tulelake from 2016 to 2019 and in several commercial wheat fields in the Klamath Basin wheat-growing area in northern California on the Oregon border (Pickett et al., 2021). The insectary in Tulelake consisted of a sequence of plots planted first to wheat in the fall followed by three adjacent plots of oats seeded about 3–4 weeks apart beginning the following April. The result was an insectary that started with young winter wheat followed by the continuous presence of early-stage oats. The undisturbed, non-sprayed plot of wheat was meant to attract the fall cohort of CLB adults and provide a bridge to young host plants for CLB and parasitoids the next year. This sequence of plantings was maintained for five years. Parasitism was based on samples taken one to two weeks apart, usually June through July, pooling on each sample date the larvae collected from all plots of oats at the UC Intermountain Research and Extension Center CLB insectary. Parasitism was measured by dissecting 2nd to 4th instar CLB larvae and recording the number of beetle larvae found with eggs or developing larvae of *T. julis*. Annual parasitism, based on the proportion of CLB larvae parasitized on each date and averaged over the summer at this insectary location, increased from 59% in 2016 (two years after releases), to 73% in 2017, and 85% in 2018. Oats were used as the host plant in these plots because of CLB's preference for this plant over other common grains (Gallun et al., 1966; Wellso and Hoxie, 1988; Roberts and Walenta, 2012).

Because parasitism reached such high levels so quickly, high numbers of CLB larvae, parasitized or otherwise, were rarely observed. Nevertheless, the UC Intermountain Research and Extension Center insectary in Tulelake was a source for naturally dispersing parasitoids in this region and for parasitized larvae that were deliberately released into nearby fields. Over several years, commercial fields of small grains in the region, located within 18.6 mi (30 km) of the field insectary at the UC Intermountain Research and Extension Center, showed a decline in numbers of CLB while parasitism was increasing (**Fig. 4**) (Pickett et al., 2021). The combined number of CLB nymphs and adults in this region was reduced by nearly 90% over the same four years (2016–2019). These fields were all located within a 25 x 25 mi (40 x 40 km) region, and the sampling results showed that *T. julis* could move several miles a year and rapidly increase its population numbers, a result that was also corroborated by Evans et al. (2015). Based on our own estimates, *T. julis* could successfully lay about six eggs per beetle larva, in agreement with other similar studies (Dysart et al., 1973). Because *T. julis* has two generations per year compared to just one for CLB, and the parasitoid can quickly move into new fields in search of hosts, it is reasonable that it can suppress beetle populations as rapidly as in a single year.

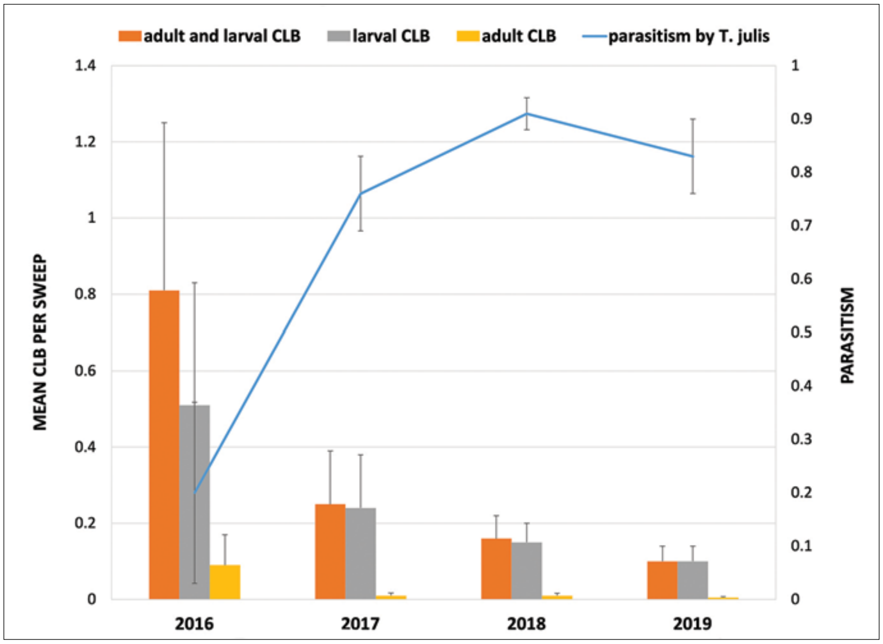

Figure 4. Changes in cereal leaf beetle, *Oulema melanopus* (CLB), numbers and portion parasitized by *Tetrastichus julis* in Modoc County, California 2016–2019. (modified from Pickett et al., 2021)

Effects on Non-Target Insects

In the 1960s, when the biological control project against CLB was started in Michigan, studies of possible impacts on non-target insects were not required by the federal government. However, in the early 2000s, post-release tests were carried out in Canada to detect potential non-target impacts (Hervet et al., 2016). Six species of beetles that were taxonomically related to CLB and shared similar life history traits were chosen from the beetle community of southern Alberta where *T. julis* had recently become established. In no-choice tests, none of these six beetle's larvae were attacked by *T. julis*, but some CLB larvae were parasitized, providing strong evidence this parasitoid is highly specialized to reproduce on CLB. Thus, work of earlier researchers who followed the standard protocols used for foreign exploration and selection of natural enemies for release in the 1960s, resulted in the establishment of an effective and safe parasitoid. In many areas of the country, *T. julis* is now highly effective in reducing populations of CLB in ephemeral grain crops. At the same time, it is highly selective as to which host species it uses for reproduction. To our knowledge, similar host specificity studies were never carried out for *A. flavipes*, the only other biocontrol agent known to widely establish permanent populations in the United States.

In our study (Pickett et al., 2021), as well as in work by Evans et al. (2013), *T. julis* populations were well synchronized with CLB during this parasitoid's first generation but not the second, resulting in a dip in parasitism levels in mid-summer. This has been attributed to increasing spring temperatures and a phenological mismatch between CLB and the parasitoid (Evans et al., 2013). Nevertheless *T. julis* has still been able to maintain high average seasonal parasitism of CLB (Roberts, 2016; Pickett et al., 2021) in the Pacific Northwest. Most likely various predators, as observed in our studies, and in laboratory and field studies by others (Shade et al., 1970; Kheirodin et al., 2019), also help control CLB in many parts of North America.

BENEFITS OF BIOLOGICAL CONTROL OF CEREAL LEAF BEETLE

The best data on the economic impact of the CLB project come from the survey by Cooperative Extension and the USDA-APHIS in Oregon. In that state, the cost of pesticides used to control CLB in Oregon, when they were causing the greatest concern in 2004, was nearly $800,000 statewide (Roberts and Rao, 2012). This information implies that in the absence of the successful CLB biocontrol program, the cost of chemical control would have amounted to nearly $16 million in just one state (in 2004 dollars) by 2022. In addition, the alternative to biological control, the annual use of insecticides to control CLB, would have resulted in unknown but likely substantial non-target effects to insects and the environment.

In the state of Washington, Roberts (2016) calculated the savings to wheat farmers attributable to the parasitoid's establishment and reduction of CLB populations. Considering application and pesticide expenditures in 2009, CLB control cost $13/acre ($33/ha). Using National Agricultural Services data, Roberts (2016) determined that if only half the wheat acreage in Washington were treated, it would cost $3.3 million annually. Again, the repeated savings of this amount annually would result in over $30 million per decade, in addition to reduced impact on the environment through the lack of these widespread annual pesticide applications.

Although this biological control effort has greatly benefited the producers of small grains in the Midwest, Pacific Northwest, and California, the same may not be true everywhere *T. julis* has spread in the United States. For example, a review of the CLB problem in the southeastern United States found little evidence for suppression of CLB by this parasitoid (Philips et al., 2011). This conclusion may be due to the lack of survey effort for *T. julis* in the region and the general reliance by growers on calendar-based pesticide schedules for CLB, rather than actual demonstrated need in particular fields.

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CHAPTER 11

Biological Control of Olive Fruit Fly in California

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NON-TECHNICAL SUMMARY

The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), was first detected in 1998 in southern California. It was soon found in all olive-growing regions in California, becoming the most destructive olive pest in this region. Control relies on frequent insecticide applications, most commonly bait formulations that target adult flies. Pest control is hampered by the large number of unmanaged olive trees that can act as sources of flies moving into treated commercial orchards. To develop better sustainable olive fruit fly management, researchers in California conducted the largest modern exploration for natural enemies across the fly's native range, imported to quarantine laboratories, evaluated potentially suitable parasitoids, and developed information needed for USDA-APHIS permits for release of selected species. Two wasp species (*Psyttalia humilis* and *Psyttalia lounsburyi*, both Hymenoptera: Braconidae) were approved for field release. For *P. humilis*, 360,240 (Kenyan strain) and 42,591 (Namibian strain) were released in seven coastal and eight interior valley counties from 2006 to 2013. Although *P. humilis* showed initial promise in quarantine studies, permanent field establishment was not detected. For *P. lounsburyi*, 22,391 (South African strain) and 64,026 (Kenyan strain) were released in 12 coastal and four interior valley counties from 2006 to 2017. *Psyttalia lounsburyi* has permanently established and expanded its range along the coast, with the highest levels of fly larval parasitism reaching 39.9–73.5% within a few years of the initial release. However, *P. lounsburyi* has not yet been recovered in interior valley counties, and its densities in coastal areas appear to be falling. At present, it does not appear that *P. lounsburyi* will significantly suppress olive fruit fly populations, especially in the important agricultural regions in the interior valleys. Continued biological control efforts for olive fruit fly may seek parasitic wasp species or strains that have biological traits better suited to California's interior valley.

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HISTORY OF INVASION, NATURE OF PROBLEM

The Species Invasion

The olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae) is monophagous; its larvae feed exclusively in olives, both wild *Olea europaea* subsp. *cuspidata* and cultivated *O. europaea* subsp. *europaea* (Daane and Johnson, 2010). *Bactrocera oleae* likely originated in sub-Saharan Africa, where the wild olive is widespread and from which the domesticated olive was derived over 5,000 years ago (Zohary, 1994). Molecular analyses suggest that the fly followed olive cultivation, with an initial northward expansion from sub-Saharan Africa into North Africa and the Mediterranean Basin, and then a westward expansion through Europe and eventually to North America (Nardi et al., 2005, 2010). Population structure and genetic analyses suggest that the origin of olive fruit fly in California is most likely from the region encompassing Cyprus, Israel, and the neighboring coast of Turkey (Nardi et al., 2005; Zygouridis et al., 2009; Nardi et al., 2010).

In California, *B. oleae* was first detected in October 1998 in West Los Angeles (CDFA, 1998). Statewide surveys suggest the fly spread rapidly, and by 2002 it was reported in almost all Californian olive-growing regions (CDFA, 2002). Its widespread geographic expansion in California, along with abundant ornamental olive trees, made a statewide eradication program unfeasible. The fly's widespread detection within such a short period of time may indicate a pre-1998 introduction (Rice, 2000). The olive fruit fly has also recently invaded Hawaii (Matsunaga et al., 2019).

Nature of the Problem

Olives were introduced into California from Mexico by Franciscan monks in the late 1700s, and the fruit was primarily used for oil before 1900 (Connell, 1994). The table olive industry began in the early 1900s, and this market gradually increased as a percentage of olive production (Ferguson et al., 1994). Currently, about 40% of California's olive production is for table olives, with the total acreage at ~36,000 acres (~14,500 ha), production at ~67,700 tons (~61,400 metric tons), and annual crop value at ~$57.9 million (see www.nass.usda.gov/ca). California olive production accounts only for ~5.8% of the total U.S. olive consumption, suggesting a market potential that is still largely untapped. While olives are grown in other U.S. states (Arizona, Florida, Hawaii, Georgia, and Oregon), California accounts for the vast majority of olive production in the United States.

Seasonal development of olive fruit fly begins with the adults that survive the winter or have emerged from overwintered pupae (Burrack et al., 2011; Yokoyama, 2015). Adult females begin to oviposit when fruit is ripe enough to support larval development, which ranges from May to early June depending on the cultivar and geographic location. Eggs are placed just beneath the fruit surface, and the newly hatched larvae feed and develop in the olive pulp. At 26°C (79°F), the fly can develop from egg to adult in 21 days, resulting in 3–5 generations per year in California (Burrack et al., 2011; Wang et al., 2012; Yokoyama, 2015). During the first fly generation of the fruiting season, larvae usually pupate inside fruit, while in later generations they often drop from fruits into the soil for pupation (Fletcher, 1987; Burrack et al., 2011). All life stages (**Fig. 1**) can be found during the winter if fruit is still present for oviposition and larval development (Burrack et al., 2011; Yokoyama, 2015). A female *B. oleae* can lay 200–500 eggs during her lifetime (Rice, 2000) and can live up to seven months under cool, humid conditions if food and water are available; however, adults live for only a few days when deprived of food and water or temperatures are high, as commonly occurs in California's interior valleys during the summer months (Wang et al., 2009a, 2013). Thus, fly populations are high in cool, humid coastal areas and low in hot, dry areas of the inland valley (Burrack et al., 2008, 2011). Eventually, climate warming could shift olive fruit fly populations northward beyond its current range (Gutierrez et al., 2009). Olive fruit flies are strong fliers and can disperse over 2 km (1.2 mi) in about 2 hours. Adults feed on available carbohydrate sources such as honeydew from black scale, *Saissetia oleae* (Hemiptera: Coccidae) (e.g., Wang et al., 2009b, 2011a).

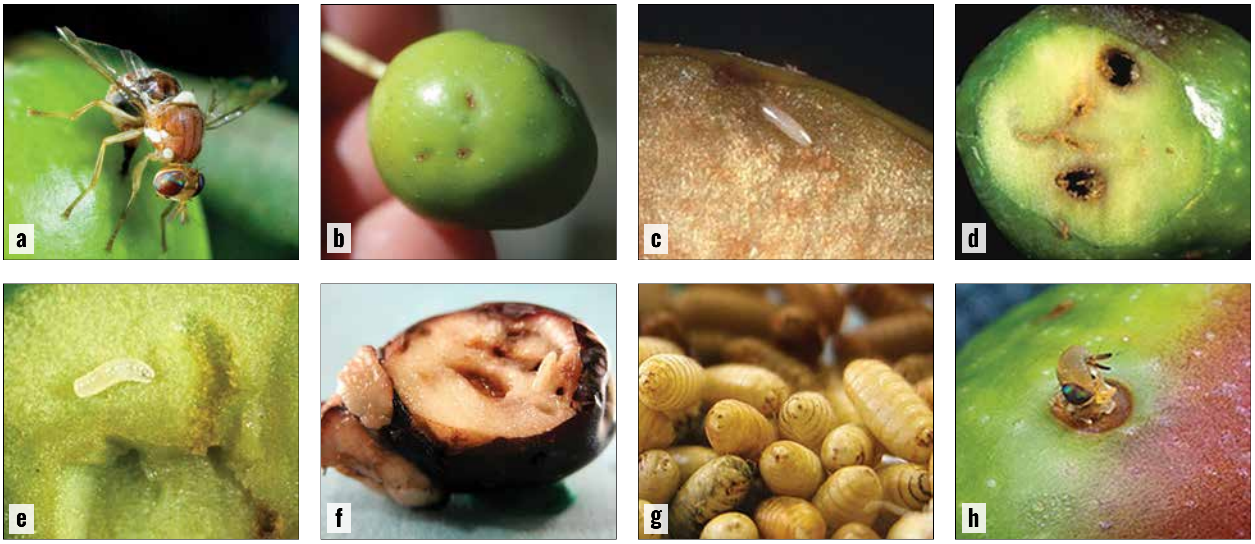

Figure 1. Life cycle of *Bactrocera oleae* and its damage on olives: (a) adult female fly; (b) oviposition stings; (c) egg; (d) feeding tunnel; (e) young larva; (f) mature larva; (g) fly puparia; and (h) adult emerging from fruit. (a–h: Marshall Johnson, UC Riverside)

Before the invasion of *B. oleae*, the major arthropod pests of olive in California were olive scale, *Parlatoria oleae* (Hemiptera: Diaspididae), and black scale. Olive scale is largely controlled by the introduced parasitoids *Aphytis maculicornis* and *Coccophagoides utilis* (both Hymenoptera: Aphelinidae) (Wilson et al., 2022), and black scale is partly controlled by a combination of natural enemies and cultural practices (Daane and Caltagirone, 1989). Therefore, *B. oleae* is now the primary threat to the olive industry. In addition, the olive psyllid, *Euphyllura olivina* (Hemiptera: Liviidae), is a species recently invasive in California with the potential to become an economical pest if it becomes established widely (Hougardy et al., 2020).

WHY CONTROL THIS INVASIVE SPECIES?

Olive crop production can be lowered by *B. oleae* oviposition or feeding on immature fruit, which may be aborted before harvest (Yokoyama, 2015). In mature fruit, *B. oleae* maggots also introduce pathogens that result in fruit deterioration. For the oil market, feeding reduces oil quality and value due to increased acidity (Rice et al., 2003). For table olives, there is a "zero tolerance" such that the presence of any maggots or pupae inside any fruit, or even oviposition marks can lead to the rejection of a grower's entire crop by table olive processors (**Fig. 1**) (Johnson et al., 2006). Rejected table olives may be used for oil production, which has a tolerance level of 10–15% infestation, although maggots in fruit can reduce oil quality and value. In areas of the world where the olive fruit fly is established, it can be responsible for loss of up to 80% of oil value and 100% of the crop for some table olive cultivars (Daane and Johnson, 2010). Non-treated olives can reach 100% infestation in California, and if olive fruit fly populations are not effectively and sustainably managed, the entire olive industry in California could be lost (Johnson et al., 2006, 2011).

Management strategies for *B. oleae* rely on frequent insecticide sprays, most commonly of an insecticidal bait (GF-120 NF Naturalyte Fruit Fly Bait, Dow AgroSciences LLC) (containing 0.02% spinosad) that attracts and kills adult flies (Johnson et al., 2006; Wilson et al., 2022). Other methods for suppressing *B. oleae* may include the use of attract-and-kill traps, particle film sprays (kaolin clay) to reduce adult oviposition, and sanitation of overwintered fruit (Wilson et al., 2022), although these techniques are not easily implemented on larger commercial operations and kaolin residues on the crop can reduce its value. The possibility of using sterile insect technique has been proposed but has not been developed due to the inability to economically rear millions of *B. oleae* individuals on an artificial diet in California or other regions (Johnson et al., 2006).

California olive growers spend $60–120 per acre ($148–297/ha) for *B. oleae* control, typically spraying the spinosad bait GF-120 once weekly or twice monthly from two weeks before olive pit hardening (early June) until fruit is harvested in the fall (for table olives) or winter (for oil production) (Wilson et al., 2022). This added expense has forced some California farmers out of olive production. Furthermore, repeated applications of GF-120 have led to resistance to spinosad in some areas (Kakani et al., 2010). GF-120 bait can also harm biological control agents (e.g., green lacewing adults and scale parasitoids) if they feed on foliar residues of the bait (Nadel et al., 2008; Wang et al., 2011b). Although studies indicate that several important tephritid fruit fly parasitoids such as *Fopius arisanus*, *Diachasmimorpha tryoni*, *Psyttalia fletcheri*, and *P. humilis* (all Hymenoptera: Braconidae) do not feed directly on fresh GF-120 residues, when the insecticide was directly applied topically to beneficial insects, high mortality resulted (Wang et al., 2005).

THE ECOLOGY OF THE PROBLEM

Insecticide-based management strategies for *B. oleae* are further hampered by movement into commercial orchards of flies from ornamental olive trees and abandoned orchards, which are often not treated (Collier and van Steenwyk, 2003). Insecticides are difficult to apply to ornamental trees in residential and public areas as well as to abandoned or volunteer trees in rough terrain. These unmanaged olives provide the fly with breeding and overwintering sites, resulting in a refuge for reinvasion to nearby commercial crops. Natural enemies, especially self-perpetuating parasitoids, may play a unique role not only because they can attack immature flies in the fruit (where pesticides are less effective), but also can reduce fly densities at the landscape level. Biological control, especially in such refuge areas, could provide a valuable ecosystem service, improve environmental quality, and lower growers' management costs.

Previously, the natural enemies attacking *B. oleae* in California were largely ineffective and consisted mainly of the generalist ectoparasitoid *Pteromalus* nr. *myopitae* (Hymenoptera: Pteromalidae) (Kapaun et al., 2010) and generalist predators such as ants (Orsini et al., 2007). This situation is similar to the Mediterranean Basin where indigenous parasitoids attacking *B. oleae* are generalist ectoparasitoids that do not sufficiently suppress fly populations (Daane and Johnson, 2010). For this reason, classical biological control in Europe has been investigated since the early 1900s. Surveys in the 1910s and later found solitary endoparasitoids, including *Bracon celer*, *Psytallia concolor*, *Psyttalia lounsburyi*, and *Utetes africanus* (all Hymenoptera: Braconidae) in collections from South Africa, Kenya, and Ethiopia (reviewed in Neuenschwander et al., 1982; Daane and Johnson, 2010; Hoelmer et al., 2011). More recent explorations showed the presence of a parasitoid complex that was closely associated with *B. oleae* and collectively contributed to *B. oleae* suppression in sub-Saharan Africa (Wang et al., 2021a). To improve sustainable management for this invasive pest, members of the University of California, California Department of Food and Agriculture, and the USDA-ARS European Biological Control Laboratory initiated a modern classical biological control project for *B. oleae* (Daane and Johnson, 2010; Daane et al., 2011, 2015; Wang et al., 2021a).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The first major attempt to introduce co-evolved parasitoids to suppress *B. oleae* populations dates to the early 1900s with the exploration in Africa for olive fruit fly natural enemies that were later released in Italy (reviewed in Wharton, 1989; Daane and Johnson, 2010). However, this initial work was constrained because imported parasitoids were difficult to rear, resulting in only small numbers being released in Italy without any documented establishment. One species, *P. concolor* obtained from Tunisia, has been repeatedly introduced into southern Europe since the early 1900s, but it has established only in the southernmost parts of Mediterranean Europe and does not provide effective control (Raspi et al., 2007; Miranda et al., 2008).

The invasion of *B. oleae* in California renewed interest in the classical biological control of olive fruit fly and led to the initiation of an importation and evaluation program in 2003 (Daane et al., 2011). Researchers conducted the largest modern exploration for olive fruit fly parasitoids, investigating sites across sub-Saharan Africa (Kenya, Namibia, and South Africa), some adjoining regions with olive fruit fly populations (Canary Islands, Morocco, Réunion Island, and Tunisia), and parts of southwestern Asia (Bon et al., 2015; Wang et al., 2021a). In sub-Saharan regions, four braconids were collected from wild olives (*B. celer*, *P. humilis*, *P. lounsburyi*, and *U. africanus*); *P. humilis* was dominant in hot semi-arid areas of Namibia, *P. lounsburyi* was dominant in more tropical areas of Kenya, and *U. africanus* was most prevalent in Mediterranean climates of South Africa. Mean parasitism levels were 30.1, 41.9, and 21.6% in Kenya, Namibia, and South Africa, respectively (Wang et al., 2021a). Collectively, these co-adapted parasitoids have contributed to maintaining a low fruit infestation rate (generally <15%) in its native range such as South Africa (Wang et al., 2021a). *Psyttalia concolor* was the only species found in the Canary Islands, Morocco, or Tunisia, while *Diachasmimorpha* nr. *fullawayi* was the only species collected from the island of Réunion (Wang et al., 2021a). In addition, *Psyttalia ponerophaga* appeared to be an effective *B. oleae* parasitoid in Pakistan, where the fly infestations are scarce, with parasitism rates of up to ~60% in Punjab Province (Bon et al., 2015). *Psyttalia humilis* is morphologically identical to *P. concolor*, but a later study suggests that all sub-Saharan populations are considered as *P. humilis* (Rugman-Jones et al., 2009), although they have been referred to as *P.* cf. *concolor* or *P. concolor* in some earlier publications (e.g., Rehman et al., 2009; Rugman-Jones et al., 2009).

All of the five major parasitoids (*B. celer, P. humilis*, *P. lounsburyi*, *P. ponerophaga*, and *U. africanus*; **Fig. 2**) were imported and evaluated at the University of California, Berkeley, quarantine facility (Daane et al., 2011). Also evaluated were three other tephritid fruit fly parasitoids, *Fopius arisanus*, *Diachasmimorpha kraussii*, and *Diachasmimorpha longicaudata* (**Fig. 2**), supplied by Dr. Russell Messing at the University of Hawaii (Sime et al., 2006abc, 2007, 2008; Nadel et al., 2009). *Fopius arisanus* is the only parasitoid that oviposits in host eggs, and all others are larval parasitoids. All species emerge as adults from host puparia. Under conditions in quarantine, *D. longicaudata* was considered the most effective. It is a generalist parasitoid of fruit flies that have been introduced from Asia into Hawaii and many other regions for biological control of various tephritids. *Diachasmimorpha kraussii* is an Australian species that was introduced into Hawaii for the control of Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) (Wang et al., 2021b).

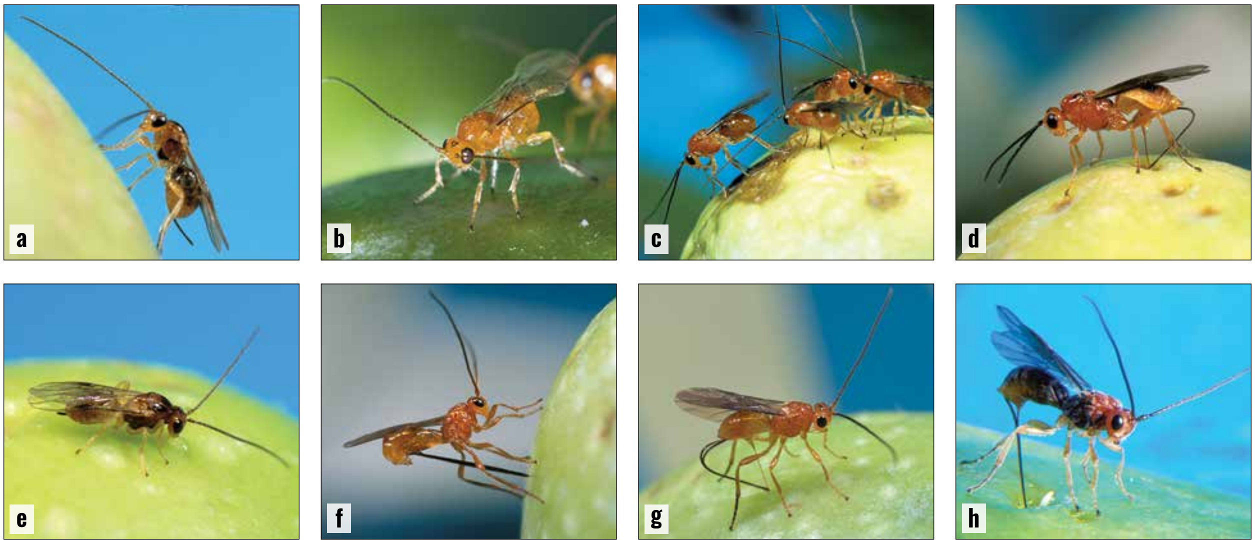

Figure 2. Parasitoids imported into California for quarantine studies include braconid parasitoids reared from wild olive fruit fly: (a) *Psyttalia lounsburyi*; (b) *P. humilis*; (c) *P. ponerophaga*; (d) *Bracon celer*; and (e) *Utetes africanus*, as well as braconid parasitoids reared on other fruit fly species, including (f) *Diachasmimorpha longicaudata*; (g) *D. kraussii*; and (h) *Fopius arisanus*. (a–h: Kent Daane [UC Berkeley] and Marshall Johnson [UC Riverside])

These parasitoids were assessed for their potential to attack several fruit fly species, including the fruit-feeding native black cherry fly (*Rhagoletis fausta*), the flower-head feeding *Chaetorellia succinea* (imported for the control of yellow starthistle, *Centaurea soltitialis*), and the gall-forming *Parafreutreta regalis* (imported for the control of stem galls in Cape ivy, *Delairea odorata*) (Daane et al., 2011). Quarantine evaluations showed that *P. lounsburyi* was the most specialized parasitoid of olive fruit fly (Daane et al., 2008). *Psyttalia ponerophaga*, *P. concolor*, and *P. humilis* were excluded because they could attack the beneficial weed biocontrol agent *P. regalis* (Daane et al., 2011), and *B. celer* could also attack and reproduce on *P. regalis* and probed other host species offered (Nadel et al., 2009). *Utetes africanus* was difficult to rear in either the target or various non-target hosts during the tests, but the literature indicates that it has been reared from several other fruit fly species. *Diachasmimorpha longicaudata* and *D. kraussii* were the most aggressive of the quarantine-screened parasitoids, probing nearly all host species presented and producing offspring from non-target, fruit-infesting species as well as from the beneficial species. These species have been reared from more than 20 fruit fly species (Wang et al., 2021b) and, therefore, they were considered not suitable for introduction. These two species are, however, already established in Hawaii and may, in the future, attack *B. oleae* there. *Fopius arisanus* was not attracted to any of the beneficial non-target species tested, which is consistent with earlier studies that showed that *F. arisanus* only attacks fruit-feeding tephritids such as *C. capitata* in quarantine tests in Hawaii (Wang et al., 2004). Most importantly, *F. arisanus* did not develop well on *B. oleae* (Sime et al., 2008).

In 2006, a USDA-APHIS permit was granted to release *P. lounsburyi* (Daane et al., 2008, 2015) and subsequently for *P. humilis*. *Psyttalia lounsburyi* populations (originating in Kenya or South Africa) and *P. humilis* (originating in Namibia) were mass-reared in France and Israel on *C. capitata* (Chardonnet et al., 2019) that had been reared on an artificial diet. Parasitoid production in 2008, 2009, and 2013–2017 was conducted at the USDA-ARS European Biological Control Laboratory in Montferrier-sur-Lez, France and in 2009–2012 at the Israel Cohen Institute of Biological Control in Bet Dagan, Israel. Adult parasitoids (1–2 weeks old) were shipped via overnight courier to California for field release (led by K. Daane and C. Pickett). All live parasitoids were fed honey and water and released within 1–2 days after their arrival. Similarly, *P. humilis* (originating in Kenya) was mass-reared on *C. capitata* at the USDA-APHIS-PPQ, MOSCAMED Parasitoid Rearing Facility at San Miguel Petapa, Guatemala and shipped to California for release (led by V. Yokoyama). Maintaining large *B. oleae* colonies throughout the season proved difficult. For this reason, the parasitoids were reared on *C. capitata* produced on an artificial diet. Use of *C. capitata* as the rearing host precluded mass-rearing these parasitoids in California, where *C. capitata* is a quarantine pest.

Releases were conducted primarily in ornamental olives or abandoned olive groves that received no insecticide treatments. Releases were not made in commercial olive orchards because they were often treated with GF-120 bait sprays. The non-treated olives at our release sites were often heavily infested by *B. oleae*, making them ideal habitats for field colonization and initial establishment of introduced parasitoids. Pre- and post-release samples were primarily taken in the spring and fall, when olive fruit fly densities were highest (Yokoyama et al., 2008; Daane et al., 2015). Before 2013, most parasitoids released were *P. humilis*, which was easier to rear and gave higher levels of parasitism in both laboratory and pre-release field cage studies than did *P. lounsburyi* (Wang et al., 2009d, 2011a). About 360,240 (approximately 50% females) *P. humilis* (Kenyan strain) parasitoids were released across six coastal counties (Los Angeles, Napa, San Diego, San Luis Obispo, Santa Barbara, and Santa Clara) and six interior valley counties (Glenn, Kern, San Benito, Merced, San Joaquin, and Tulare) from 2006 to 2011 (Yokoyama et al., 2008, 2010, 2011, 2012). Similarly, 42,591 *P. humilis* (Namibian strain) parasitoids were released in five coastal counties (Napa, Sonoma, San Diego, San Luis Obispo, and San Mateo) and two interior counties (Butte and Yolo) from 2008 to 2013 (Daane et al., 2015). Both strains of *P. humilis* (Kenyan and Namibian) were recovered consistently within the same fruit seasons, and the highest levels of parasitism reached 30–40%. However, this parasitoid was not recovered in subsequent years (Daane et al., 2015). Because of its lack of establishment, releases of *P. humilis* ended in 2013.

A total of 22,391 (3,155 males) and 64,026 (9,728 males) of the South African and Kenyan *P. lounsburyi* were released, respectively, in 12 coastal counties (Alameda, Los Angeles, Marin, Mendocino, Napa, San Diego, San Luis Obispo, San Mateo, Santa Barbara, Santa Cruz, Sonoma, and Ventura) and 4 interior counties (Butte, Riverside, Solano, and Yolo), which included 42 coastal and four interior release sites from 2006 to 2017 and a total of 138 releases.

HOW WELL DID IT WORK?

In California's Central Coast region, where releases were first made, *P. lounsburyi* was recovered in 2010 in San Luis Obispo County a year after it was first released, indicating that the parasitoid had successfully overwintered (Daane et al., 2015). Since then, *P. lounsburyi* has been recovered at many Central Coast sites in San Luis Obispo and nearby counties. For example, *P. lounsburyi* was found at 30 out of 31 sampled sites in Santa Barbara, San Luis Obispo, and Ventura Counties in 2015. The highest levels of parasitism were 63.0% in Santa Barbara County, 73.5% in San Luis Obispo County, and 36.9% in Ventura County. Across all sample sites in San Luis Obispo County, mean parasitism increased from 3.3% in 2012 to 32.2% in 2015 (Wang et al., unpub. data).

Further north near the San Francisco Bay Area, *P. lounsburyi* was first recovered in San Mateo County in 2011 after being released there in 2010 (Daane et al., 2015; Wang et al., unpub. data). Since then, the parasitoid has been found at many sites in each subsequent fruiting season, with mean parasitism increasing from 5.2% in 2011 to 12.7% in 2015; recoveries have been made at 23 out of 25 randomly sampled locations in San Mateo, Santa Clara, San Cruz, and San Francisco Counties, with the highest levels of parasitism being 34.6% in San Mateo and 37.3% in Santa Clara Counties. In other northern coastal counties, the parasitoid was recovered in Marin County in 2014 following its first release there in 2013 (Daane et al., 2015). By 2015–2016, *P. lounsburyi* was recovered from most sites surveyed in Marin (0–42.5% parasitism), Alameda (0–47.7% parasitism), Sonoma (2.8% parasitism) and Napa (0.9% parasitism) Counties, although only 1–3 sites were sampled in each region.

In southern California, early releases of *P. lounsburyi* started in 2010 in San Diego, and the parasitoid was first recovered in 2016 (0.1% parasitism) and again in 2017 (0.3% parasitism). *Psyttalia lounsburyi* was released for the first time in Riverside County in 2015 and in Los Angeles County in 2016, but post-release sampling in 2017 did not find this parasitoid established at these release sites. It was found, however, in one sampled non-release site in Los Angeles County (23.3% parasitism) that bordered Ventura County (Daane et al., 2015; Wang et al., unpub. data).

There are some release areas of California where *P. lounsburyi* has not been recovered. North of the San Francisco Bay Area, *P. lounsburyi* has not yet been recovered in Mendocino County, nor has it been recovered from any interior valley counties (Butte, Solano, and Yolo) after multiple years of releases (Daane et al., 2015; Wang et al., unpub. data).

These results demonstrate that *P. lounsburyi* has permanently established in California's coastal regions and may over time expand its range in this mild, temperate region. Surveys in 2018 showed that the overall numbers of recovered wasps and recovery sites were lower when compared to previous years, with highest parasitism being 26.7% in San Luis Obispo County and 29.5% in Alameda County (Wang et al., unpub. data). However, fly larval density was also lower, dropping from 0.43 to 0.25 larvae per fruit between 2010 and 2018. Surprisingly, *P.* nr. *myopitae* was found at most sampled sites, and its parasitism seemed to have increased over the years, with the highest rates of parasitism (42.8 and 50.9%) in August to September in San Luis Obispo and San Mateo Counties, respectively. Several other native parasitoids, including *Eurytoma* sp., and *Eupelmus* sp., were also reared from *B. oleae* (Daane et al., 2015; Wang et al., unpub. data).

In summary, many factors could have affected establishment of *P. humilis* and *P. lounsburyi* in California. The first consideration is likely the climatic adaptability of a species (and strain), as neither *P. humilis* nor *P.*

lounsburyi appears to have a winter diapause, and only *P. lounsburyi* was able to overwinter at any of the sampled sites (Wang et al., 2013). Laboratory studies suggest that *P. lounsburyi* is more cold-tolerant than *P. humilis*, and its low temperature tolerance is a better match with that of *B. oleae* than that of *P. humilis* (Wang et al., 2012; Daane et al., 2013). The coastal regions of California are characterized by year-round mild temperatures, while the interior valleys are characterized by hot, arid summers and cold winters (Wang et al., 2011b, 2013). In addition, the California olive fruit fly population appears to have originated from the Mediterranean Basin, and the long-term separation of the parasitoids released in this program from their host historically might have led to some divergence in thermal performance between the fly and these two African *Psyttalia* species (Wang et al., 2012). *Psyttalia ponerophaga* from Pakistan, as described previously, may be more cold-tolerant than these two tropical species (Daane et al., 2013) and could be an important addition that would complement the activity of *P. lounsburyi*. Second, seasonal host availability is another key factor given that in Africa *B. oleae* larvae are present throughout the year in many regions, which ensures a constant supply of hosts for parasitoids (Wang et al., 2021b). However, in California the phenology of domesticated olives, periodic drought, and other climate extremes may restrict the availability of *B. oleae* larvae to limited seasonal periods. For successful biological control, the introduced parasitoids must be able to find hosts year-round, and yet *B. oleae* larvae are scarce during summer and winter periods in many California olive regions.

A third potential factor is that domesticated olives are much larger than the wild olives present in the fly's native range, allowing fly larvae in commercial fruit to tunnel and feed deeper inside the fruit, thereby providing a refuge from parasitoids with a relatively short ovipositor (Wang et al., 2009d). In other words, co-adapted larval parasitoids (e.g., *P. humilis*, *P. lounsburyi*, *P. ponerophaga* and *U. africanus*) may have ovipositors too short to reach fly maggots feeding deep within the larger olives (Sime et al., 2007; Wang et al., 2009cd). Other larval parasitoids seeking to attack concealed maggots, such as *Anastrepha* spp. larvae in mangoes in Mexico, face the exact same issue with respect to accessing larvae in larger cultivated fruits vs smaller wild species of fruits (e.g., López et al., 1999; Sivinski et al., 2001). Although *D. longicaudata* and *D. kraussii* reproduced well on cultivated olives due to their very long ovipositors (Sime et al., 2006c), their host ranges are too broad to be considered for introduction.

The modern practice of classical biological control strongly emphasizes minimal non-target impacts of introduced agents, which consequently reduces the number of potential agents. Olive domestication may also alter other aligned or inherent tri-trophic relationships (e.g., presence of key chemical cues for parasitoid foraging), potentially disrupting the parasitoids' host-searching and host location success. A final factor potentially affecting this project's results may be interspecific competition among parasitoids. *Pteromalus* nr. *myopitae*, as an ectoparasitoid, may have a competitive advantage over larval endoparasitoids (such as species of *Psyttalia*) (Wang et al., unpub. data).

BENEFITS OF BIOLOGICAL CONTROL OF OLIVE FRUIT FLY

To date, the benefits of this program lie largely in the area of developing the groundwork, rather than having already achieved suppression of the target pest. This project has imported and evaluated several parasitoids of olive fruit fly, of which the three most promising larval parasitoids are *P. lounsburyi*, *P. humilis*, and *P. ponerophaga*. Both *P. humilis* and *P. lounsburyi* were approved and released widely, while *P. ponerophaga* has yet to be permitted for field release. Permanent establishment of *P. humilis* was not achieved, although augmentative field releases of *P. humilis* showed some impact on the pest (e.g., Yokoyama et al., 2010). However, it is unlikely that augmentative releases of *P. humilis* will ever be a commercially viable option due to costs and difficulties of mass-rearing this species. In contrast, *P. lounsburyi* has become permanently established in most coastal olive-growing regions. Genetic analyses of recovered specimens show that the South African strain of *P. lounsburyi* has established more widely than the Kenyan strain (Bon et al., 2017), even though the latter was released in greater numbers.

The permanent establishment of *P. lounsburyi* is a major step in the development of a successful classical biological control program against *B. oleae* in California and marks the first-ever establishment of a specialized *B. oleae* parasitoid worldwide outside of its native range (Daane et al., 2015). At present, it is too early to predict whether this parasitoid will significantly suppress *B. oleae*. To date, parasitoid populations have fluctuated strongly among sample years, perhaps due to recent California droughts, which reduce fruit availability in ornamental olives. Future work might consider establishing different commercial olive cultivars that have either fruiting periods that expand the seasonal availability of fly larvae or smaller fruit size that increases the accessibility of fly larvae in the pulp. This may be most feasible for olive oil production where fruits are relatively small, and some fly damage can be tolerated. For table olives, reducing olive fruit fly populations that act as sources of invasive flies entering commercial fields through biological control should help to make other integrated pest management strategies (e.g., trapping and killing, bait sprays) more efficient and economical. Therefore, continued biological control efforts against *B. oleae* should consider these ecological constraints and seek parasitoid species or strains with better inherent abilities to survive both climatic extremes and periods of low host density. There is also a need to introduce new parasitoids or strains that are better suited to California's interior valleys. The long-term economic benefit of the project would reduce pesticide use and enhance the California olive industry.

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CHAPTER 12 Successful Biological Control of Asian Citrus Psyllid, *Diaphorina citri*, in California

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NON-TECHNICAL SUMMARY

Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae: Diaphorinini), is an invasive pest of citrus in California, United States. *Diaphorina citri* was first detected in California in 2008 and presents a significant threat to the long-term viability of the California citrus industry because of its ability to vector a phloem-limited bacterium, *Candidatus* Liberibacter asiaticus (*C*Las), which causes a lethal citrus disease, huanglongbing. *Candidatus* Liberibacter asiaticus was first detected in urban-grown citrus in 2012. In California, *D. citri* has been the target of a classical or introduction biological control program with two species of parasitoids, *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), sourced from part of the presumptive native range of *D. citri*, the Punjab province of Pakistan. *Tamarixia radiata* established readily, and *D. aligarhensis* failed to establish following release in California. In California-grown citrus, *D. citri* benefits from a disruptive food-for-protection mutualism that has developed with another invasive pest, the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Ants protect *D. citri* from natural enemies, and in return, psyllid nymphs provide ants with honeydew, a feeding waste product that is a source of nutritive sugars. Since the inception of the biological control program targeting *D. citri* in California, pest densities have declined by approximately 70% through a combination of parasitism of nymphs by *T. radiata* and predation by generalist predators, of which syrphid fly larvae are key predators. Control of Argentine ant significantly increases the efficacy of natural enemies against *D. citri* and other honeydew-producing sap-sucking pests (e.g., soft scales and mealybugs) that ants protect from natural enemies. A potentially important consequence of this successful biological control program is the substantial reduction in *D. citri* densities in urban areas, which may have caused a significant slowing of the spread of *C*Las in southern California into commercial citrus production areas.

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HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae: Diaphorinini), (see Burckhardt and Ouvrard, 2012 for revised classification) (**Fig. 1**), was described in 1908 from specimens collected in Taiwan from citrus (Kuwayama, 1908) where it was likely non-native (Beattie et al., 2008). The presumed native range of *D. citri* is thought to be the Indian subcontinent (Halbert and Manjunath, 2004; Beattie et al., 2008; Grafton-Cardwell et al., 2013). This supposition is based on observations that *Diaphorina* spp. exhibit ecological preferences for xeric habitats in the Old World and have radiated on plant species that evolved in these areas (e.g., arid regions of the Mediterranean Basin, the Middle East, and the Indian subcontinent) (Hollis, 1987).

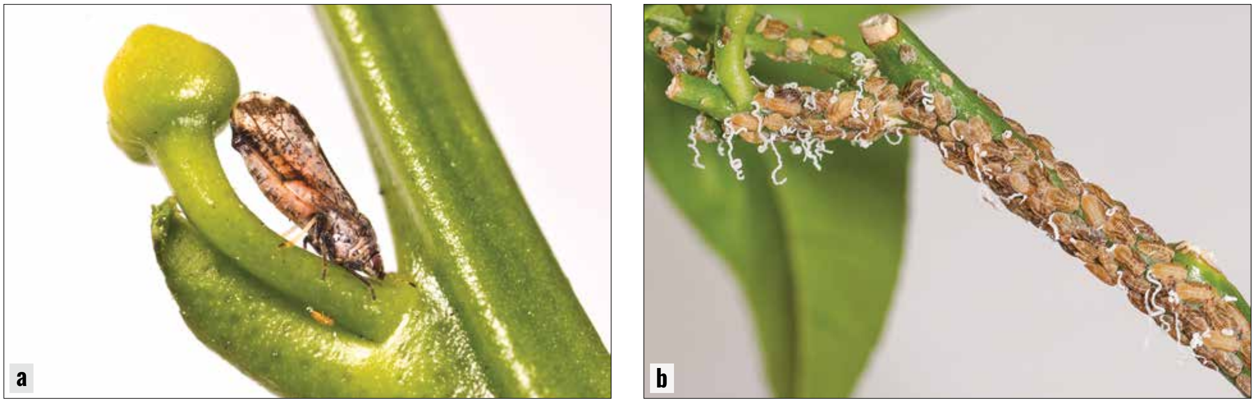

Figure 1. Asian citrus psyllid, *Diaphorina citri*, adult (a) and nymphs (b) infesting citrus. The white ribbon-like material hanging from nymphs are waxy tubules that direct honeydew away from the nymphs. This honeydew is harvested by Argentine ant, *Linepithema humile*. (a,b: Mike Lewis, UC Riverside)

In August 2008, *D. citri* was first detected in Imperial and San Diego Counties in southern California. Initial detections were relatively close to the California-Mexico border (Anon., 2010), suggesting that in these areas *D. citri* may have invaded California via northwards dispersal out of Mexico, where *D. citri* is widely established (Atkins, 2010). In 2009, surveys by the California Department of Food and Agriculture (CDFA) detected large and widespread populations of *D. citri* in residential areas in Orange and Los Angeles Counties (Grafton-Cardwell, 2010). These populations, especially the heavy infestations in Los Angeles County, were disjunct and widely separated (>122 miles [>195 km]) from initial *D. citri* detections close to the California-Mexico border. This suggested a separate and undetected introduction of *D. citri* had occurred in Los Angeles, likely before 2008 (see *D. citri* distribution map in Anon., 2010). The establishment of *D. citri* in southern California is generally viewed as the most serious threat to California's multibillion dollar citrus industry because of this pest's capacity to spread *Candidatus* Liberibacter asiaticus (*C*Las), the presumed causative agent of a lethal bacterial disease of citrus, huanglongbing, which is also known as citrus greening (Anon., 2010). *C*Las was first detected in residential citrus in Los Angeles County in 2012 (Kumagi et al., 2013).

Nature of the Problem

The first published association of *D. citri* with the lethal demise of citrus was by Husain and Nath (1927), who worked on citrus pest management in Sargodha, Lyallpur (now Faisalabad), and Gujranwala, formerly located in the Punjab area of India, but following partition in 1947, these locations are now in Pakistan. At this time, it was unknown that a bacterium, later identified as *C*Las and vectored by *D. citri*, was the likely agent responsible for mortality of citrus trees infested by *D. citri* as observed by Husain and Nath (1927). The area of origin of *C*Las is controversial. Beattie et al. (2008) argue that this bacterium (and its relative, *Candidatus* Liberibacter africanus [*C*Laf]) evolved in Africa and *C*Las was spread into India then into China and later globally via trade in citrus and citrus relatives. Halbert and Manjunath (2004) suggest that *C*Las has an area of origin similar to that of its principal vector, *D. citri*, the Indian subcontinent. Conversely, Bové (2006) and Taylor et al. (2019) state that the area of origin for *C*Las is Asia.

*C*Las-infection symptoms include leaves with irregular chlorotic mottling, misshapen fruit, bitter-tasting juice, premature fruit drop, branch dieback, and tree death within 5 to 8 years of infection (Halbert and Manjunath, 2004; Bové, 2006). Detection of *C*Las in infected trees can be difficult, even with molecular techniques, which makes early pre-symptomatic management decisions (e.g., removal of infected trees) difficult. Consequently, this provides a window for *D. citri* to reproduce and feed on infected trees, which results in nymphs and adults that are infected with *C*Las. Nymphs that acquire *C*Las are infective for life and are more efficient vectors of this bacterium as adults compared to *D. citri* individuals that acquire the bacterium as adults. Low rates (~2–6%) of *C*Las transmission can occur transovarially (Pelz-Stelinski et al., 2010). The more *C*Las-infected *D. citri* that feed on a citrus tree, the greater the likelihood of infection with *C*Las (Pelz-Stelinski et al., 2010). Suppression of *D. citri* populations to low levels is considered essential to reduce spread of *C*Las and subsequent disease impacts in citrus (Bassanezi et al., 2013), and mathematical models suggest that increased levels of natural enemy activity targeting vectors can significantly reduce rates of spread of plant pathogens (Stella and Ghosh, 2019).

Diaphorina citri-*C*Las can cause substantial economic losses, and impacts have been especially significant in Florida. *Diaphorina citri* was first detected in Florida in 1998 (Halbert and Manjunath, 2004), and in 2005 *C*Las was detected (Farnsworth et al., 2014). Currently, the incidence of *C*Las in Florida citrus orchards is high with >90% of the acreage having disease incidence that is affecting >80% of planted trees (Alvarez et al., 2016). Poor cooperation in managing *D. citri* and *C*Las infections among affected grower groups is thought to be, in part, responsible for the severity of this vector-pathogen combination in Florida, especially when outcomes are compared to other large citrus-producing areas, like Brazil, which have managed this dual problem more effectively (Singerman and Rogers, 2020).

In Florida, the economic costs of *D. citri*-*C*Las (in terms of reduced acreage in commercial production, subsequent loss of jobs, decline of support industries, and increased management costs) are estimated to be in the hundreds of millions to billions of dollars (Hodges and Spreen, 2012; Farnsworth et al., 2014; Moss et al., 2014; Alvarez et al., 2016). For example, Hodges and Spreen (2012) estimate that over the period 2006/07–2010/11, *D. citri*-*C*Las cost the Florida citrus industry ~$4.54 billion in output losses. This estimated total loss is comprised, in part, of a total value of ~$2.72 billion from reduced fruit production and a loss in labor income of ~$1.75 billion, which resulted from an estimated loss of ~8,257 jobs as the citrus bearing acreage decreased due to tree mortality and higher production costs.

Alvarez et al. (2016) assessed the economic benefits/costs of biological control of *D. citri* for reducing *C*Las impacts on citrus in Florida. Field studies suggest that *Tamarixia radiata* (Hymenoptera: Eulophidae), sourced from southeast Asia and released initially as part of a classical biological control program for *D. citri* in Florida in 1999, may be a minor contributor to *D. citri* population suppression. Generalist predators such as coccinellids may play a more important role in *D. citri* control in Florida citrus orchards (Michaud 2001, 2004; Halbert and Manjunath, 2004; Qureshi and Stansly, 2009; Alvarez et al., 2016). However, the significance of the role of intraguild predation by coccinellids on *D. citri* nymphs parasitized by *T. radiata* is not currently known, and the potential impact of *T. radiata* may be underestimated as a result (Halbert and Manjunath, 2004).

If the conclusion that the widely occurring populations of *T. radiata* in Florida are relatively ineffective against *D. citri* in insecticide-managed commercial orchards is correct, then augmentative releases of mass-reared *T. radiata* at critical times of the year may be important for localized suppression of *D. citri*. The impacts of carefully timed releases of *T. radiata* to coincide with the *D. citri* nymphs that are most vulnerable to parasitism (i.e., third through fifth instars) and host feeding may make important contributions to *D. citri* control. These releases would have to be made during windows of chemical control of *D. citri* when applications are non-existent or low, or if compatible insecticides are being applied to avoid killing parasitoids. The potential efficacy of augmentative *T. radiata* releases in Florida citrus may be especially important given the increasing likelihood that this pest is developing resistance to commonly used insecticides (Tiwari et al., 2011; Kanga et al., 2016). Alvarez et al. (2016) concluded that insufficient data on the efficacy of the augmentative biological control program targeting *D. citri* with *T. radiata* are available and therefore cannot be adequately evaluated, even though the costs of mass producing and releasing *T. radiata* as part of an augmentative biological control approach is well documented (i.e., it costs $361,529 to produce three million parasitoids, a cost of $0.11 per individual of which ~50% will be female).

WHY CONTROL THIS INVASIVE SPECIES?

The well-documented, negative impacts of *D. citri*-*C*Las on the Florida citrus industry has guided, to some degree, the development of management plans for *D. citri*-*C*Las in California. One aspect of this management plan has been the development of the classical biological control program targeting *D. citri* with *T. radiata* and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) in California. The primary aim of this project was to import and establish parasitoids sourced from part of the home range of *D. citri* that had a good climatic match with citrus production regions of California. Backyard gardens with citrus were identified as major breeding areas for *D. citri*, from which adults and *C*Las could spread into agricultural production areas. High levels of public resistance to government-run spray programs that treated residential citrus for *D. citri*, and the eventual cessation of these programs, made it possible to target this pest with natural enemies in urban habitats that are largely free of insecticide use (Hoddle and Pandey, 2014). The urgency for controlling *D. citri* populations in urban citrus intensified when the first trees infected with *C*Las were found in backyard gardens in Los Angeles County in 2012, four years after the initial detection of *D. citri* in San Diego County (Kumagi et al., 2013). The remainder of this chapter will focus on the biological control program targeting *D. citri* in California.

THE ECOLOGY OF THE PROBLEM

Diaphorina citri is the only species of psyllid infesting citrus in California, and it is the sole vector of *C*Las. Presently, *D. citri*-*C*Las is almost exclusively a problem of urban citrus. Urban citrus production is highly heterogenous in comparison to the homogeneity of commercial citrus production areas. For example, urban backyard citrus plantings tend to be small and often of mixed varieties (e.g., oranges, mandarins, lemons, limes, and grapefruit) that may be composed of just a few types of citrus (e.g., a mixture of just one or two each of an orange, lemon, mandarin, and grapefruit). Trees in urban areas are typically not treated with insecticides, and, in many instances, they are well watered. Fertilization and pruning practices are highly variable, which can result in strong asynchronous bursts of flush growth (this is the plant growth stage *D. citri* needs for oviposition) at varying times of the year across residential properties with citrus. In comparison, commercial citrus orchards tend to be monocultures of one variety (e.g., oranges), flush growth is highly synchronous and limited to specific times of the year, frequent insecticide use is likely, and fertilization and pruning practices are applied uniformly in orchards. We speculate that the urban citrus ecosystem, with its high levels of heterogeneity (especially with respect to the availability of flush growth used by *D. citri* for oviposition) and its very low levels of insecticide use, may provide favorable conditions for patchily distributed but persistent existence of natural enemy populations. If true, this could help maintain relatively stable, widespread, low-density populations of *T. radiata*. This situation could persist because the asynchronous flush of new leaves would support a mosaic of localized outbreaks of *D. citri* nymphs that would be irregularly distributed throughout residential areas but available to *T. radiata* for host feeding and oviposition.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Classical biological control of *D. citri* in California started in September 2010 with exploratory trips to India and Pakistan by MSH and CDH to determine where best to base natural enemy collection efforts. The Department of Entomology, University of Agriculture, Faisalabad (UAF) in Punjab, Pakistan was selected as the project's home base. Faisalabad is in the probable home range of *D. citri*, and the area has about a 70% climate match with the major citrus-producing areas of the Central Valley in California. Also, UAF is located close to Sargodha, Gujranwala, and Toba Tek Singh, major citrus production areas that predominantly grow "Kinnow" mandarins, where *D. citri* could be collected (Hoddle, 2010, 2012). Five foreign exploration trips to collect *D. citri* natural enemies were made from March 2011 to April 2013. Collections of parasitized *D. citri* resulted in the collection of two primary parasitoids, *T. radiata* (an arrhenotokous ectoparasitoid) (**Fig. 2a**) and *D. aligarhensis* (**Fig. 2b**) (an arrhenotokous endoparasitoid) (Hoddle et al., 2014) and several species of associated hyperparasitoids (Hoddle et al., 2013; Bistline-East and Hoddle 2014, 2016).

Figure 2. (a) *Tamarixia radiata* and (b) *Diaphorencyrtus aligarhensis* are primary parasitoids attacking nymphs of *Diaphorina citri*; (c) *D. aligarhensis* adults emerge from the posterior region of parasitized *D. citri* nymphs (left), while *T. radiata* adults emerge from the anterior region (right). Emergence hole location is a very useful field diagnostic for accurate determination of parasitoid species identity. (a–c: Mike Lewis, UC Riverside)

To preserve genetic diversity of the collected parasitoids, isocage lines were set up in quarantine (Hopper et al., 1993; Roush and Hopper, 1995). A total of 17 isocage lines were set up for *T. radiata*, and four isocage lines were maintained for *D. aligarhensis*. Each isocage represented a different collection event identifiable by date and location (Hoddle and Hoddle, 2013) (**Fig. 3**). Theory supporting genetic preservation of natural enemies in quarantine suggests that inbreeding of populations maintained in isocages results in some loss of genetic diversity, alleles become fixed as homozygosity is reached, and adaptation or domestication of natural enemies to prevailing quarantine conditions is stalled once homozygosity is attained and further gene loss is not possible. Each isocage has some random unknown amount of genetic diversity preserved through this inbreeding process until homozygosity results. These preserved "genetic snapshots" undergo reconstitution when individual males and females from each isocage are introduced into a panmictic breeding cage where random mating occurs, and some unknown but presumably increased level of genetic variation is attained in the resulting hybrid offspring. It is unlikely that the complete level of genetic variation originally collected from the field is preserved with this approach. However, resulting genetic variation is thought to be greater than what would occur from cage colonies comprised of mixtures of natural enemies collected from different areas and time. It is assumed that these mixed-type colonies result in populations that become adapted (i.e., domesticated) to quarantine conditions and may therefore be poorly suited to the field conditions in which they are released.

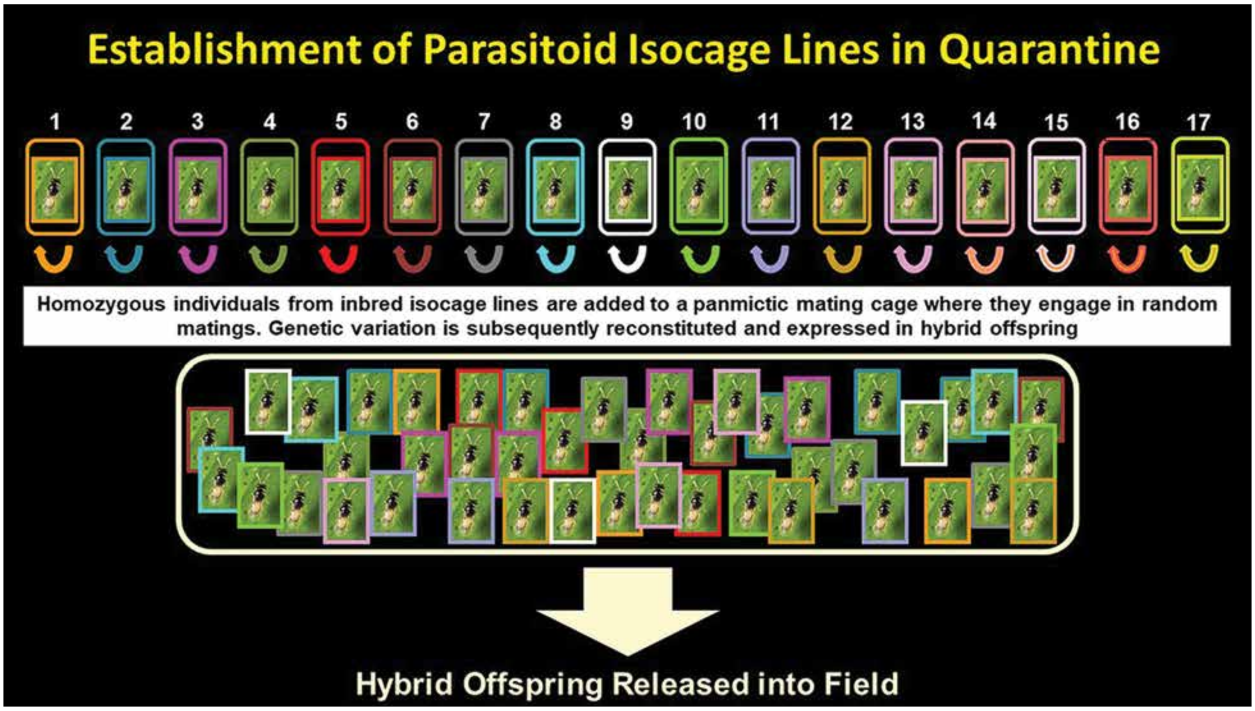

Figure 3. Schematic of 17 isocage lines set up in quarantine for maintaining populations of *Tamarixia radiata* that were collected at different locations and times from Punjab, Pakistan. (M. Hoddle, UC Riverside)

Hybrid offspring from random matings between isocage line adults are released into the environment, and natural selection preserves genetic combinations that are best suited to the local conditions. Poorly adapted offspring die off, and those inferior genetic combinations are lost from the population (Hopper et al., 1993; Roush and Hopper, 1995). Although this approach is well supported theoretically, there are no field data to support the underlying assumption that isocage lines preserve superior levels of genetic diversity in comparison to traditional maintenance of colonies where a few cage populations with individuals of mixed origins are repeatedly bred with themselves before release. The use of isocage lines to maintain genetic diversity in laboratory colonies of natural enemies warrants serious investigation to determine if this labor-intensive approach is justified.

Following the completion of host range and host specificity testing for *T. radiata* (Hoddle and Pandey, 2014) and *D. aligarhensis* (Bistline-East et al., 2015), permission to release these parasitoids was received from the USDA-APHIS in December 2011 and December 2014 for *T. radiata* (Hoddle and Hoddle, 2013) and *D. aligarhensis* (Hoddle, 2015), respectively. As of January 2022, >23 million *T. radiata* have been released at >19,000 release sites in California, and >700,000 *D. aligarhensis* have been released at >300 sites (D.J.W. Morgan, pers. obs.; Milosavljević et al., 2022). Additional details on the parasitoid release programs are provided in the following section.

HOW WELL DID BIOLOGICAL CONTROL WORK?

Establishment

Initial releases of *T. radiata* and *D. aligarhensis* into urban citrus in southern California were made by the Applied Biological Control Laboratory at the University of California Riverside. The mass rearing and release program then transitioned to the California Department of Food and Agriculture's *D. citri* biological control program at the Mt. Rubidoux, Riverside, California facility, significantly increasing parasitoid production numbers and translating to increased release sizes and frequencies (Hoddle et al., 2016).

Tamarixia radiata established almost immediately in southern California after the initial releases in urban areas of Riverside and Los Angeles Counties from December 2011 through February 2012, with parasitoid recoveries being made within a couple of months at release sites. Rapid establishment, especially over winter, may have been due, in part, to extremely high *D. citri* densities at release sites, which parasitoids could use for host feeding and parasitism. In fall 2015, following the release of ~2.55 million parasitoids into urban citrus, a large establishment survey was conducted across Riverside, San Bernardino, San Diego, Orange, Imperial, and Los Angeles Counties. A total of 100 sites, a combination of 72 release and 28 non-release sites, found *T. radiata* at 91 sites (i.e., 91%). Of the 28 non-release sites, 24 (86%) had *T. radiata*, indicating that the parasitoid dispersed successfully into non-release areas. Some *T. radiata* recoveries were as far as eight miles (~13 km) away from the nearest release site (Hoddle et al., 2016). Field-collected parasitoids were subjected to molecular analyses and confirmed as being of Pakistani origin. This result indicated that *T. radiata* populations recovered from field collections had established from material deliberately released as part of the classical biological control program targeting *D. citri*. This finding confirmed that parasitoids were not of unknown origin and were not self-introduced with the founding pest invasions into San Diego and Los Angeles Counties.

Conversely, releases of >700,000 *D. aligarhensis* from 2014 to 2018 across >300 sites failed to result in parasitoid establishment, and mass rearing and release of this parasitoid ended in 2019 (Milosavljević et al., 2022). Similarly, a uniparental strain of *D. aligarhensis* also failed to establish in Florida (Rohrig et al., 2012). Reasons for establishment failure of this species in California are uncertain but may be attributable to competition from *T. radiata* that established at *D. aligarhensis* release sites (Milosavljević et al., 2022). Laboratory studies indicated that *T. radiata* is a superior competitor to *D. aligarhensis* for *D. citri* nymphs (Vankosky and Hoddle, 2017, 2019a,b). In Punjab, Pakistan, where *D. aligarhensis* was sourced (as was *T. radiata*), this parasitoid exhibited a very strong association with *Diaphorina aegyptiaca* infesting leaves of Assyrian plum, *Cordia myxa* (S.Z. Khan, C.D. Hoddle, and M.S. Hoddle, pers. obs.). *Diaphorina aegyptiaca* on *C. myxa* was also reported as a host for *D. aligarhensis* in India where *D. aligarhensis* is also native (Hayat, 1981). *Cordia myxa* is a fruit-bearing tree that may be grown in close proximity to citrus orchards or interspersed through orchards. Infestations of *D. aegyptiaca* could act as reservoirs from which *D. aligarhensis* disperse and attack less preferred or less suitable hosts like *D. citri* infesting neighboring citrus trees. A preferred alternative psyllid host, such as *D. aegyptiaca* for use by *D. aligarhensis*, is lacking in California (Milosavljević et al., 2022). Additionally, in comparison to *T. radiata*, *D. aligarhensis* is more likely to be attacked by hyperparasitoids, of which at least 17 species are known from Southeast Asia and Pakistan (Qing, 1990; Hoddle et al., 2014; Bistline-East and Hoddle, 2014, 2016; Milosavljević et al., 2020), and at least eight of these hyperparasitoid genera are present in California (Bistline-East and Hoddle, 2016). Although not documented, an additional impediment to establishment of *D. aligarhensis* may have been attack by hyperparasitoid species already present in California. One other impediment to *D. aligarhensis* establishment may have been the Argentine ant, *L. humile* (Hymenoptera: Formicidae), which was present year round at all release sites (Milosavljević et al., 2022).

Following confirmation of establishment of *T. radiata* in the urban landscape of southern California, a critical issue that needed addressing was determination of the impact *T. radiata* and other natural enemies were having on *D. citri* population densities.

Impact of *Tamarixia radiata* on Asian Citrus Psyllid Populations

To assess the impacts of *T. radiata* and other natural enemies on *D. citri* populations infesting urban citrus in southern California, three different research avenues were pursued; (1) population density counts over time, (2) life table studies, and (3) videography of field-deployed cohorts of *D. citri* infesting small potted citrus trees.

Population density counts

Population counts of *D. citri* life stages infesting backyard citrus in southern California were conducted across multiple years and sites (Kistner et al., 2016a; Milosavljević et al., 2021). Phenology studies indicated that *D. citri* population densities declined over time, and that pest densities could vary substantially across years, sites, and citrus varieties. Densities consistently peaked during the fall and spring when flush growth, the required oviposition substrate for *D. citri*, was present (Kistner et al., 2016a; Milosavljević et al., 2021). Initial studies between 2012 and 2014 found that average parasitism levels by *T. radiata* were low (about 5%), but that in Riverside and Los Angeles Counties, *D. citri* densities declined ~50–70% following *T. radiata* releases and establishment (Kistner et al., 2016a). However, Kistner et al. (2016a) were unable to conclude that *T. radiata* was solely responsible for observed population declines. A subsequent larger study, from 2015 to 2018 over a much larger geographic area, confirmed that *D. citri* densities infesting urban citrus had declined by ~75% and that parasitism by *T. radiata* was a significant mortality factor, often exceeding 60% during peaks of parasitoid activity. Importantly, statistical analyses indicated that *T. radiata* parasitism was density dependent, and subsequent reductions in *D. citri* densities lagged behind observed parasitism rates by about one year (Milosavljević et al., 2021). Additionally, Milosavljević et al. (2021) determined that presence of the invasive pest ant, *L. humile*, resulted in a 3-fold increase in *D. citri* densities in residential citrus. This finding supported previous observations in urban citrus (Tena et al., 2013) and strongly suggests that controlling *L. humile* in urban and especially commercial citrus will be critical if the full potential of *T. radiata* and other natural enemies is to be realized (see below for more on control efforts targeting *L. humile* in commercial citrus).

Life table studies

Life table studies were conducted to determine the impacts of natural enemies on survivorship rates of cohorts of *D. citri* nymphs infesting small potted citrus plants. Artificially infested plants were deployed over spring through winter in urban citrus, and survivorship rates of *D. citri* nymphs were visually assessed every other day. *Tamarixia radiata*, predatory syrphid flies, and lacewing larvae were the key natural enemies killing *D. citri* nymphs. Collectively (depending on time of year), parasitism and predation of *D. citri* nymphs reduced psyllid reproduction by 55–95% (Kistner et al., 2016b).

Videography of field deployed cohorts

Videography studies of cohorts of *D. citri* nymphs on small potted citrus plants deployed in urban citrus confirmed the importance of *T. radiata*, syrphid fly larvae (*Allograpta* sp.), and lacewing larvae (*Chrysoperla* sp.) as key natural enemies of nymphs (Kistner et al., 2017). A grand total of 19,200 hours of digital video revealed that *T. radiata* is a diurnal natural enemy with activity periods peaking around 13:00 hours. Syrphid fly adults oviposited on *D. citri* patches during the day, and syrphid fly larvae tended to feed most actively on *D. citri* nymphs at night. Although observed less frequently than syrphid larvae, lacewing larvae tended to consume greater numbers of *D. citri* nymphs when patches were discovered. Spiders were seldom observed attacking *D. citri* (Kistner et al., 2017), similar to results from surveys in Pakistan (Vetter et al., 2013). Intraguild predation events were observed; coccinellids consumed syrphid fly larvae, pirate bugs (Anthocoridae) killed lacewing larvae, and surprisingly, brown garden snails (*Cornu asperum*) consumed mummified *D. citri* nymphs parasitized by *T. radiata* (Kistner et al., 2017). Importantly, *L. humile* was the most common arthropod associated with *D. citri* patches, and ants reduced the abundance and efficacy of natural enemies, especially *T. radiata* and syrphid fly larvae (Kistner et al., 2017).

A Shift in the *Tamarixia radiata* Release Strategy

The first releases of *T. radiata* in 2011 were made in urban areas where *D. citri* infestations were confined to relatively small geographic areas (**Fig 4a**). As *D. citri* spread, a new *T. radiata* release strategy was adopted in 2014 with the goal of establishing *T. radiata* across all 4,000 square miles (10,360 km^2) of urban citrus grown in California. A 1-mile square grid pattern (2.6 km^2) was overlain on this large, infested area and used to guide parasitoid releases. Every month, mass-reared *T. radiata* were released into every fifth square mile cell in the grid, and releases were made in a staggered pattern across the grid area (**Fig 4b**). Areas considered to be at high risk of *C*Las occurrence had a greater number *T. radiata* released (600–1,000 parasitoids per 1-square mile grid), while low risk areas received fewer parasitoids (200–600 parasitoids per 1-square mile). Areas at high and low risk of *C*Las infestation were determined using an algorithm developed by the USDA (Gottwald et al., 2014; McRoberts et al., 2019) (**Fig. 4c,d**). This release pattern of concentrating *T. radiata* releases in urban areas with high *C*Las risk was continued for three consecutive years.

In 2017, a new *T. radiata* release strategy was adopted that identified areas at high risk for spread of *D. citri* from urban areas into commercial citrus production zones. Areas considered as high risk for *D. citri*-*C*Las spread were (1) border areas of nearby states (i.e., Arizona) or countries (i.e., Mexico) with active *D. citri* infestations, (2) corridors along major trade routes (i.e., state and interstate highways) through areas where citrus is grown or agricultural equipment is regularly transported, (3) newly detected urban infestations outside previous *T. radiata* release areas, and (4) areas were *C*Las-positive trees have been identified. In the last of these cases, *T. radiata* releases at higher rates and at more frequent intervals were employed as an augmentative management strategy with the aim of boosting parasitoid numbers near *C*Las quarantine areas. The goal of more intense release efforts in these *D. citri*-*C*Las buffer zones was for *T. radiata* to drive down *D. citri* populations, reducing vector density and reducing further *C*Las spread. The buffer zone releases are part of an IPM strategy that also includes *C*Las-positive citrus tree removal, intensified *C*Las surveys, and insecticidal treatment of citrus trees in close proximity to *C*Las-positive trees. This latter group of trees was also treated with insecticides to suppress *D. citri* infestations. As of 2022, approximately 75% of *T. radiata* released in California were released into *D. citri*-*C*Las buffer zones surrounding *C*Las quarantine areas (**Fig. 4d**).

To assess the impacts of augmentative releases of *T. radiata* made in *D. citri*-*C*Las buffer zones, a monitoring study was initiated in 2018. Results suggest that in the first year of the study (i.e., 2018–2019) a significant correlation existed between the number of *T. radiata* released into an area and subsequent reductions in *D. citri* population densities. Analysis of data suggested that, to achieve psyllid suppression, parasitoid releases need to exceed 4,000 *T. radiata* per 0.4 square miles (i.e., ~1 square kilometer) per month. With this level of *T. radiata* release, *D. citri* densities become negligible (i.e., an average of <0.02 *D. citri* per shoot of citrus in flush). Field surveys from 2018 to 2021 in *D. citri*-*C*Las buffer areas found that use of this augmentative *T. radiata* release regimen maintained *D. citri* populations at very low densities and that *C*Las spread was significantly slowed.

Enhancing Biological Control of *Diaphorina citri* through Control of Argentine Ant

The invasive Argentine ant, *L. humile*, invaded California in 1905, and thereafter it rapidly developed disruptive food-for-protection mutualisms with honeydew-producing hemipteran pests in citrus and other crops (Markin, 1970). From their associated Hemiptera (e.g., aphids, soft scales, mealybugs, and whitefly nymphs), *L. humile* collects and feeds on honeydew, which is a sugary waste product that results from these insects' phloem feeding. In return, Argentine ants protect their micro-livestock from natural enemies, which increases the densities of these sap-sucking pests. In addition to tending mealybugs, soft scales, and aphids (which produce liquid honeydew), *L. humile* harvests the waxy filaments that are covered in honeydew from *D. citri* nymphs (**Fig. 5a**).

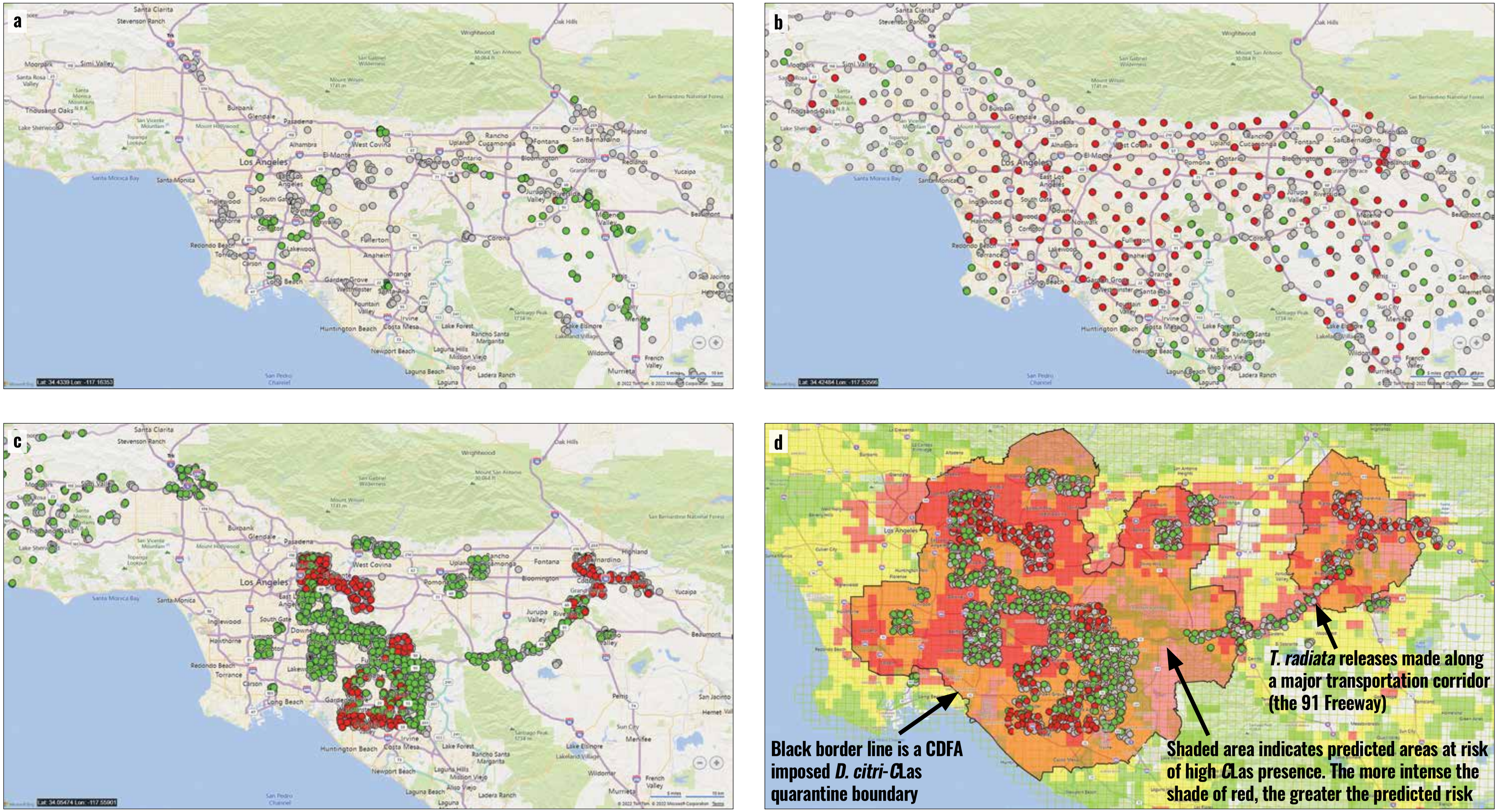

Figure 4. (a) Initial releases (i.e., 2013) of *Tamarixia radiata* into urban areas of southern California by the University of California Riverside and the California Department of Food and Agriculture (CDFA) were made in widely spread urban gardens that had citrus trees infested with *Diaphorina citri*. (b) In 2014, CDFA initiated the 1-square mile (2.6 km^2) grid system to guide geographically uniform releases of *T. radiata*. Parasitoid releases were made at one or more sites within grids on urban properties with *D. citri* infested citrus. (c) In 2017, CDFA reoriented the *T. radiata* release strategy, and emphasis was placed on areas predicted to be at high risk of both *D. citri* and *C*Las invasion. For example, releases were concentrated along major transportation corridors. (d) *T. radiata* releases concentrated in areas predicted to be at high risk of *C*Las establishment (red and pink areas contained with a black border which designates a *D. citri*-*C*Las quarantine boundary established by CDFA). Map Legend: Green dots <600 *T. radiata* released, red dots >600 parasitoids released, grey dots indicated previous releases the year before. The irregularity of release patterns in some grids is due to either a lack of citrus trees or site inaccessibility.

In citrus, approximately 55–66% of *D. citri* colonies are tended by Argentine ants (Tena et al., 2013; Schall and Hoddle, 2017). When *T. radiata* are encountered by *L. humile* on patches of *D. citri* nymphs, up to 95% of foraging parasitoids are harassed and chased off the host patch, or captured and killed (Schall and Hoddle, 2017) (**Fig. 5b**). These negative interactions with ants significantly reduce rates of host feeding and parasitism by *T. radiata* (Schall and Hoddle, 2017). Consequently, this food-for-protection mutualism leads to a positive feedback cycle—ant protection causes pest outbreaks, which produce more honeydew for ants, and ant colony size increases. This cycle increases pest densities and plant damage, reduces yields, and in the case of *D. citri*-*C*Las, potentially increases disease incidence in citrus orchards (Schall and Hoddle, 2017).

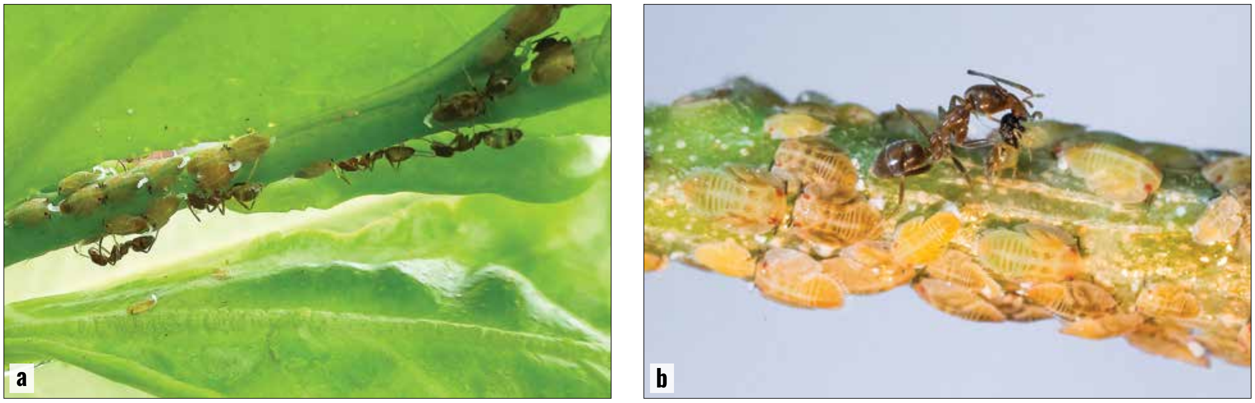

Figure 5. (a) Argentine ants, *Linepithema humile*, harvesting wax filaments with honeydew from a colony of *Diaphorina citri* nymphs infesting urban-grown citrus; (b) *Linepithema humile* worker attacking a female *Tamarixia radiata* on a patch of *D. citri* nymphs. (a: M. Hoddle, UC Riverside; b: Mike Lewis, UC Riverside)

If the full potential of *D. citri* natural enemies is to be realized, control of *L. humile* is necessary. Deployment of containers with liquid bait composed of a 25% sucrose solution with ultra-low concentrations of insecticide (e.g., 0.0001% thiamethoxam) rapidly reduces populations of *L. humile*. Ant workers consume the toxic bait, transporting it back to subterranean nests, where it is fed to nest mates and queens. In comparison to non-treated control plots, a 75–90% reduction in foraging ant densities is achieved within three days of bait deployment, and ants are held at near-zero densities when bait stations are replaced every two weeks (Schall and Hoddle, 2017). When ants were controlled in study plots, successful bouts of host feeding and parasitism by *T. radiata* increased two-fold, and densities of generalist predators (e.g., syrphid fly and lacewing larvae) increased 1.5–3.5 times on newly flushed citrus infested with *D. citri* (Schall and Hoddle, 2017). Within about eight weeks of successful suppression of *L. humile* populations, densities of honeydew-producing pests were reduced to very low levels, and natural enemy activity maintained psyllids at non-damaging levels for the duration of the ant management program (Schall, 2019).

Despite the efficacy of slow-acting toxic liquid baits for control of *L. humile*, deployment of liquid bait in plastic bait stations is expensive and time consuming. Bait stations are expensive to purchase, they need to be collected, cleaned, replenished, and redeployed monthly, and must be deployed at high densities (>100 per acre) (Nelson and Daane, 2007; Cooper et al., 2008; Daane et al., 2008; McCalla et al., 2020). An alternative approach to the deployment of liquid bait in plastic containers for *L. humile* control is the use of biodegradable alginate hydrogels (Tay et al., 2017), which can be broadcast directly onto the soil beneath citrus trees where ants form foraging trails that ascend tree trunks to reach colonies of honeydew-producing pests (**Fig. 6a**) (McCalla et al., 2020).

Biodegradable hydrogel beads are miniaturized bait stations that hold sugar water and insecticide. Ants imbibe the toxic solution directly from beads and transport the poisoned liquid sugar to the nest (**Fig. 6b**). Within a few days, the gels degrade and are no longer attractive to ants (irrigation events may help increase the longevity of the beads by as much as one extra day as beads are capable of rehydration). However, within the relatively short time the beads are active, ants recruit to them in significant numbers, and ant populations rapidly decline (Schall et al., 2018). Repeating applications every three weeks leads to density reductions of 91% of foraging ants compared to non-treated plots (McCalla et al., 2020).

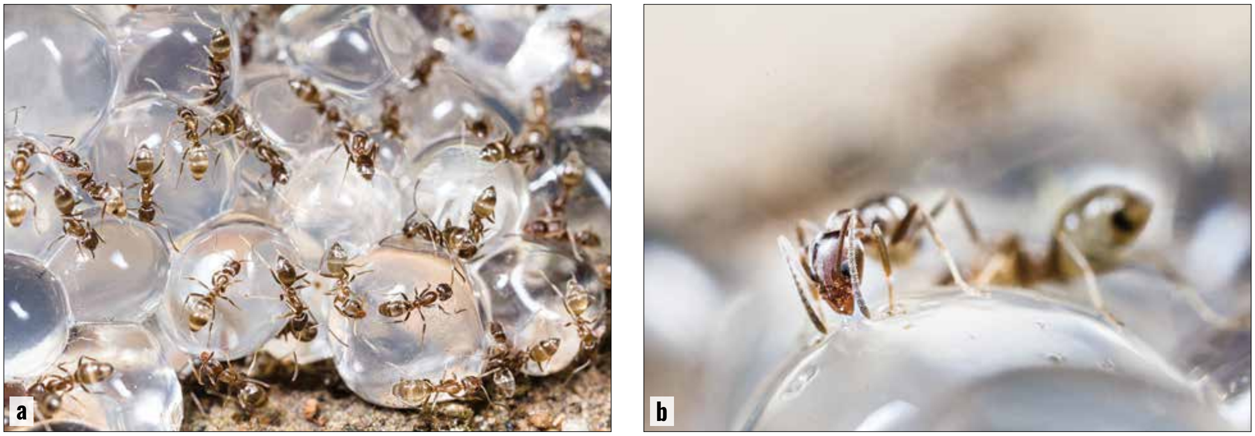

Figure 6. (a) Argentine ants, *Linepithema humile*, feeding on insecticide-laced sugar water contained within biodegradable hydrogel beads deployed on soil under citrus trees; (b) Argentine ant worker drawing solution out of a hydrogel bead (note also 'chunks' of missing bead; ants may have excised pieces of bead to return to the nest to feed to nest mates and queens). (a,b: Mike Lewis, UC Riverside)

Automated ant monitoring has the potential to greatly improve estimates of densities of foraging ants, which can inform decision-making with respect to initiating ant control treatments. Experimental observations suggested that more than 20–30 ants ascending a citrus tree trunk per minute caused biological control services in the canopy to decline. Visually counting ants is labor intensive and inaccurate when ant densities are high (**Fig. 7a**). To improve accuracy and reduce labor inputs, an automated ant-counting tool was developed. Automating ant counts was possible because of a highly stereotypical behavior that *L. humile* exhibits in citrus orchards. Ants use soil surface irrigation pipes as highways to move back and forth from subterranean nests to trees infested with colonies of honeydew-producing insects (**Fig. 7b**). Foraging workers prefer to move in straight line routes (Yates and Nonacs, 2016) over terrain that is relatively smooth (Clifton et al., 2020), and irrigation pipes satisfy both of these requirements. Therefore, infra-red (IR) sensors clamped to irrigation pipes (**Fig. 7c**) count ants as they move along the upper surface of the pipe. IR sensors can be outfitted with GPS tags, humidity and temperature sensors, and are powered by batteries (solar-powered operation is also possible). The device turns itself on for one minute each hour to count ants on pipes. Hourly, field sensors upload data to a LORA (i.e., long range, ~5 miles [8 km] radius) Gateway, which connects to the cloud via LTE cellular networks, and data are transferred to a virtual repository where they can be accessed and viewed on a smart device application (e.g., AntCount by FarmSense). Summary ant count data from each orchard sector can be assessed, treatment decisions made, and treatments applied when and where needed. Importantly, sensors count ants day and night; videography by Kistner et al. (2017) found that *L. humile* is also active on *D. citri* patches at night, and ants can interfere with nocturnally-foraging natural enemies.

Automated ant counting with IR sensors has the potential to greatly improve management decisions and to automate the entire ant control program. For example, GPS-guided deployment of biodegradable hydrogel beads by autonomous land drones could occur at night when human workers are absent, and reentry intervals could be met safely. Additionally, deployment of beads at night would enhance their longevity as temperatures are relatively lower and humidity is higher than during the day. Highly efficient, cost-effective ant control would promote enhanced biological control of *D. citri* and other hemipteran pests infesting California citrus.

Figure 7. (a) Visually estimating *L. humile* densities ascending trunks of citrus trees; (b) Argentine ants using irrigation pipes as highways to move from subterranean nests over uneven soil terrain to tree trunks; (c) an infra-red sensor attached to an irrigation pipe with a 3D-printed clamp. (a–c: M. Hoddle UC Riverside)

BENEFITS OF BIOLOGICAL CONTROL OF *DIAPHORINA CITRI*

The two primary goals of the classical biological control program targeting *D. citri* in California were (1) to establish host specific natural enemies from part of the home range of this pest (Punjab, Pakistan) and (2) to significantly reduce pest densities in backyard citrus growing in urban areas with imported biological control agents. The key desired outcomes of these efforts were attained; *T. radiata* released in 2011 established widely in California, and *D. citri* densities in urban citrus declined by more than 70%. These achievements have likely contributed to lower rates of spread of *D. citri* and the citrus-killing pathogen *C*Las from infested urban areas into neighboring commercial citrus.

In the intervening 10 years since the discovery of *C*Las in urban southern California, this vector-pathogen combination has not moved aggressively into commercial citrus production areas. This crop protection is possibly due, in part, to significant reductions in *D. citri* in urban citrus by the classical biological control agent *T. radiata* and native natural enemy species such as predatory syrphid fly larvae. These events have possibly led to a long period of containment (i.e., >10 years) of *D. citri*-*C*Las in urban areas and the prevention of rapid and widespread movment into commercial citrus. Slowing the invasion of *D. citri*-*C*Las in this manner has not previously been achieved. This observed containment of *D. citri* predominantly in urban areas strongly suggests that in this case, biological control of a pathogen's vector can substantially reduce spread of the vector (i.e., *D. citri*), the pathogen it spreads (i.e., *C*Las), and subsequent disease incidence in a crop (i.e., huanglongbing [HLB] in citrus). This outcome contradicts the widely held belief that classical biological control of insect vectors that transmit plant pathogens cannot control or slow the spread of a plant disease.

Work on *D. citri* biological control in urban areas identified the ubiquitous Argentine ant, *L. humile*, as a significant factor reducing natural enemy efficacy against *D. citri* nymphs. Consequently, research was pursued on developing new methods to monitor and control *L. humile*. These efforts have resulted in the development of efficacious management tools for *L. humile* in commercial citrus in advance of the anticipated widespread establishment of *D. citri*-CLas in major production areas. These efforts led to two important advances: (1) the development of biodegradable hydrogel beads that deliver ultra-low concentrations of insecticide to foraging ants and (2) the use of infra-red sensors for automated monitoring of ant densities in orchards to optimize timing and location of hydrogel bead applications. Work completed so far clearly indicates that suppression of *L. humile* in commercial citrus grown under a low input IPM program (i.e., minimal insecticide use) greatly enhances natural enemy activity, which significantly reduces densities of *D. citri* and other species of honeydew-producing pests (e.g., mealybugs, soft scales, and aphids). Control of *L. humile* in urban areas would also improve efficacy of natural enemies in residential citrus. However, in southern California, this urban environment is huge, and residential ant control would require homeowners to pay for treatments that extend beyond barrier sprays commonly applied around houses. Instead, one management approach could focus on control of *L. humile* at the urban-agricultural interface as both *D. citri* and *L. humile* tend to exhibit greater activity at these boundaries, and ants may penetrate from surrounding borders where they tend to be abundant (Anastasio et al., 2021). Management of *L. humile* at urban-agricultural interfaces could be incorporated into pest management plans for citrus orchards as opposed to being a residential-based control program.

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GROUP 2
PROTECTING LANDSCAPE PLANTS

Healthy blooming *Lilium michiganensis*, a species native to North America that is attacked by the introduced lily leaf beetle, *Lilioceris lilii*. Three parasitoid species have been successfully introduced to North America for the the control of the lily leaf beetle. (Jeff Steele, iNaturalist.org CC BY-NC 4.0)

CHAPTER 13

Reduction of Euonymus Scale, a Pest of Landscape Shrubs

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NON-TECHNICAL SUMMARY

Euonymus scale (*Unaspis euonymi*) (Hemiptera: Diaspididae) is a problem to the nursery industry because it makes growing euonymus shrubs for sale more costly and requires the use of pesticides for the scale's control. It is a problem for homeowners, small businesses, and apartment owners, who often use euonymus shrubs to landscape their properties because the scale makes the plants grow poorly and increases the number of plants that die prematurely. The lady beetle *Chilocorus kuwanae* (Coleoptera: Coccinellidae) reduced the number of heavily infested shrubs by about half (down from 20.9% pre-project to 11.6%) within 12 years (1989/1990 to 2002). The parasitoid *Coccobius* nr. *fulvus* (Hymenoptera: Aphelinidae) also caused 21% mortality of adult female scales by 2006/2007. The combined impacts of this parasitoid with predation from the lady beetle have not been evaluated but are likely to have further reduced scale density since the last evaluation in 2002.

Most euonymus species used in landscaping are from Asia, so this project was not intended to protect native biodiversity. However, a U.S.-native euonymous species (*Euonymus aericansus*) is damaged by the scale and benefits from this biocontrol project. There is no impact of the project on the winged euonymous (*Euoymus alatus*), an invasive plant in eastern U.S. forests because it is not attacked by the scale in the first place. Conversely, two Asian euonymus species on which the scale feeds (*Euonymus fortunei* and *E. japonica*) are sometimes invasive (Parker and Acevedo-Rodriguez, 2017). These two invasive species benefited from the esablishment of *C. kuwanae* and *C.* nr. *fluvus*.

HISTORY OF INVASION AND NATURE OF PROBLEM

Scales (various families of insects, including armored scales, soft scales, and others) are among the most highly invasive of insect groups because they are highly fecund, tightly adhered to plants, inconspicuous, occur in groups on their host plants, and some species are all females. Because of this final characteristic, a single insect can start a new population. Human movement of plants among the world's continents has led

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Van Driesche, R. G. 2022. Reduction of Euonymus Scale, a pest of landscape shrubs, pp. 145–150. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

to many scales being spread to new countries. When only a few scales are transported, it is very easy for the specialized parasitoids that keep most scales in check to be left behind. Population explosions of the scales follow, which can be highly damaging because imported scales are usually not suppressed by local generalist natural enemies. In the United States, at least 19 species of invasive scales in six families have been targeted for biological control by introducing missing natural enemies. Most of these pests (11 species) are armored scales, many of which attack citrus (Clausen, 1978). One of these eleven armored scales is the euonymus scale, *Unaspis euonymi* (Hemiptera: Diaspididae), a pest of landscape woody plants.

Euonymus scale (**Fig. 1a,b**) was first described in 1881 as *Chionaspis euonymi* from scales collected in Norfolk, Virginia, on *Euonymus latifolia* (Scalenet, 2020). This scale is now found in many parts of the United States and around the world, except in Australia. The native range of this scale is believed to be Japan and China. It was accidentally imported into the United States in the 19th century, likely on imported woody nursery plants (Johnson and Lyon, 1991). While it is most widely known as a pest of the evergreen species of *Euonymus*, such as *E. japonica*, it also feeds on species of many other shrubs used to landscape homes and businesses, including plants in the genera *Camelia, Buxus, Celastrus, Daphne, Eugenia, Hedera, Hibiscus, Ilex, Jasminum, Ligustrum, Lonicera, Olea, Paxistima, Pachysandra, Solanum*, and *Prunus* (Johnson and Lyon, 1991). Heavy infestations can defoliate and kill host plants. As an aside, it is worth noting that winged euonymus (*Euonymus alatus*), which invades forests in the northeastern United States (Ecological Landscaping Alliance, 2020), is not a host of euonymus scale. A polyphagous scale parasitoid, *Encarsia citrina* (Hymenoptera: Aphelinidae), commonly parasitized this scale before the initiation of this biocontrol project. While this parasitoid was both widespread and abundant (e.g., Matadha et al., 2003), it did not suppress euonymus scale density (R. Van Driesche, pers. obs.).

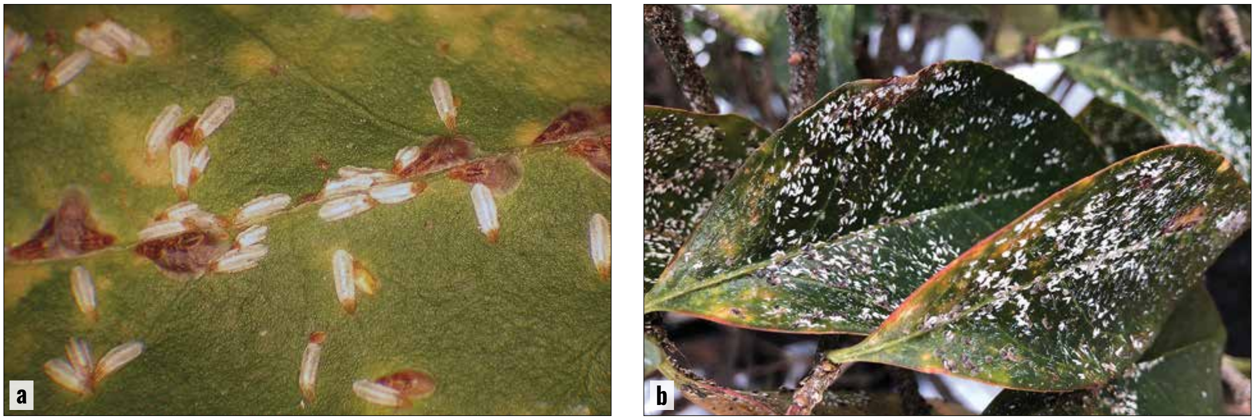

Figure 1. Euonymus scale (*Unaspis euonymi*) on leaf (a) close-up of white males and larger brown females; (b) white males, brown females, and yellow leaf chlorosis. (a: Lisa Ames, University of Georgia, Bugwood.org CC BY US 3.0; b: Susan J. Hewitt, iNaturalist.org CC BY-NC 4.0)

From 1991 to 1994, the impact of euonymus scale on survival of *Euonymus fortunei* plants in commercial and residential landscapes in southern New England (Massachusetts, Connecticut, Rhode Island) was assessed by multi-year field assessments of year-to-year survival of tagged plants. Plants that were heavily infested with euonymus scale suffered 12.1% mortality within the following year, a 4-fold increase compared to non-infested or less infested plants. Of 3,549 landscape euonymus plants in Massachusetts examined from 1989 to 1992, 20.9% had heavy scale infestations. Using this information, the annual economic loss from euonymus scale in Massachusetts (at $22.50 per replacement plant, value from the time of this study) was estimated as $355,568, or for southern New England as a whole, $711,135, in dollars of that period (Van Driesche et al., 1998a).

WHY CONTROL THIS INVASIVE SPECIES?

The goal of this biological control project was to lower scale density on landscape euonymus plants, as only heavy infestations (**Fig. 2**) defoliate and kill plants (Van Driesche et al., 1998a).

THE ECOLOGY OF THE PROBLEM

The ecology of this system is simple: an exotic plant was imported for commercial use as a landscape plant; later, one of the pests in its native range (euonymus scale) was accidentally imported as well. The pest spread throughout the United States, both naturally through wind dispersal of young scales and by shipping of infested nursery stock. The desired goal was to suppress the pest enough to continue the commercial use of euonymus plants, which are the scale's preferred hosts.

Figure 2. Euonymus shrub with dead patch killed by heavy euonymus scale (*Unaspis euonymi*) infestation. (© Chrisky, stack.exchange.com)

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Efforts to suppress euonymus scale with natural enemies from Asia occurred three times: once in the 1980s in the Mid-Atlantic states, again in the 1990s in New England, and again from 1987 to 2000 in New Jersey. The first effort was based on importation from Korea by Robert Hendrickson of the USDA of two predatory beetles found feeding there on euonymus scale. The larger beetle was the lady *Chilocorus kuwanae*[1] (Coleoptera: Coccinellidae; **Fig. 3a**). The second imported species was a much smaller beetle in the family Cybocephalidae called *Cybocephalus* nr. *nipponicus* (**Fig. 3b**). The lady beetle is a relatively large predator that seeks out high-density patches of scale, where the adult feeds on scales and lays its eggs. Lady larvae then

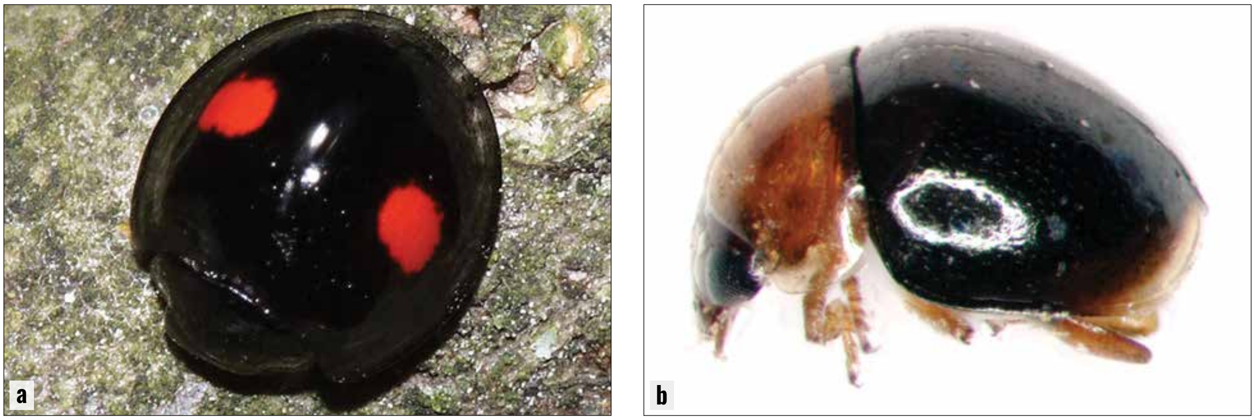

Figure 3. (a) The introduced Asian lady beetle *Chilocorus kuwanae*; (b) *Cybocephalus* nr. *nipponicus*. (a: Tom Murray, Bugwood.org, CC BY US 3.0; b: Pennsylvania Department of Conservation and Natural Resources, Bugwood.org, CC BY US 3.0)

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[1] A recent morphological paper suggests this species is the same as *C. renipustulatus*, which is found in western Europe (Bieńkowski and Orlova-Bienkowskaja, 2020). However, this finding was based solely on morphological measurements, without DNA analysis. Consequently, it is unclear if this suggested synonymy should be accepted.

also feed on scales, eating many throughout their development. In contrast, the smaller *C.* nr. *nipponicus* lays its eggs on individual scales. The larvae of *C.* nr. *nipponicus* then consume scales sequentially, requiring an average of 20 scales to grow to adults (Alvarez and Van Driesche, 1998).

Chilocorus kuwanae and *C.* nr. *nipponicus* were both collected in Korea and released in the United States, starting in 1984. Both species successfully established in several states, including Massachusetts and Mid-Atlantic states (Drea and Carlson, 1987, 1988), and *C. kuwanae* was later widely redistributed in the eastern United States (Hendrickson et al., 1991). However, no detailed assessments were reported for the impact of these releases on euonymus scale populations in the region.

A second round of importations of euonymus scale natural enemies was made in New England from 1991 to 1994, during which the same two predatory beetles plus three parasitoid species were imported from China by USDA-APHIS and Mike Rose of Texas A & M University. These species were released in New England through Roy Van Driesche of the University of Massachusetts/Amherst (Van Driesche et al., 1998b). Both predators, *C. kuwanae* and *C.* nr. *nipponicus*, established in Massachusetts—especially *C. kuwanae*, which was recovered throughout the state. In addition, two parasitoids of euonymus scale, *Encarsia* sp. nr. *diaspidicola* and *Coccobius* nr. *fulvus*[1] (both Hymenoptera: Aphelinidae) were released and initially recovered at some sites. A third parasitoid, *Aphytis yanonensis*[2] (Hymenoptera: Aphelinidae) was also released but did not appear to have become established (Van Driesche et al., 1998b).

A third effort was conducted by the New Jersey State Department of Agriculture in cooperation with APHIS. From 1987 to 2001, they mass produced and released the five agents previously collected from China (the two predators, plus the three parasitoids *E.* nr. *diaspidicola*, *A. yanonensis* [at the time known as *A. proclivia*], and *Coccobius* [at the time, *Physcus*] nr. *fulvus*) throughout New Jersey. Both *C. kuwanae* and *C.* nr. *nipponicus* became widely established, but the parasitoids were not recovered (Matadha et al., 2003).

HOW WELL DID IT WORK

The only follow-up studies on the effects of natural enemy releases against euonymus scale that went beyond simple confirmation of establishment were done in New England (Van Driesche et al., 1998c; Van Driesche and Nunn, 2003; O'Reilly and Van Driesche, 2009). Before the Massachusetts biocontrol program, surveys in planted residential and commercial landscapes in southern New England from 1989 to 1992 found that 20.9% of *E. fortunei* shrubs examined had heavy euonymus scale infestations (Van Driesche et al., 1998a). The impact of the predator *C. kuwanae* was first evaluated in research plots (1991–1995) at 14 sites where releases of this species were made, with these plots being compared to 13 similar control sites where releases had not been made (Van Driesche et al., 1998c). The predator suppressed scale numbers at nine of the 14 release sites. At three sites, *C. kuwanae* did not become abundant, and at two sites, the owners removed the shrubs during the test. Of the 13 control sites, three developed large populations of *C. kuwanae* due to natural spread of the predator during the experiment. Of the 10 control sites where *C. kuwanae* remained absent or rare, scale numbers on average did not change (going up at four locations, down at four sites, and staying the same at two).

In the second experiment in our study, we released beetles at a large apartment complex landscaped with 110 euonymus shrubs on a 32-ha (79-acre) site (Van Driesche et al., 1998c). Within four months, *C. kuwanae* spread to 64% of the euonymus plants on the property, and within one year, the proportion of plants with heavy scale infestations dropped from 46% to only 13%.

The third evaluation of *C. kuwanae* in Massachusetts was based on two statewide surveys, one done in 1994 and the other in 2002. In 1994, we found no significant change had yet occurred in the proportion

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[1] *Coccobius* nr. *fulvus* may be the same as *Coccobius fulvus*. This is consistent with a recent revision of Chinese *Coccobius* (Wang et al., 2014). However, direct genetic comparisons have not been made.

[2] Some literature refers to this species as *Aphytis proclia*; for species description see DeBach and Rosen, 1982.

of euonymus that had heavy scale infestations (Van Driesche et al., 1998c). However, in 2002, we found that the percentage of shrubs with heavy scale infestations had dropped from 20.9% in the pre-project survey in 1989/1990 (Van Driesche et al., 1998a), to 11.6% in 2002 (Van Driesche and Nunn, 2003), a drop of 44%. In 2002, *C. kuwanae* was present on 43.1% of all plants in our survey that had heavy scale infestations.

A fourth evaluation, done in 2006/2007, focused on estimating the rate of parasitism from the parasitoid species released from 1991 to 1994, at the beginning of the project. Of the three species introduced, *C.* nr. *fulvus* was well established in 2006/2007 and was common in urban areas of the Connecticut River Valley of Massachusetts (Van Driesche and O'Reilly, 2009). *Coccobius* nr. *fulvus* was found at 87% of all locations sampled and parasitized an average of 21% of the adult female scales (the stage examined). *Encarsia* nr. *diaspidicola*, which was presumed established from 1991 to 1994, was not detected in 2006/2007. No further assessments of this pest and its control have been made since 2007.

BENEFITS OF BIOLOGICAL CONTROL OF EUONYMUS SCALE

The benefits of this project consisted of reduced costs to homeowners and businesses to replace euonymus shrubs killed by scale. Because these plants are not native, there were no obvious ecological benefits. In southern New England, various species of euonymus (especially *E. fortunei*) are commonly used for landscaping. In the late 1990s, the number of evergreen euonymus shrubs in the landscape was estimated at 821,846. (Van Driesche et al., 1998a). At that time, 20.9% (171,766) of these plants were heavily infested with euonymus scale and were likely to suffer about 12% mortality annually due to the scale. This would result in the deaths of about 15,803 euonymus plants per year. The cost to consumers to replace these 15,803 dead plants was estimated (at $22.50 per replacement plant) as $355,568 in Massachusetts alone (Van Driesche et al., 1998a). By 2002, the percentage of heavily infested plants had declined to 11.6% (Van Driesche and Nunn, 2003), reducing the number of plants dying per year by nearly 6,000 (down from 15,803 per year) producing a savings in that year alone of $127,756. Further declines in the rate of heavily infested shrubs may have occurred since, leading to further savings, but no surveys have been run since 2002.

REMAINING WORK

No assessment of this system has been made since 2002, a current gap of 19 years. It would be useful to update knowledge of this project by remeasuring the percentage of euonymus shrubs with heavy scale infestations. Also, in 2007, surveys showed that one of the introduced parasitoids, *C.* nr. *fulvus*, had increased and spread. The status and the degree of impact of this parasitoid need to be remeasured throughout the region.

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Chapter 14

Successful Management of the Pink Hibiscus Mealybug, *Maconellicoccus hirsutus*, in the Coachella Valley of California

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NON-TECHNICAL SUMMARY

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), is a highly polyphagous pest with hosts ranging from forest trees to ornamental landscape plants to a wide variety of agricultural commodities. During feeding on the phloem of its hosts, PHM injects a salivary toxin that can distort the new shoots, creating an unsightly 'bunchy top' appearance. Heavy infestations cause defoliation and lead to plant death. Furthermore, heavy honeydew deposition mixes with the waxy filaments produced by the mealybug, resulting in a sticky, waxy mess on infested plants. The sugary honeydew supports the growth of sooty mold fungi, which causes indirect damage by reducing plant photosynthesis.

A native of southern Asia, PHM has become widely established in the tropical and subtropical regions of the globe and was discovered in the Western Hemisphere in 1983 in Hawaii. It was first detected in the continental United States in 1999, becoming established in southern California's Imperial Valley, where it was successfully managed through a focused effort of rearing and releasing parasitoids. In 2014, PHM was found to be widespread in the urban regions of the Coachella Valley of California, killing landscape trees and shrubs. The importance of finding a solution to this problem was further emphasized by the proximity of the urban area to agricultural production areas of citrus, dates, grapes, and vegetable crops, all known to be hosts of PHM.

Given the past effectiveness of introductions of natural enemies against PHM, which date back to the early 1900s, and the success of classical biological control efforts in the Imperial Valley, we turned to this strategy for a solution in the Coachella Valley. For two years we released large numbers of what was believed at the time to be *Anagyrus kamali* and a small number of *Gyranusoidea indica* (both Hymenoptera: Encrytidae), primarily in the urban areas of the valley. Just one year after we started releasing these parasitoids, we documented a 97% decrease in the numbers of PHM. Parasitoids reared from field collections of PHM were predominantly "*A. kamali*," and numbers of this parasitoid have continued to increase since the time of introduction.

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The management of such a potentially devastating pest across the plant-diverse urban and agricultural landscape could not have been accomplished using the other strategies commonly employed in integrated pest management (IPM) programs (including insecticides, resistant plants, cultural control, pest exclusion, or quarantine). The establishment of highly effective natural enemies in a classical biological control program was the best solution for this pest problem. An additional benefit of this program was the scientific investigation that led to the taxonomic clarification of several parasitoids useful in biological control and the proper identification of what we thought was *A. kamali*. This parasitoid was eventually recognized as a new species and was described as *Anagyrus callidus*, a species that originated in Taiwan and that has provided excellent biological control of PHM.

HISTORY OF INVASION AND NATURE OF PROBLEM

History of Pest Invasion

Figure 1. Pink hibiscus mealybug, *Maconellicoccus hirsutus*, on hibiscus. (T.M. Perring, UC Riverside)

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) (**Fig. 1**), is a tropical and subtropical pest with a long history of invasion and establishment in new regions across the globe. This mealybug is believed to be native to southern Asia and first was described from India (Green, 1908; Williams, 1996; Williams, 2004; Roltsch et al., 2006). Not long after its description, PHM was discovered in Egypt, having invaded the African continent (Hall, 1926), and it became widely established throughout tropical Africa (Williams, 1986). In 1983, it was discovered in Hawaii (Beardsley, 1985; Kairo et al., 2000) and later quickly spread in the Western Hemisphere. In the one hundred years since its first detection outside of its native range, PHM has spread and become established in new tropical and subtropical regions as well as some warm temperate areas worldwide (**Fig. 2**).

Within the continental United States, PHM was first discovered in 1999 in the Imperial Valley (Imperial County) of southeastern California (Kairo et al., 2000; Roltsch et al., 2001; Roltsch et al., 2006); this invasion also extended south into the Mexicali Valley of Mexico. Within the United States and its associated territories, PHM is established in Hawaii, California, Florida (Hoy et al., 2002), Puerto Rico, the U.S. Virgin Islands and the Mariana Islands (Reddy et al., 2009), and infestations have been reported from several additional states including Louisiana (W. Spitzer, pers. comm.), Texas (Bogran and Ludwig, 2007), Georgia (Horton, 2008), North and South Carolina (Chong, 2009), as well as Oklahoma, Alabama, Tennessee, and New York (EPPO, 2021). It was introduced into the southern Coachella Valley (Riverside County) of California in 2008, but it remained at relatively low abundance until 2014 when it was found to be widely established on landscape plants in the northwest urban parts of the valley, posing a serious threat to the agricultural industries in the southeast region of the valley.

Nature of the Problem

If left unmanaged, the PHM poses a significant risk to horticulture, forestry, and agriculture. PHM is a sap-sucking pest that is highly polyphagous, feeding on over 330 plant species (Chong et al., 2015). This

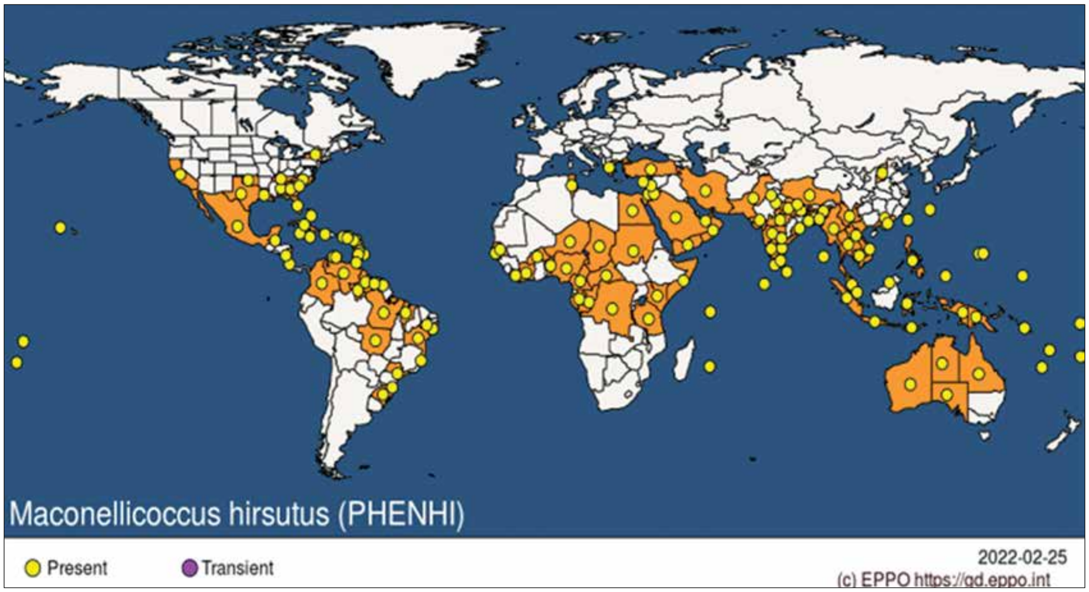

Figure 2. Global distribution of *Maconellicoccus hirsutus.* (EPPO, 2021)

extensive host list includes forest trees, woody ornamentals, fruit trees, vegetables, and other food and fiber crops. Uncultivated plants surrounding field crops and orchards can act as reservoir plant hosts that support PHM development and enable eventual mealybug movement onto economically important plants.

Feeding and development on host plants can cause both direct and indirect damage. As a piercing-sucking hemipteran, the PHM injects its saliva into host plants during feeding. This saliva is phytotoxic to some hosts and causes distortion of developing shoots and leaves resulting in 'bunchy top' symptoms (Williams, 1996; Kairo et al., 2000). In heavy infestations, defoliation and plant death can occur (Sagarra and Peterkin, 1999). Indirectly, PHM damages plants via the production of honeydew, a sugary substance excreted by the mealybugs as they feed on plant phloem. Honeydew supports growth of sooty mold, which can interfere with plant photosynthesis.

WHY CONTROL THIS INVASIVE SPECIES?

PHM is a highly fecund species with individual females reportedly producing as many as 600 eggs under optimal environmental conditions (Ghose, 1972; Hoy et al., 2002). Furthermore, the life cycle takes only 23–30 days (Hoy et al., 2002), so that multiple generations per year are common. These biological characteristics, combined with the insect's extensive host range and ease of both short and long-range dispersal, make prevention and eradication from favorable environments nearly impossible. PHM crawlers (or 1st-instar nymphs) are highly mobile and can move from plant to plant or be carried by the wind, insects, or birds. PHM can also be spread on landscaping tools, plant trimmings, or live plants, which can disseminate all life stages throughout regions such as the Coachella Valley. Dissemination based on movement of infested plant material causes international spread (Chong, 2009). All of these routes of spread become more likely if PHM densities are high.

THE ECOLOGY OF THE PROBLEM

The Coachella Valley (CV) includes about 675 square miles (1,758 km^2), of which approximately 120 square miles (310 km^2) are predominately urban and 100 square miles (259 km^2) are used for agriculture (Coachella

Valley Water District, 2019); the rest is in natural vegetation. The urban area is located in the northwest part of the valley, where the cities of Palm Springs, Rancho Mirage, Cathedral City, Indian Wells, and La Quinta are located. Many of the homes in this area have highly manicured landscapes, and landscape workers routinely move infested plants or trimmings throughout the CV. The agricultural region of the CV is adjacent to the southeastern portion of the urban area (**Fig. 3**). The CV had gross farm receipts of just over $700 million in 2020 (Riverside County Agriculture Production Report, 2020). The major commodities produced are dates, grapes, citrus, turf, and vegetables.

The CV is in the Colorado Desert section of the Sonoran Desert. Mid-summer daily temperatures (July) average 93°F (33.9°C) (high 107°F [41.7°C] and low 78°F [25.6°C]) and mid-winter daily temperatures (December) average 53°F (11.7°C) (high 69°F [20.6°C] and low 41°F [5°C]); the average annual rainfall is about 6 in (15.2 cm) (Weatherspark, 2022). The predominant wind direction is from northwest to southeast (from urban to agricultural regions), which is an important consideration for blowing small PHM nymphs or influencing movement of the insects and birds on which PHM nymphs travel.

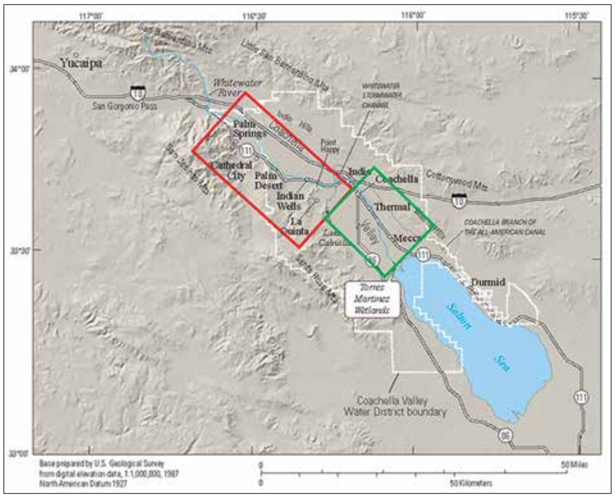

Figure 3. Coachella Valley showing the close proximity of the urban region in the northwest (red) and the agricultural region in the southeast (green). (map created by T.M. Perring using USGS base map [USGS, 2022])

Figure 4. Dates infested with *Maconellicoccus hirsutus* in Coachella Valley, 2014. (T.M. Perring, UC Riverside)

In 2014, our team was called by a date packer to investigate an insect infestation in 30,000 pounds (13,600 kg) of dates he had received from a grower. Upon inspection, we identified PHM (**Fig. 4**), which prompted an investigation into the source of the infested dates. We determined that the dates had been harvested from date palms in La Quinta in an area where date palms from a discontinued date ranch had been left as landscape trees when the ranch was converted into a residential area. These palms had been managed for fruit production, but even so, the dates were heavily infested with PHM, and the entire 30,000 pounds had to be destroyed. Further investigation found PHM widely distributed throughout the northwest urban area of the valley on many species of landscape plants. Infestations caused deformed foliage on carob trees (*Ceratonia silique*), and pink hibiscus mealybug was killing hibiscus bushes (*Hibiscus rosa-sinensis*) and silk oak trees (*Grevillea robusta*). The silk oaks had been widely planted on golf courses, and many large specimens had to be removed because of damage from PHM.

With such a widespread infestation on so many plant species in an urban area, there was a high likelihood of rapid spread into the valley's agricultural crops, all of which can host PHM. Consequently, we needed a quick solution to this crisis. Clearly, we could not spray all of the landscape and backyard trees in the affected cities, so we initiated a classical biological control approach.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

From the PHM invasion of Egypt in the early 1900s (Hall, 1926), the value of introducing natural enemies with a narrow host range to control this invasive mealybug has been evident (Moursi, 1948a; Mani, 1989; Cross and Noyes, 1996). Based on this historical information, soon after PHM was discovered in the early 1990s in the Caribbean Islands, efforts began to identify and collect natural enemies known to control PHM. While the predatory beetle *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) was among these biological control agents, the main focus was on two primary parasitoid species, *Anagyrus kamali* and *Gyranusoidea indica* in the family Encyrtidae (Hymenoptera), and the species *Allotropa* sp. nr. *mecrida* in the family Platygastridae (Hymenoptera).

A review was carried out of the likely efficacy and safety of *A. kamali*; in general, species in the genus *Anagyrus* are relatively narrow in their host specificity. *Anagyrus kamali* is recorded primarily as a parasitoid of PHM, but it is known to occasionally parasitize mealybugs in other genera (Cross and Noyes, 1996). A host suitability study for *A. kamali* that compared PHM with eight other mealybug species (in seven genera) (Sagarra et al., 2001) found that PHM was the only species suitable for the parasitoid's complete development. Furthermore, Roltsch et al. (2006) made many collections of two local mealybug species in southern California, and none were parasitized by either *A. kamali* or *G. indica* after these parasitoids had been released in high numbers in Imperial County.

Anagyrus kamali was successfully introduced to Egypt from Java in Indonesia on several occasions in the 1930s (Kamal, 1951), and the species was described from specimens that were field-collected in Egypt (Moursi, 1948ab). This species was later released, with highly successful results, throughout the United States and Mexico (Klotz et al., 2002; Roltsch et al., 2006; Valencia-Luna et al., 2007; Santiago-Islas et al., 2008; Van Driesche et al., 2022). *Gyranusoidea indica* also has been released along with *A. kamali* (Roltsch et al., 2006; Van Driesche et al., 2022). From 1999 to 2004, both of these species were reared and released in the Imperial Valley (Imperial County), California (Roltsch et al., 2006). In late summer of 2004, following successful parasitoid establishment and effective control of PHM in the Imperial Valley, the insectary there was scheduled to close. However, the USDA-APHIS requested that it continue to operate. At that time, startup samples of both *A. kamali* and *G. indica* were received from an insectary in Puerto Rico. The *A. kamali* sample included a strain that had recently been collected from Taiwan, an important fact that will be discussed later in this chapter.

In 2009, PHM was found by the Riverside County Agricultural Commissioner's Office in a jujube (*Ziziphus jujuba*) field in the southern end of the Coachella Valley; this infested field was removed. There were three additional isolated detections of PHM between 2009 and 2014, when we were alerted to the infested dates mentioned earlier. At this time, two approaches were used to obtain *A. kamali* and *G. indica*. First, potted hibiscus plants infested with 2nd- and 3rd-instar PHM nymphs (the appropriate life stages to be parasitized) were stationed in several residential yards in the Imperial Valley as sentinel plants. The plants were left for approximately five days to enable the parasitoids to detect and parasitize the PHM on the plants, after which the plants were then taken to the Insectary and Quarantine facility at the University of California, Riverside, where hosts were held for parasitoid emergence. From these sentinel plants, over 600 *A. kamali* and 150 *G. indica* females were collected and used to start the colonies that supplied the parasitoids released in the Coachella Valley. Second, arrangements were made with Mexican government scientists to ship parasitoids to California from the Regional Laboratory for Reproduction of Biological Control Agents in Valle de Banderas, Nayarit, Mexico.

From November 2015 through December 2017, we released just under 250,000 individuals of *A. kamali*, and, from April 2017 through December 2017, we released approximately 15,000 *G. indica*. These releases were made at 349 sites in the Coachella Valley. At the time of our releases, we thought we were releasing *A. kamali*, but during the course of our studies, we realized we were releasing a different species (see following pages).

Figure 5. (a) Hibiscus, *Hibiscus rosa-sinensis*, and (b) hibiscus infested with pink hibiscus mealybug; (c) carob tree, *Ceratonia silique*, and (d) carob tree branch infested with pink hibiscus mealybug; (e) mulberry tree, *Morus nigra*, and (f) mulberry branch terminal infested with pink hibiscus mealybug; (g) silk oak tree, *Grevillea robusta*, among three palm trees, and (h) a silk oak branch infested with pink hibiscus mealybug. (a–h: T.M. Perring, UC Riverside)

HOW WELL DID IT WORK?

To assess parasitoid success, we sampled four plant species that were severely infested with PHM. We sampled hibiscus bushes (**Fig. 5a,b**), as well as carob (**Fig. 5c,d**), mulberry (*Morus nigra*) (**Fig. 5e,f**), and silk oak trees (**Fig. 5g,h**) in the fall of 2015, 2016, 2017, and 2018. For parasitoid sampling, we collected four 5-in (12.7 cm) branch terminals that had obvious infestations of PHM from each host plant at various locations. These samples were processed by isolating 2nd- and 3rd-instar mealybug nymphs or pre-oviposition adult females into individual gel caps and holding them for up to four weeks to obtain emergence of parasitoids. The species of each parasitoid was determined, and the number was divided by the total number of isolated mealybugs to calculate the percentage parasitism. This procedure followed Roltsch et al. (2006) and was used in 2015, 2017, and 2018. In 2016, an estimate of total parasitism (independent of species) was determined by dividing the total number of mummified hosts in each sample by the total number of late 2nd- and 3rd-instar nymphs and adult females of PHM collected. To determine the impact of releases on the PHM population, we collected four randomly selected 5-in (12.7-cm) branch terminals from each of the plant species that were sampled for parasitoid assessment, but without assessing the PHM status before picking the terminal to be collected. All life stages of PHM were counted on the entire terminal.

Nearly all the parasitoids that emerged from our sampling were classified by us as *A. kamali*; we recovered only eight *G. indica* over the course of the study. The small number of *G. indica* recovered likely resulted from the smaller number of releases (only 15,000) that we made of *G. indica*, compared to 250,000 for *A. kamali*. Releases of *G. indica* were made for only nine of the 25 months of the study. *Anagyrus kamali* established very quickly, and parasitism increased each year from none in 2015 to an average per sample of 6, 16, and 63% in 2016, 2017, and 2018, respectively. The average number of mealybugs per terminal dropped precipitously in the first year after the initial parasitoid release, from an average of 213 per terminal in 2015 (before release) to an average of just nine PHM per terminal (a 97% decline). The density of PHM on plants remained low through the end of our study in 2018.

BENEFITS OF BIOLOGICAL CONTROL OF PINK HIBISCUS MEALYBUG

There were two goals to this project. First, we had to suppress the abundance of PHM on landscape plants that was causing severe losses in the urban areas of the Coachella Valley. Second, we hoped that suppression in urban areas would prevent the movement of PHM into the agricultural regions of the valley. We accomplished both goals and did so without insecticide applications in either environment. While insecticide use was not an option in the urban landscape, there are systemic materials registered in grapes, citrus, and vegetable crops that are effective against mealybugs. However, there are no such materials available for date palms. In response to that situation, we established an IPM approach for use in dates that used augmentative releases of PHM parasitoids, expanding on the successful management of PHM in the urban regions using classical biological control. For this date IPM program, growers were trained to recognize PHM-infested date bunches during harvest in the fall (**Fig. 6**). A very low number of date bunches are infested every fall, the result of a single bunch becoming infested by mealybugs that presumably blow into the bunch or are carried by insects, birds, or farm workers. The normal production practice in dates is

Figure 6. Date bunch infested with *Maconellicoccus hirsutus*. (T.M. Perring, UC Riverside)

to enclose bunches in mesh bags to prevent insect infestation and protect the dates from rain (Perring and Nay, 2015). However, these bags also prevent parasitoids from entering bunches infested with PHM before bag installation. When this happens, the PHM population develops unchecked, which renders the dates unmarketable. When workers remove the bag and see an infestation, they are instructed to bury the bunch or put the bag back on the bunch and set it out in the sun to solarize the fruit and kill the PHM. The workers are also instructed to note the location of such infested bunches, which is then communicated to our laboratory. The following spring, while the new bunches are setting fruit, we release parasitoids in the vicinity of the infested bunch. These parasitoids locate any PHM that may have infested the crown of the date palm since the previous fall.

Our work in the Coachella Valley, in addition to controlling PHM there, detected and resolved a taxonomic error in the mass rearing of the key parasitoid. About a year into the PHM biological control program, we were releasing what we believed were *A. kamali* from two sources: (1) our colony that we established from collections in Imperial Valley and (2) the colony being reared in the Nayarit, Mexico facility. For a related project aimed at distinguishing these two populations, we ran genetic comparisons that found the two colonies were genetically and morphologically identical, but that both differed from the true *A. kamali* (Triapitsyn et al., 2007; Andreason et al., 2019). We determined that parasitoids we released in the Coachella Valley were, in fact, a new species, *Anagyrus callidus* (**Fig. 7**) and not *A. kamali*.

Figure 7. *Anagyrus callidus* (Hymenoptera: Encyrtidae). (C. Dominguez, UC Riverside)

This raises two questions, how did *A. callidus* get introduced into the Imperial Valley and why is it the predominant parasitoid? In answer of the first question, Triapitsyn et al. (2019) presented evidence that *A. callidus* originated in southern Taiwan, and it was moved from Taiwan in 2000 through various agencies to the USDA rearing facility in Puerto Rico. From Puerto Rico, insects thought to be *A. kamali* (but now known to be *A. callidus*) were sent to Florida in 2002 and also to the Imperial Valley CDFA facility in 2005 to start colonies for the re-commissioned rearing program for USDA-APHIS. A recent review of the records has shown that approximately 15,000 *A. callidus* were released in the summer and fall of 2007 before the closing of the Imperial Valley Insectary. In addition, *A. callidus* was sent from the Puerto Rico facility to the Nayarit lab in Mexico (Valencia-Luna et al., 2007; Santiago-Islas et al., 2008), where it seems to have displaced *A. kamali* in the rearing facility, and was released as *A. kamali* in Mexico, including in the Mexicali Valley, which is in close proximity to the Imperial Valley. From either (or both) source(s), *A. callidus* became established in the Imperial Valley in the seven years between its introduction in 2007 and our collection in 2014; all *Anagyrus* parasitoids recovered on our sentinel plants were *A. callidus*. The answer to the second question about the displacement of *A. kamali* by *A. callidus* is unknown at the present time. We suspect that *A. callidus* has biological characteristics that enabled it to outcompete *A. kamali*. This is the topic of ongoing research in the Perring laboratory.

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CHAPTER 15

Successful Biological Control of the Lily Leaf Beetle, *Lilioceris lilii*

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NON-TECHNICAL SUMMARY

The lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae: Criocerinae), native to Eurasia, was discovered in Canada in 1943 and found in the United States in 1992. It has since spread widely throughout northeastern United States and eastern Canada, with satellite populations in several western states and provinces. Throughout its invaded range, it causes widespread damage to cultivated and native lilies. Protecting natural populations of native lilies from defoliation by this pest is impractical, and many gardeners stopped growing lilies in infested areas. Consideration of a biological control program against this pest was based on past successes in North America against several of the pest's close relatives in the same subfamily; the cereal leaf beetle, *Oulema melanopus*, and two asparagus beetles, *Crioceris asparagi* and *C. duodecimpunctata* (all Coleoptera: Chrysomelidae: Criocerinae).

Initial exploration in Europe in 1996 found several parasitoids of lily leaf beetle, and beginning in 1998, CABI of Delémont, Switzerland, was contracted for European research on this pest. CABI scientists identified seven parasitoids of *L. lilii*; four were selected for further evaluation of their chemical ecology and host specificity, including parasitism of closely related species. Additional host range testing in quarantine at the University of Rhode Island showed that three species had adequate host specificity for release in North America. With USDA and Canadian approval, *Tetrastichus setifer* (Hymenoptera: Eulophidae) was released in Massachusetts in 1999 and in Ontario in 2010. Based upon European parasitoid distributions, *Lemophagus errabundus* (Hymenoptera: Ichneumonidae) was released in coastal areas of the northeastern United States, and *Diaparsis jucunda* (Hymenoptera: Ichneumonidae) was released at inland sites. Between 1999 and 2006, we made 44 releases of *T. setifer*, 28 releases of *L. errabundus*, and 46 releases of *D. jucunda* adults in New England. Later, we made releases in New York and Washington State, as well as 25 releases of *T. setifer* in Canada, including Ontario, Quebec, Alberta, and Manitoba.

By 2016, *T. setifer* was established at release sites in New England, Ontario, and Alberta. *Lemophagus errabundus* established in Massachusetts and Rhode Island, and *D. jucunda* did so in Massachusetts, Rhode Island, Connecticut, and Maine. All three parasitoids had spread at least 10 km (6.2 mi) from release sites

Casagrande, R. A., L. Tewksbury, and N. Cappuccino. 2022. Successful biological control of the lily leaf beetle, *Lilioceris lilii*, pp. 161–171. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

and caused substantial reduction in *L. lilii* numbers (Tewksbury et al., 2017). An updated survey of lily growers in 2021 showed considerable decline in damage caused by the beetle in eastern Massachusetts and Rhode Island, as well as the cities of Ottawa and Montreal and their surrounding suburbs.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Lily leaf beetle, *Lilioceris lilii* (Coleoptera: Chrysomelidae: Criocerinae), occurs throughout Europe, North Africa, and parts of Asia, from Siberia to Morocco (Slate, 1953; Labeyrie, 1963), and in China (Lu and Casagrande, 1998). It was first reported in North America near Montreal, Canada in 1943 (LeSage, 1992), and in successive years, the beetle spread slowly from that area. However, in 1992 the beetle was detected in many distant sites, including Cambridge, Massachusetts, apparently imported with bulbs from Europe, and it was then moved along with plants within North America (Dieni et al., 2016). By 2016 the beetle was present throughout all of New England and much of the northeastern United States, with localized populations in the midwestern United States and Washington State. In Canada by 2016, it was widely distributed in the Maritime Provinces, Newfoundland, Quebec and Ontario, and also found in Manitoba, Saskatchewan, and Alberta (Tewksbury et al., 2017). The beetle's natural spread by flight has been assisted by movement of infested lilies within Canada and the United States (Lesage and Elliott, 2003; Cappuccino et al., 2013; Tewksbury et al., 2017). Current distribution records (iNaturalist, 2021) show that the beetle is well-established in southeastern Pennsylvania, and it has populations near Washington, D.C. and in parts of eastern Tennessee.

Nature of the Problem

Lily leaf beetle overwinters as an adult, and in spring it feeds and then lays its eggs on the undersides of leaves (**Fig. 1**). The larvae cover themselves with their fecal material, which apparently protects them from predators (Jolivet and Verma, 2002; Keefover-Ring, 2013). However, these fecal shields are used by parasitoids to locate beetles and identify them as hosts (Schaffner and Müller, 2001). Larvae complete four instars and then pupate in the soil (Haye and Kenis, 2004). Adults emerge in late summer and feed for a few weeks before moving to overwintering sites. There is one generation per year. *Lilioceris lilii* is a serious pest of both native and cultivated lilies in North America (LeSage and Elliott, 2003). Adults and larvae both feed on lily leaves, buds, and flowers (Ernst, 2005), often completely defoliating plants. After a few years of defoliation, plants cease flowering and many die. Perhaps more commonly, gardeners who are unwilling to tolerate defoliated lilies covered with fecal-coated larvae (**Fig. 2**) simply remove the plants (LeSage, 1992; Stocker, 2002). Despite availability of several new cultivars, lily sales have declined by more than 50% concurrent with the spread of the beetle throughout the United States (White Flower Farm, pers. comm.).

Figure 1. Lily leaf beetle (*Lilioceris lilii*) laying eggs. (Andrea Brauner, Agriculture and Agri-Food Canada)

Lily leaf beetle's threat to native plants stems from its host-range expansion onto native lilies and related species. The beetle has been reported from Canada lily (*Lilium canadense*) (Bouchard et al., 2008), Michigan lily (*Lilium michiganense*) (Blackman et al., 2016), Turk's cap lily (*Lilium superbum*) (Livingston, 1996), and

Figure 2. Lily leaf beetle (*Lilioceris lilii*) larva with fecal material. (Andrea Brauner, Agriculture and Agri-Food Canada)

rose twisted stalk (*Streptopus lanceolatus*) (Blackman et al., 2016). Infestations on wood lily (*Lilium philadelphicum*) have not been reported (Bouchard et al., 2008; N. Cappuccino, pers. obs.); however, the species is an excellent laboratory host for lily leaf beetle (Ernst et al., 2007; Bouchard et al., 2008). No estimates of the impact of the beetle on plant fitness have been published, although mean defoliation levels as high as 65% have been reported for *L. canadense* (Bouchard et al., 2008). The conservation status of native lilies is tenuous for many species. Of the 24 native lily species in North America, four are federally listed as threatened or endangered (USDA Plants Database, 2021; ECOS Environmental Conservation System, 2021), and many other species are listed as rare, threatened, or endangered by individual states. Modeling of habitat suitability predicts that the beetle will be able to spread throughout most of North America, and its distribution will eventually overlap with that of most endangered, threatened, rare, or sensitive species in *Calochortus, Fritillaria, Lilium, Medeola*, and *Streptopus* (Freeman et al., 2020).

WHY CONTROL THIS INVASIVE SPECIES?

The lily leaf beetle is susceptible to many insecticides but out of concern for pollinators, gardeners and natural area managers are reluctant to use insecticides on flowers. It was at the request of Master Gardeners from the Boston, Massachusetts area that we began investigating biological control of *L. lilii*, based on our experience with the closely related cereal leaf beetle, *Oulema melanopus* (Casagrande et al., 1977). The cereal leaf beetle, the common asparagus beetle, *Crioceris asparagi*, and the spotted asparagus beetle, *C. duodecimpunctata* (all Coleoptera: Chrysomelidae: Criocerinae), invaded North America from Europe. All became serious pests, but all three species were largely controlled by releases of their European parasitoids (Haynes and Gage, 1981; Hendrickson et al., 1991; Evans et al., 2006; Poll et al., 1998; see also Chapter 10 on cereal leaf beetle in this volume).

THE ECOLOGY OF THE PROBLEM

In classical biological control of insects, it is customary to look to the center of origin of the pest and its host plants for insights. Lilies are in the genus *Lilium*, which apparently originated in the Himalayas, with species diversifying about 12 million years ago as they spread throughout Eurasia and into North America (Patterson and Givnish, 2002). At present, about 55 of the roughly 100 species in this genus exist in China, which has the greatest diversity of wild *Lilium* in the world (Rong et al., 2011).

Lily leaf beetle is in the genus *Lilioceris*, which contains 142 species, mostly concentrated in China (Yu et al., 2001). Five species are reported in Europe (Schmitt, 2013), but none are native to North America. *Lilioceris lilii* is the most widespread species of its genus in Europe (Casagrande and Kenis, 2010), and its spread from Asia may have been assisted by moving ornamental lilies into Europe about 400 years ago (Orlova-Bienkowskaja, 2013). Based on this information, it would be reasonable to expect to find natural enemies of the lily leaf beetle in China. However, we are not aware of any such records. In Europe, despite its widespread distribution, the lily leaf beetle is not generally known as a serious pest of native lilies or the commonly grown ornamental varieties (Salisbury, 2003). This led to the expectation that, like the cereal leaf beetle and two asparagus beetles, this pest might have been introduced into North America without the natural enemies that regulated densities in Europe. If so, like those species, lily leaf beetle would be a good candidate for biological control. Livingston (1996) found no native parasitoids and no predation among *L. lilii* populations near Boston, Massachusetts. A literature review revealed very few studies of this pest in Europe. Lataste (1932) mentioned an unidentified gregarious parasitoid of *L. lilii* in France, and Fox-Wilson (1942) referred to a larval parasitoid in England. Given the widespread distribution of *L. lilii* in Europe, the scarcity of studies there on its natural enemies suggests that it is relatively unimportant as a pest.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The first step of the biological control project (foreign exploration) started in 1996/1997 with surveys in France that revealed lily leaf beetle was often present, but it occurred in low numbers in home gardens and small commercial lily fields. We found two parasitoids—*Tetrastichus setifer* (Hymenoptera: Eulophidae) and *Lemophagus errabundus* (Hymenoptera: Ichneumonidae)—attacking lily leaf beetle during this survey (Gold et al., 2001). Based on these discoveries, we enlisted for assistance an international biocontrol institution, CABI, through its station in Delémont Switzerland, to undertake further agent discovery, agent evaluation, and rearing.

Surveys run by CABI researchers found a generally high rate of parasitism in *L. lilii*, ranging from 25 to 78%, with regional and seasonal variation in parasitoid complexes (Haye and Kenis, 2004). CABI entomologists also found five additional parasitoids of *L. lilii*. Two tachinid flies were eliminated from consideration in this program because they were also known from other hosts. Similarly, an egg parasitoid was determined to have inadequate host specificity because it overwinters in an alternate host species (Haye and Kenis, 2004). The remaining four species were deemed worthy of consideration for lily leaf beetle biological control in North America, and in initial studies Haye and Kenis (2004) evaluated their biology and distribution, as briefly summarized below.

(1) *Tetrastichus setifer* was found to be widely distributed in Europe, from the United Kingdom to Bulgaria and from northern Germany through Italy. Adult parasitoids paralyze host larvae for several minutes while they insert their eggs (**Fig. 3**). An average of seven parasitoid larvae develop per host larva, and they overwinter in the host cocoon in the soil. In spring, adults emerge over several weeks and attack all four larval stages of *L. lilii*. There is one parasitoid generation per year.

(2) *Diaparsis jucunda* (Hymenoptera: Ichneumonidae) was the dominant parasitoid found in Switzerland, Austria, and Italy, but it was rare in the coastal regions of Western Europe and northern Germany. Like the other ichneumonid parasitoids under consideration, it overwinters as a young teneral adult inside the host's pupal case. It has one generation per year.

(3) *Lemophagus errabundus* (Hymenoptera: Ichneumonidae) was the dominant parasitoid found in the United Kingdom,

Figure 3. *Tetrastichus setifer* ovipositing in lily leaf beetle (*Lilioceris lilii*) larva. (Andrea Brauner, Agriculture and Agri-Food Canada)

the Netherlands, western France and northern Germany, but it was rare at greater distances from the coast. It is a solitary parasitoid, producing one larva per host with one generation per year.

(4) *Lemophagus pulcher* (Hymenoptera: Ichneumonidae) was found widely across Europe (except in the United Kingdom), but it was the dominant species only in Bulgaria. It has one full generation and a partial second each year. It is commonly attacked by the hyperparasitoid *Mesochorus lilioceriphilus* (Hymenoptera: Ichneumonidae), which also occasionally attacks *L. errabundus*.

These four parasitoids were evaluated in Europe through laboratory host range testing and studies of field parasitism of other species of *Lilioceris* from the same areas (Scarborough, 2002), as well as through investigation of the species' chemical ecology (Schaffner and Kenis, 1999; Schaffner and Müller, 2001; Schaffner, 2002). Afterwards, further host range testing was continued in quarantine in the United States by Gold (2003) who evaluated these parasitoids against three species of Criocerinae found in North America (*O. melanopus*, *C. asparagi*, and *Lema trilineata*), as well as non-Criocerinae species of chrysomelids (e.g., *Plagiodera versicolora, Leptinotarsa decemilineata, Galerucella calmariensis,* and *G. pusilla*). One coccinellid, the Mexican bean beetle, *Epilachna varivestis*, was also tested.

These three research approaches (field studies of parasitism of related host species, chemical ecology, and laboratory screening) produced generally similar results that together gave a clear picture of these parasitoids' host specificity (Casagrande and Kenis, 2010). *Diaparsis jucunda* is the most specialized of the group, showing a clear preference for *L. lilii* over *L. merdigera* and *L. tibialis* in the field. It is attracted to lily foliage that has been damaged by *L. lilii* and is stimulated to oviposit by fecal shields from *L. lilii* larvae. Furthermore, in quarantine trials, it never attacked any other species, while in the same tests it consistently parasitized *L. lilii*.

At the other extreme, *L. pulcher* was the least specialized of the group. In natural field populations, it was more commonly found parasitizing *L. tibialis* and *L. merdigera* than nearby *L. lilii*. Chemical screening in the laboratory showed it to be attracted to *L. lilii* larvae, their fecal material, and lily leaves damaged by these larvae, but it also showed attraction and oviposition responses to extracts from the North American species *L. trilineata*. Finally, in quarantine, *L. pulcher* attacked *L. trilineata* as readily as *L. lilii*, and it also attacked *C. asparagi*. This species is clearly the least specific of the four species investigated, and based on these tests, it is not presently being considered for release in North America.

The other two parasitoids showed intermediate results in these evaluations. Neither species was very common in sympatric populations of congeneric species, but *T. setifer* was more common in *L. tibialis* than in *L. lilii*. Neither species responded to an olfactory bioassay, but *T. setifer* was more attracted to fecal material from *L. lilii* than from *L. merdigera*. Quarantine studies showed *L. errabundus* to attack nothing but *L. lilii*, and *T. setifer* attacked only a single *L. trilineata* (of 150 exposed). Thus, *L. errabundus* and *T. setifer* have host preferences within the genus *Lilioceris*, but they are unlikely to attack insects outside of that genus. Therefore, they were determined safe to release in North America (Casagrande and Kenis, 2010) because there were no other *Lilioceris* species in North America at that time. This changed when *Lilioceris cheni* was established in Florida in 2012 as a biological control agent of air potato, *Dioscorea bulbifera* (Overholt et al., 2016). Fortunately, collaboration between the lily leaf beetle and air potato biocontrol projects (Lake et al., 2020) demonstrated little potential for parasitoids of *L. lilii* to interfere with air potato biological control.

Following USDA and state approvals based on data described above, *T. setifer* release was approved and began in 1999. Initial releases were made for three years in Wellesley and Waltham in Massachusetts, and Cumberland in Rhode Island into relatively large plots (6 x 6 m [~20 x 20 ft] with approximately 800 lilies) to allow destructive sampling. Within-season sampling showed parasitism of *L. lilii* larvae as high as 63% within these plots. However, there was no overwintering survival in any plot for two seasons until a 5 cm (2 in) layer of cedar bark mulch was removed from two plots. Following mulch removal and continued parasitoid releases, parasitoids became permanently established in the Wellesley and Cumberland plots, but never established at Waltham, which remained mulched. We have not mulched lily plots used for releases since then. No additional parasitoids were released in the Wellesley plot where larval parasitism was found to increase annually. *Lilioceris lilii* populations decreased to near zero in this plot by 2008, while remaining relatively constant in a control plot 3 km (1.9 mi) away (Gold, 2003; Tewksbury et al., 2017). Based upon this success and similar results in Cumberland, additional releases of *T. setifer* were made at 27 sites (mostly home gardens) through 2016, including three in Rhode Island, one in New Hampshire, four in Maine, two in Massachusetts, 16 in Connecticut, and one in Ontario, Canada (where 88 *T. setifer* adults were released into a 2 x 2 m (6.5 x 6.5 ft) plot in Ottawa in 2010 [Tewksbury, 2014]). By 2016, *T. setifer* was found to be established in every state where it was released and to be spreading roughly 1.5 km (0.9 mi) per year, with associated reductions in both *L. lilii* populations and damage to lilies (Tewksbury et al., 2017). Similar results, but with somewhat slower dispersal, were observed in Ottawa, Ontario, where *T. setifer* had dispersed 3.5 km (2.2 mi) in six years following the initial release and was parasitizing up to 100% of beetle larvae at sites where it was present (Blackman, 2017). After 2016, *T. setifer* was released at eight sites in New York (2017–2018) and one site in Vermont. In northwestern Washington State, *T. setifer* was released at four sites (2018–2019). In addition, in Canada, *T. setifer* was released at 25 sites, in Ontario, Quebec, Alberta, and Manitoba.

After obtaining USDA release permits and state approvals, *L. errabundus* and *D. jucunda* releases were begun in 2003. *Lemophagus errabundus* releases were concentrated in coastal areas, with initial release sites in Middleboro and Plainville, Massachusetts, and Kingston, Rhode Island. By 2005, it was confirmed to have established in a garden 1.2 km (0.75 mi) from the release site in Plainville, and subsequently at six sites in southern Rhode Island. As with *T. setifer*, increasing parasitism by *L. errabundus* was associated with reduction in *L. lilii* populations, and the parasitoid was found to have spread approximately 1.5 km (0.9 mi)/year (Tewksbury et al., 2017). There were two releases of *L. errabundus* in New York from 2017 through 2018.

Starting in 2003, *D. jucunda* was released in Cumberland and Kingston, Rhode Island, two sites in New Hampshire, and one in Maine. It was first recovered in a garden 5 km (3.1 mi) from the Cumberland release site four years after release. In Orono, Maine, it was confirmed to have established one year after its release. This parasitoid has spread 4–5 km (2.5–3.1 mi)/year away from release sites (Tewksbury et al., 2017). There were two releases of *D. jucunda* in New York from 2017 through 2018 and four in Washington State in 2018/2019.

HOW WELL DID BIOLOGICAL CONTROL WORK?

In the spring of 2021, we used an online survey to determine the status of the lily leaf beetle and its parasitoids in North America. We asked participants what year they had first noticed the lily leaf beetle in their gardens, what year they had experienced the worst damage caused by the beetle, and how they would describe the observed trend in damage levels (increasing, decreasing, remaining at a high level, or remaining at a low level) over the past year and over the past five years. We also asked them to tell us what methods they had used to control the beetle. A total of 649 individuals responded to the survey. Most people (55%) who responded to the question about control methods replied that they hand-picked lily leaf beetles from their plants. Nearly 10% reported giving up growing lilies and removing the defoliated plants from their gardens.

Respondents from Massachusetts and Rhode Island, where the first U.S. releases took place, commonly reported that lily beetle damage in their gardens had declined over the last five years (green dots in **Fig. 4**) or had remained at low levels (yellow in **Fig. 4**). A few people from previously infested parts of Massachusetts reported having never seen a lily leaf beetle in recent years. Gardeners from the adjacent states of Connecticut and New York were more likely to report increasing damage from lily leaf beetle (**Fig. 4**). Parasitoids were first released in Connecticut in 2016 and New York in 2017. As parasitoids only move 2–4 miles per year, many years are required for parasitoids to spread long distances from release sites.

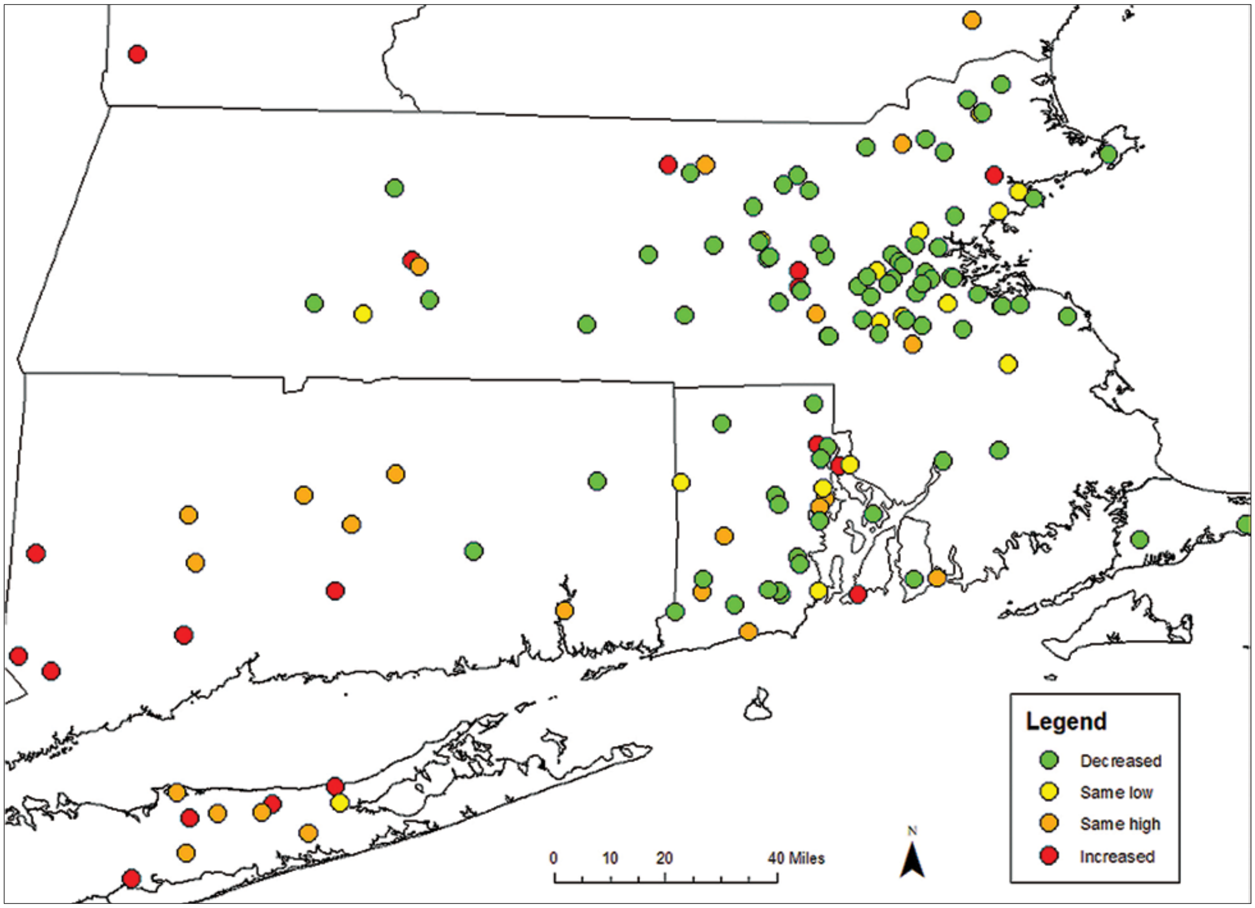

Figure 4. Survey results for southern New England, showing reduced lily leaf beetle (*Lilioceris lilii*) numbers in Massachusetts and Rhode Island, locations where parasitoids were first released. (Map: Alana Russell, University of Rhode Island)

Similarly, respondents from Ottawa and Montreal, where releases were made in 2010 and 2013, respectively, commonly reported declining or low levels of damage (**Fig. 5**). No lily leaf beetles or damaged plants were observed at the Ottawa release sites and several adjacent sites in July 2021. Beetle populations were generally found to decline wherever parasitoids had been released, including release sites in western Canadian provinces where growers reported declining beetle populations around release sites near Winnipeg, Manitoba and from Calgary to Edmonton, Alberta.

In addition to carrying out the online survey, for further evaluation we collected larvae from nine sites in Massachusetts or Rhode Island and dissected them for parasitism. Seven of those sites (77.8%) had larvae that were parasitized by either *L. errabundus*, *D. jucunda*, or both (**Table 1**). Parasitism, when present, ranged from 28–100%. None of the sites we sampled had serious leaf damage, and in general, effort was required to find larvae, except for those Massachusetts sites that were far from parasitoid releases.

Given results of earlier sampling efforts (Tewksbury et al., 2017), we were not surprised to find low populations of *L. lilii*. However, we did expect to find *T. setifer*, which was known to be widely established throughout the sampled area. The absence of this parasitoid from our results may indicate that we sampled too early for *T. setifer*. Haye and Kenis (2004) found that the ichneumonds (*D. jucunda* and *L. errabundus*) emerged before *T. setifer* in Europe, and Gold (2003) found that established populations of *T. setifer* were most abundant around mid-June near Boston, Massachusetts. Most of our samples in 2021 (**Table 1**) were taken before mid-June. Parasitoid dispersal rates may also have affected our results. Tewksbury et

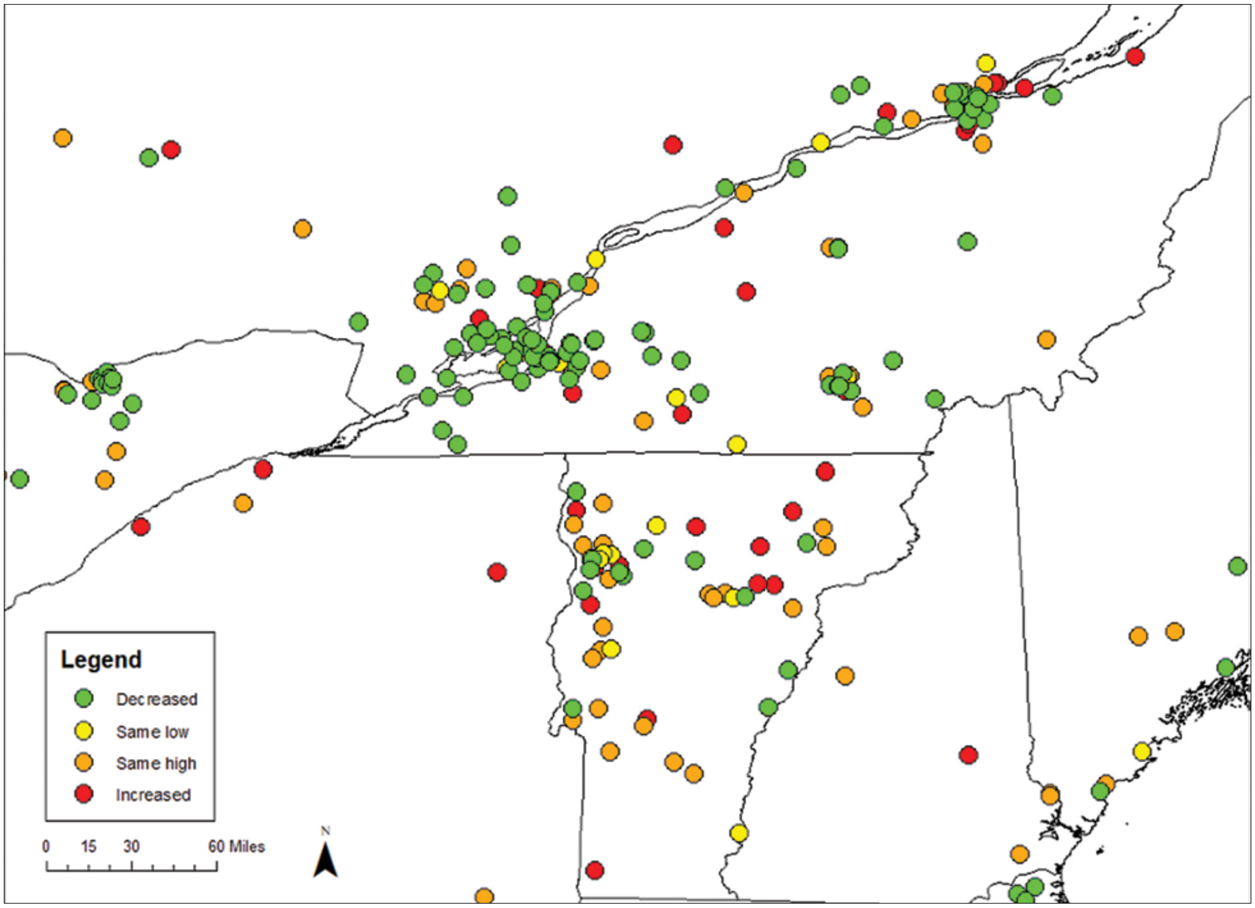

Figure 5. Clusters of decreasing lily leaf beetle (*Lilioceris lilii*) populations near Ottawa, Montreal, Quebec City, and Burlington, Vermont, all sites where parasitoids were released. (Map: Alana Russell, University of Rhode Island)

Table 1. Levels of parasitism in lily leaf beetle (*Lilioceris lilii*) larvae at sites sampled for introduced parasitoids in spring of 2021. Abbreviations: TS – *Tetrastichus setifer*, DJ – *Diaparsis jucunda*, LE – *Lemophagus errabundus*, RI – Rhode Island, USA, MA – Massachusetts, USA.

Site	Date	# Larvae Dissected	# Parasitized by TS	# Parasitized by DJ	# Parasitized by LE	% Parasitism
URI Kingston, RI	June 1–12	20	0	6	3	45
Wakefield, RI	June 10	19	0	0	15	79
Charlestown, RI	June 11	3	0	1	0	33
Charlestown, RI	June 12–30	14	0	0	0	0
Richmond, RI	June 23	16	0	0	0	0
Cumberland, RI	June 2	2	0	2	0	100
Cumberland, RI	June 2	18	0	0	5	28
Lancaster, MA	June 5	112	0	58	0	52
Holliston, MA	June 5	23	0	20	0	87

al. (2017) found *D. jucunda* to disperse more rapidly than the other parasitoids, and it may have been the first parasitoid species to reach the sites in Holliston and Lancaster, Massachusetts. It is also possible that *D. jucunda* and *L. errabundus* have displaced *T. setifer*. Haye and Kenis (2004) found that although *T. setifer* is widespread in Europe, it only dominates in northern Germany and Sweden. Scarborough (2002) found that *T. setifer* was best suited to parasitize small larvae—a disadvantage for a parasitoid emerging relatively late in the season. Further, Gold (2003) found that when *T. setifer* oviposited into a larva previously attacked by either of the ichneumonids *D. jucunda* or *L. errabundus*, the ichneumon prevailed. Although our limited sampling season may have caused us to miss *T. setifer*, it does show that both *D. jucunda* and *L. errabundus* are widely distributed and likely contributing significantly to the regional decline of *L. lilii* populations and damage.

Declines of lily leaf beetle in Ottawa, where we had previously collected *T. setifer* (Blackman, 2017), were also likely due to the activity of *T. setifer*. We do not have dissection results to document establishment of *T. setifer* at the other 25 sites where *T. setifer* was released in Ontario, Quebec, Alberta, and Manitoba, but Tewksbury et al. (2017) showed that *T. setifer* was established at most sites where it had been released. *Lilioceris lilii* populations have declined near Canadian sites where this parasitoid was released, while beetle populations have increased elsewhere in the United States and Canada.

BENEFITS OF BIOLOGICAL CONTROL OF LILY LEAF BEETLE

Control of the lily leaf beetle has been of obvious benefit to gardeners. Several survey respondents from Massachusetts, Rhode Island, Ottawa, and Montreal included a note of gratitude that they can once again grow lilies without the constant vigilance that was previously necessary to hand remove beetle life stages before defoliation became severe. In recent years, as the lily leaf beetle has become rare in much of southern New England, lilies are once again becoming common in residential landscapes. In recognition of the success of this biological control program, the North American Lily Society in June 2021 presented R. Casagrande with their E.H. Wilson award that is "given to an individual who has made an outstanding contribution to the genus *Lilium*."

Although releases have been made in populations of native lilies, including *L. canadense* in Granby, Quebec, and *L. michiganense* in Burlington, Ontario, follow-up sampling has not been done to determine if the parasitoids established and prevented damage. The protection of populations of native lilies, especially species of conservation concern, is arguably the most important benefit stemming from our biocontrol efforts. Overall, the biological control program for the lily leaf beetle has already been quite successful in the parts of eastern North America where the beetle and its parasitoids have been established longest. The established parasitoid species will continue to spread on their own, filling in many of the gaps between release sites. Additional releases will be needed in the western states and provinces as it would be difficult (or take decades) for the parasitoids to spread naturally against the prevailing winds. To date, *T. setifer* looks very successful in Canada, and *D. jucunda* and *L. errabundus* may be considered for future releases.

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GROUP 3
PROTECTING SHADE TREES, NATIVE TREES, AND FORESTS

Healthy green ash (*Fraxinus pennsylvanica*) tree, a species native to North America that is attacked by the introduced emerald ash borer, *Agrilus planipennis.* Four parasitoid species have been successfully introduced to North America for the control of emerald ash borer. (Alex Zorach, iNaturalist.org CC BY-NC 4.0)

Chapter 16

Successful Suppression of the Birch Leafminer, *Fenusa pumila,* in the Northeastern United States

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NON-TECHNICAL SUMMARY

Historically, the uncontrolled international movement of plants allowed pests to enter the United States at a rapid rate. U.S. plant importations were first regulated by the 1912 Plant Quarantine Act, and since then, restrictions and inspections have slowed the accidental introduction of plant-feeding pests, even though some new introductions still happen. Among the pests that invaded the United States in the early 1900s was the birch leafminer, *Fenusa pumila* (Hymenoptera: Tenthredinidae). Established in Connecticut sometime before 1924, this pest spread from New England to Alaska, causing annual defoliation of white and gray birches in urban and forest landscapes for over 60 years before being brought under biological control in the 1980s.

In the 1970s, the birch leafminer was identified as a good candidate for classical biological control (as defined and described in Chapter 1), and specialized natural enemies that hold this pest in check in Europe were introduced into the United States by USDA scientists. Of these imported beneficial insects, *Lathrolestes nigricollis* (Hymenoptera: Ichneumonidae) proved most successful. It readily established and spread naturally through forests and urban landscapes, eventually lowering birch leafminer numbers across the region. By 2000, birch leafminer was reduced to a non-pest in at least the northeastern United States, eastern Canada, and Alberta. No harmful effects occurred from this introduction, and its benefits were more attractive birch trees in the landscape, reduced need for pesticides around homes, and healthier birches in the forest.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Fenusa pumila (Hymenoptera: Tenthredinidae) (**Fig. 1**), formerly called *Fenusa pusilla*, is distributed from Ireland through Siberia, China, and Japan (Digweed et al., 2009). It was first found in North America in

Van Driesche, R. G. and R. A. Casagrande. 2022. Successful suppression of the birch leafminer, *Fenusa pumila*, in the northeastern United States, pp. 173–178. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

Connecticut in the early 1920s, and by 1931 it was present throughout New England, as well as New York, New Jersey, and the Canadian province of New Brunswick (Friend, 1931). It now occurs from Newfoundland south to Maryland, and west to Alberta, the Great Lake States, and Iowa, with isolated populations in Oregon, Washington, British Columbia, and Alaska (Drooz, 1985; Snyder et al., 2007; Digweed et al., 2009).

In the northeastern United States, it most commonly attacks gray birch (*Betula populifolia*) and paper birch (*Betula papyrifera*). In Europe, the native silver birch (*Betula pendula*) is a key host. *Betula pendula* is also a host in North America where it has been introduced as an ornamental (Fuester et al., 1984), and this may have been how the leafminer reached North America.

Figure 1. Adult birch leafminer (*Fenusa pumila*). (Andrey Ponomarev, iNaturalist.org CC BY-NC 4.0)

NATURE OF THE PROBLEM

While the extensive damage caused by birch leafminer (**Fig. 2a**) has long been recognized, there are no specific studies of the consequences of its feeding on the health of birch trees. Birch leafminer does not kill trees, but its leaf-mining (akin to defoliation) destroys much of the photosynthetic tissue of the plant (**Fig. 2b**), especially on the new shoots which are the preferred oviposition site. Damage occurs early in the season (mid-May to mid-June in Massachusetts). In the northeastern United States, moderate to heavy mining of leaves of gray and white birch (**Fig. 2c**) occurred in many areas, year after year from the 1930s through at least the 1980s (Fuester et al., 1984). While not studied specifically for this leafminer, insect defoliation or leaf-mining can reduce tree growth, accelerate die-back, and reduce reproductive output (Rose, 1958; Kulman, 1971; Long, 1988; Muzika & Liebhold, 2001; Thalmann et al., 2003).

WHY CONTROL THIS INVASIVE SPECIES?

Compared to gypsy moth, *Lymantria dispar*, which can defoliate whole forests over hundreds of thousands of acres, birch leafminer is a relatively unimportant forest pest. Its damage, usually most intense along forest edges, is too limited to merit control in commercially managed forests. The leafminer's damage typically produces a general browning of birches in June (in Massachusetts) when the first generation of mining larvae mature and consume the foliage. The poor appearance of heavily mined trees along city streets, in private yards, and in commercial nurseries led to regular use of many pesticides for control of birch leafminer, including foliar applications (Schread, 1954; Nielsen and King, 1992), soil applications (Cheng and LeRoux, 1968, Scheer and Johnson, 1970), and tree injection (Marion et al., 1990). Biological control of this species was intended to reduce pesticide use and improve the appearance of white birch in the suburban landscape.

In addition to landscape uses, gray and white birch are important pioneer species that are among the first trees to establish in abandoned fields or forest openings. Also, in Pennsylvania and other coal-mining areas, gray birch often revegetates strip-mined areas, and it can be planted to speed up recovery of such sites (Anon., 1975; Davidson, 1977, 1979).

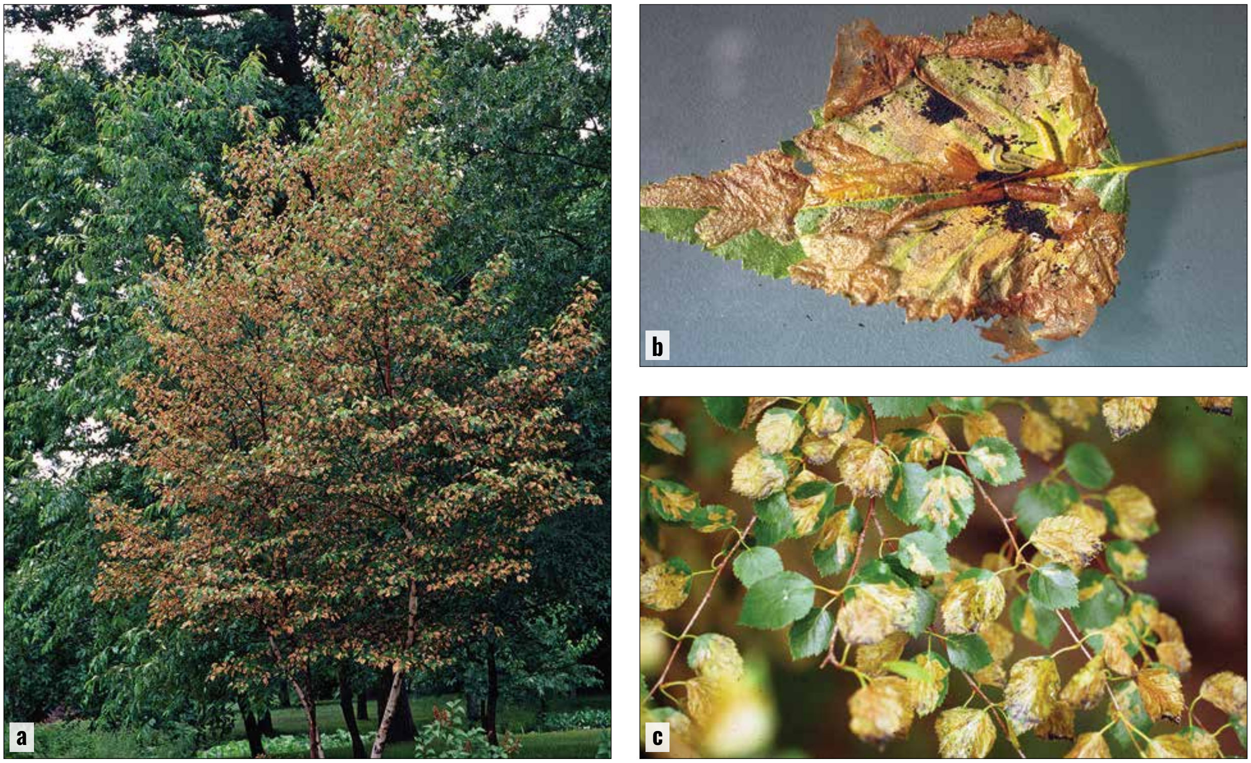

Figure 2. (a) Birch leafminer (*Fenusa pumila*) damage at the whole tree level; (b) heavily mined birch leaf with mature larvae exiting the leaf; (c) moderate to heavy damage to individual birch leaves. (a: Steven Katovich; b: E. Bradford Walker, Vermont Department of Forests, Parks and Recreation; c: Whitney Cranshaw, Colorado State University; a–c: Bugwood.org CC BY-NC 3.0 US)

THE ECOLOGY OF THE PROBLEM

Immature leafminers are found within leaves, and small larvae or lightly infested plants can escape detection by horticultural inspectors. The pupae are in the soil, which allows for easy movement of the pest if trees are shipped in other than a bare root condition. Movement of nursery stock was likely the route of invasion for *F. pumila* into North America, and indeed, all but one of the *Fenusa* species found in North America are invasive European species (Eichhorn and Pschorn-Walcher, 1973).

Populations of many leafminers are strongly suppressed by specialized natural enemies called parasitoids (e.g., Andersen et al., 2021). In small samples, such as plants with only a few leafminers, parasitoids may be present or absent, by chance. When such plants are moved overseas, leafminers, unrestrained by specialized parasitoids, may reach outbreak populations unless controlled by local natural enemies or the co-invasion of their own specialist enemies (Kirichenko et al., 2019). When the birch leafminer was accidentally introduced into North America, its native (European) parasitoids were left behind. While later surveys in North America found 15 species of local generalist parasitoids attacking *F. pumila* in Quebec, they collectively caused <10% mortality (Cheng and LeRoux, 1969). In Europe, 17 parasitoid species are found attacking *F. pumila*, but larval parasitism was much higher (38–47%). The most effective species causing most of the mortality were not present in North America (Eichhorn and Pschorn-Walcher, 1973; Digweed et al., 2009). In Europe, these more effective species not only caused high mortality, they also attacked few if any other leafminer species (Eichhorn and Pschorn-Walcher, 1973). Such specialized species are unlikely to harm populations of native leafminers in North America, where there is only one native *Fenusa* species, which attacks a rose (Smith and Eiseman, 2017).

The key steps needed to suppress *F. pumila* to much lower levels were to select the most promising parasitoids (based on their abundance and wide distribution in Europe), and then release them to determine if one or more were able to thrive under North American climatic conditions. Climates in North America differ from those of Europe in various ways because the Gulf Stream warms western Europe to much higher latitudes than in North America. This changes the relationship between photoperiod and temperature in ways that can be damaging to parasitoids adapted to European conditions.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Figure 3. *Lathrolestes nigricollis* on a mined leaf. (R.A. Casagrande, University of Rhode Island)

Efforts to suppress the birch leafminer in eastern North America started in 1968, when the Canadian Forest Service requested the Commonwealth Institute of Biological Control (an international organization dedicated to biological control) to begin surveys in Europe for parasitoids of *F. pumila* (Eichhorn and Pschorn-Walcher, 1973). Those surveys took place in Switzerland, Germany, Austria, France, and Denmark. While many species of parasitoids were found attacking *F. pumila* in its native range, two wasps, *Lathrolestes nigricollis* (**Fig. 3**) and *Grypocentrus albipes* (both Hymenoptera: Ichneumonidae), were selected for introduction to North America because they caused high rates of mortality and were widely distributed in Europe (Eichhorn and Pschorn-Walcher, 1973). Host-ranges of these parasitoids were estimated from observations made in field surveys of various species of leafminers on birch or alder in Europe. Those field observations only recorded *G. albipes* from *F. pumila*, while *L. nigricollis* seemed also to only attack *F. pumila* or perhaps some other species of *Fenusa*.

Releases of *L. nigricollis* and *G. albipes* in Canada were made in Newfoundland and Labrador (1973), then Quebec (1974–1978), and Alberta (1994–1996) (Digweed et al., 2009). Releases in the Mid-Atlantic U.S. states (especially Pennsylvania) were made in 1976–1982 (Fuester et al., 1984). *Lathrolestes nigricollis* was moved from Pennsylvania to Massachusetts and Rhode Island in 1979 (as adults) and then again (as parasitized larvae) in 1989–1990 (Van Driesche et al., 1997).

In Canada, *G. albipes* became established near Quebec City (Quednau, 1984) and in Alberta (Langor et al., 2000, 2002), but in general did not quickly increase in abundance (Quednau, 1984). Similarly, in the United States, *G. albipes* was rarely recovered (Fuester et al., 1984). In contrast, *L. nigricollis* established quickly at most locations and spread rapidly (Van Driesche et al., 1997; Digweed et al., 2009).

HOW WELL DID IT WORK

Following the release and rapid spread of *L. nigricollis* in New England and parts of Canada, the level of damage from *F. pumila* (as % leaves mined) declined dramatically. In the United States, intensive follow-up studies were done in Massachusetts (Van Driesche et al., 1997) and Rhode Island (Casagrande et al., 2009). At the release site in Amherst, Massachusetts, the percentage of leaves mined by *F. pumila* declined from 50–54% in 1979 (the year of the first release of *L. nigricollis*) to 1–3% in 1990–1995. Concurrently, parasitism rates at the release site increased from undetectable in 1979–1980 to 30–80% in 1990–1995 (Van Driesche et al., 1997). In Massachusetts in this same study, it was observed that by 1995, parasitism within

4 km (2.5 mi) of the original release site was 28% compared to only 14% at sites 15–20 km (9–12 mi) away, and this difference in parasitism rate was associated with an increase in damage from 6% near the release site to 33% at sites 15–20 km away. Within 4 km of the original release site, leaf-mining had been reduced to inconsequential levels by 1995 (Van Driesche et al., 1997). Similar events were also observed at a release site in Rhode Island, where the percentage of leaves mined fell from 87% in the 1990s to about 3% in 2004–2008, as parasitism by *L. nigricollis* rose from 6% in the 1990s to 38% in 2004 (Casagrande et al., 2009).

To learn if control had also occurred at a much larger scale over the northeastern United States, a survey was conducted in 2007 at 183 sites spread out over seven states (Massachusetts, Connecticut, Rhode Island, New York, Pennsylvania, New Jersey, Delaware). This survey found that birch leaf miner levels (as % of leaves mined in spring) had declined to barely detectable levels in five states (Massachusetts, Connecticut, Rhode Island, New York, Pennsylvania) but was still at damaging levels in southern New Jersey (ca 50% leaves mined) despite high parasitism levels; this area was near the southern limit of white and gray birch (Casagrande et al., 2009).

These findings were further corroborated by work in Canada. In Alberta, the same two parasitoids (*L. nigricollis* and *G. albipes*) were released against *F. pumila* in 1994–1996 in Edmonton. Five years after the parasitoids' introduction, at a location where establishment of *L. nigricollis* had occurred, 78% of the larvae of the spring generation were parasitized (Langor et al., 2000). Later, surveys across Canada found that *F. pumila* was rare or absent at most locations visited (Digweed et al., 2009).

BENEFITS OF BIOLOGICAL CONTROL OF BIRCH LEAFMINER

Defoliation of gray and white birch in the northeastern United States is now uncommon, instead of being the norm. The benefits of this change include a general lack of any need for pesticide treatment of yard or street white birch for leafminer control. Also, white birch around homes, along streets, or trees used for landscaping at commercial sites now have increased aesthetic value because they no longer turn brown each spring. Forest stands of white and gray birch may have improved growth rates compared to years with defoliation because the stress of defoliation, which forced trees to grow a new set of leaves just a month after production of spring leaves, has been removed. This should lead to healthier trees being able to grow quicker and better perform their normal ecological functions in regional forests.

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CHAPTER 17

Biological Control of Ash Whitefly in California

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NON-TECHNICAL SUMMARY

Ash whitefly, *Siphoninus phillyreae* (Hemiptera: Aleyrodidae), once a highly visible pest on ash and ornamental pear street trees in California, is now difficult to find. First discovered in California in 1988, this whitefly pest spread rapidly throughout southern California, building to high numbers in residential neighborhoods and filling the air with adult whiteflies during the warm afternoons of 1988, 1989, and 1990. The air was so thick with this insect that people were advised to stay indoors during summer afternoons because some were experiencing respiratory problems. Many people wore surgical masks when outside. Stories on the clouds of whiteflies filled the evening news and local newspapers. Knowledge that whiteflies are good targets for classical biological control set the stage for a major biological control project directed at ash whitefly. Within one year of this whitefly's discovery in California, *Encarsia inaron* (a stingless wasp, Hymenoptera: Aphelinidae) and *Clitostethus arcuatus* (a lady beetle, Coleoptera: Coccinellidae) were imported from Europe and Israel into the University of California, Riverside Quarantine facility. Ash whitefly occurs naturally across Europe into the Middle East and beyond to India, where it is uncommon and rarely a pest. The first releases of *E. inaron* and *C. arcuatus* were in late 1989. Upon close examination of the more important natural enemy, *E. inaron*, we learned this wasp could cause high levels of mortality to the ash whitefly population. *Encarsia inaron* was mass reared and released throughout California from 1989 to 1992. Soon whiteflies were difficult to find on the same trees that had been dripping with honeydew (whitefly excrement) one to two years earlier. The benefit to cost ratio for wholesale value of street trees was $270 gained for each dollar spent on the project. Total savings for the state in 1990, based on the same metrics, was assessed at $324,171,888.

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HISTORY OF INVASION, NATURE OF PROBLEM

The Species Invasion

Figure 1. Adult and third instar of ash whitefly, surrounded by droplets of honeydew. (C. Pickett, CDFA)

Ash whitefly, *Siphoninus phyillyreae* (Hemiptera: Aleyrodidae), also known as the pomegranate whitefly (**Fig. 1**), has a natural distribution from India, west to the Middle East and on to North Africa and from Turkey across Europe to Ireland (Sorensen et al., 1990). In its native range, ash whitefly is rarely reported as a pest, despite its feeding on common species such as ash (*Fraxinus* spp.), apple (*Malus domestica*), pear (*Pyrus* spp.), pomegranate (*Punica granatum*), peach (*Prunus persica*), and olive (*Olea europaea*) (Mound and Halsey, 1978). Outbreaks of ash whitefly had been reported before it arrived in the United States, but they were considered the result of disruption of control by natural enemies due to pesticide use (Tremblay, 1969, 1973) or its invasion of new areas lacking its natural enemies (Costacos, 1963; Kolev, 1973). Four years after *S. phillyreae* was found in Greece, three predators and three parasitoids were discovered causing high levels of mortality (Mentzelos, 1967), and this species was never again reported as a pest in this country. Its reputation as an urban and agricultural pest began with its arrival in California, first being detected in Los Angeles County in August of 1988 (Mound and Halsey, 1978; Gould et al., 1992a). It has since invaded Arizona, Nevada, New Mexico (Gerling et al., 2004), North Carolina (Perlman et al., 2006), and Oregon (Oregon Department of Agriculture, 2015). Internationally it has invaded South Africa, New Zealand, Australia, Japan, and Mexico (Giliomee and Millar, 2010).

Nature of the Problem

Ash whitefly feeds on the leaves of a wide range of trees, of which many are commonly planted on city streets of California, such as the ash trees *Fraxinus uhdei* and *Fraxinus velutina*, and the ornamental pear trees *Pyrus calleryana* and *Pyrus kawakamii*. Other, less preferred hosts include hawthorn (*Rhaphiolepis indica*), firethorn (*Pyracantha* sp.), and tulip tree (*Liriodendron tulipifera*) (Sorensen et al., 1990; Pickett et al., 1996). During its outbreak, one street inventory in California reported 17% of the urban landscape affected by ash whitefly (Pickett et al., 1996). Agricultural crops known to be attacked by ash whitefly in California include pomegranate, apple, pear, Asian pear (*Pyrus pyrifolia*), and citrus, and in Spain, olives (Pedemonte et al., 2013). The only known economic crop loss in California was reported on pomegranate and Asian pears (Pickett et. al., 1996; Pickett and Pitcairn, 1999). During the first two to three years after the whitefly's initial discovery, populations of ash whitefly exploded throughout southern California. Like other whiteflies, ash whitefly has a high reproductive capacity if left unchecked (Gerling, 1990; Leddy et al., 1995). Unique to this insect invasion was its impact on the general population of people in southern California. Ash whitefly quickly spread and built up to high populations in various urban neighborhoods. It also spread throughout the state's urban centers, north to Oregon. The outbreaks were repeatedly reported by the media, which focused on damage to street trees and respiratory problems in humans. People were advised to stay indoors during the afternoon, and outdoor barbecuing was discouraged. In addition to the whiteflies themselves, there were reports of other particulates in the air, which turned out to be microdroplets of honeydew in the air (Davis et al., 1993) (**Fig. 2a**). Honeydew, the sticky excrement of the whiteflies, covered the leaf surfaces and promoted the growth of black sooty mold fungus which reduced photosynthesis of the plants (**Fig. 2b**). The honeydew and sooty mold also fell on sidewalks near the infested trees (**Fig. 2c**), where it became slick when wet causing hazardous walking conditions.

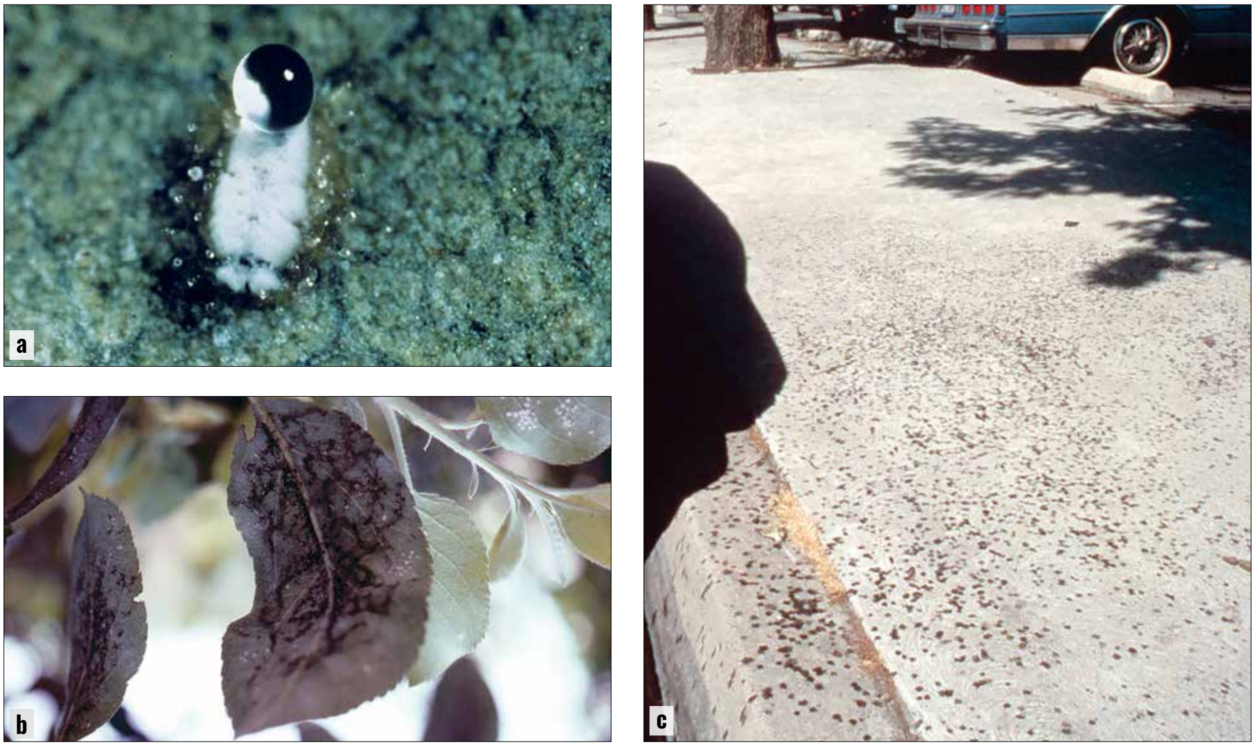

Figure 2. (a) Late-instar ash whitefly, *Siphoninus phillyreae*, with droplet of honeydew at the rear of the body; (b) apple tree leaves covered in black sooty mold from ash whitefly; (c) droplets of honeydew on sidewalks below ash whitefly-infested trees. (a: C. Pickett, CDFA; b,c: J. Gould USDA-APHIS-PPQ)

WHY CONTROL THIS INVASIVE SPECIES?

High population densities of ash whitefly needed to be reduced to non-damaging levels first to protect the health of city street trees. Without sources of mortality, whitefly populations would have greatly increased in size (Gould et al., 1992a). Southern California has a year-round mild to hot climate perfect for insect population growth. The warmer areas of this region benefit from street trees, which provide a valuable cooling service from their shade. They also have an aesthetic value enhancing the property values of buildings with street or yard trees. Furthermore, trees are today recognized as another way to capture carbon dioxide, critical to mitigating global warming effects. Secondly, ash whitefly was a potential threat to several California crops, and damage would likely have occurred to pomegranate, pear, or even citrus if the insect had been left unchecked. Finally, human health was being affected by the high numbers of the whiteflies and their honeydew because of inhalation of insect parts or waste products and the unsafe sidwalks caused by honeydew deposits.

THE ECOLOGY OF THE PROBLEM

Whiteflies as a group (Aleyrodidae) frequently invade new regions and become separated from the specialized parasitoids that regulate their numbers. Other examples of invasive whiteflies in California include Japanese bayberry whitefly, *Parabemisia myricae* (Rose et al., 1981), wooly whitefly, *Aleurothrixus floccosus* (DeBach and Rose, 1976; DeBach and Rosen, 1991), tobacco whitefly, *Bemisia tabaci* (Gould et al., 2008; Pickett et al., 2013), and the giant whitefly, *Aleurodicus dugesii* (Pickett et al., 2011). Whiteflies like citrus blackfly (*Aleurocanthus woglumi*), tobacco whitefly, and greenhouse whitefly (*Trialeurodes vaporarium*) are problematic not only in the United States but also worldwide (Dowell et al., 1981; Summy et al., 1983; Gerling, 1990; Gould et al., 2008).

Ash whitefly, like many others in the family, has traits that are amendable to high levels of parasitism. Their nymphs, the stages that parasitoids attack, are largely sessile and exposed to parasitoid oviposition on the surface of the host plant's leaves. Although the ash whitefly has many host plants (Sorensen et al., 1990; Bellows et al., 1990), its largest populations in California were on its preferred hosts, mainly ash and pear (Pickett et al., 1996). Ash whitefly reproduces at higher rates on those trees compared to other species of ornamentals (Leddy et al., 1993). Ash and pear are commonly planted as street trees, and in urban areas they provide a stable, favorable habitat because they are perennial and rarely treated with insecticides. Although both ornamental pear and ash trees are deciduous, Pickett and Pitcairn (1999) found that ash whitefly can survive over the winter in the duff under these trees.

Quickly following its invasion, ash whitefly reached high densities capable of defoliating street trees in California. In addition, these whitefly numbers posed a health threat to people in cities, and they had the potential to harm several high value crops, especially pomegranate (Pickett and Pitcairn, 1999), apple, Asian pear, and citrus. The total value of these crops in 1994 in California was over $2 billion. Furthermore, initial observations indicated only generalist predators and no specialist parasitoids were attacking the whitefly in California (Bellows et al., 1990; Gould et al., 1992a). Because most ash whiteflies initially occurred on street and yard trees, where conditions were stable and favorable, biological control was highly feasible because the same conditions that favored ash whitefly favored the reproduction and survival of the specialized parasitoid of the pest.

Indeed, at the very onset of the statewide biocontrol program, a promising parasitoid, *Encarsia inaron* (formerly *Encarsia partenopea*; Hymenoptera: Aphelinidae) (**Fig. 3**) had already been discovered in the native range of the ash whitefly. Further research suggested that this parasitoid would likely have a high impact on ash whitefly's population reproduction and density (Gould et al., 1992a,b). Both sexes of *E. inaron* are primary parasitoids of whiteflies and are not autoparasitic (a complicated form of host use that is common for many species of *Encarsia* in which female eggs are laid on whiteflies, but male eggs are laid on immature female parasitoids).

Figure 3. *Encarsia inaron* adult on ash whitefly-infested leaf. (J. K. Clark)

In 1988 when ash whitefly was first reported in California, studies demonstrating a parasitoid's safety to native, non-pest insects were not required for field release. Regulatory rules for importing biocontrol agents were beginning to change by 1990, but safety was still primarily determined by the practitioners or scientists carrying out the project (Van Driesche et al., 2008). At that time, importation of a new biocontrol agent was usually based on the agent's efficacy and specialization, its relatedness to known, effective biocontrol agents as reported in the literature, and climatic matching between its place of origin and the invaded region. Therefore, *E. inaron*'s potential impact on other related whiteflies was of little concern at that time because other whiteflies would be regarded as plant pests. The decision to import *E. inaron* in 1990 was largely made by the entomologists conducting the foreign exploration for the project, T. Bellows of the University of California (UC) Riverside and L. Bezark, California Department of Food and Agriculture

(CDFA). The most critical concern during importation was preventing the establishment of undesirable hyperparasitoids, which are parasitoids that attack other parasitoids.

At the time, there was no agreement among biocontrol practitioners as to whether to import more than one agent and, if several were imported, the order in which they should be released (Huffaker et al., 1974). As mentioned earlier, only one parasitoid was commonly recovered from ash whitefly, and one predator was found eating ash whitefly. Ultimately, it was decided to collect both natural enemies, each from two regions (Italy and Israel). These two regions were chosen to match the range of climatic conditions in whitefly infested areas in California, from mild coastal areas to dry, hot inland regions.

Funding was available because a variety of interests were being damaged, including residences of state legislators in Sacramento (the state's capitol), the agricultural industry (which foresaw increased pesticide costs for whitefly control in some crops), and homeowners (some of whom had to cut down their ash trees because of whitefly damage [C. H. Pickett, pers. obs.]).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Until ash whitefly invaded in California, it was just another whitefly among the 1,156 species and 126 genera occurring throughout the world (Mound and Halsey, 1978). Most whiteflies are not agricultural pests (Byrne et al., 1990), and it is highly likely that natural enemies play a key role in maintaining these whitefly species well below economically damaging levels (Gerling, 1990). When first reported in California in 1988, ash whitefly populations were widespread throughout the southern region of the state, and the whitefly could be found in very high numbers on individual trees, suggesting they had been in California for at least two years (Bellows et al., 1990). Efforts to import natural enemies of ash whitefly began immediately, with plans to collect from Italy and Israel, which were both countries with entomologists specializing in whitefly pest management. At the same time, observations made on infested trees in California suggested that existing local natural enemies had little if any impact on this new whitefly (Bellows et al., 1990; Gould et al., 1992a). By 1989, three promising natural enemies had been sent to the UC Riverside quarantine for biological studies: two populations of *E. inaron* (one from Israel collected by D. Gerling, Tel Aviv University and a second from Italy collected by L. Bezark, CDFA) and the coccinellid beetle *Clitostethus arcuatus* from Israel (Bellows et al., 1992a). In late 1989, *E. inaron* (**Fig. 3**) was cleared for release from the UC Riverside Quarantine facility, and the first releases were made at an undisclosed site in southern California. The parasitoid *E. inaron* was next released in 1990 in Riverside County as part of a field study to measure its impact on ash whitefly (Gould, 1992a,b). Recoveries of *E. inaron* were made in field plots in 1990, and subsequent life table analyses provided strong evidence that this parasitoid alone would significantly reduce ash whitefly populations. Initially, the two populations of *E. inaron* were maintained at UC Riverside, each in separate greenhouses. Differences in recoveries between the two biotypes within one year of release suggested the Israeli biotype was having a greater impact on ash whitefly. Therefore, a decision was made by both CDFA and UC Riverside to focus on releases of the Israeli biotype (L. Bezark, pers., comm.). Mass rearing of this single biotype was conducted by CDFA in Sacramento for releases in central and northern California, while UC Riverside provided parasitoids for release in southern California.

The second natural enemy of ash whitefly that was imported into California was the coccinellid *C. arcuatus* (**Fig. 4a,b**). The first releases of this lady beetle were made spring 1990 in San Diego County (Bellows et al., 1992a). The beetle was reared at the University of California Riverside and later released in central and northern California. Other than one laboratory study on its biology (Bellows et al., 1992b), quantitative studies on its release and impact were not carried out. Eventually it was recovered in field studies in northern California, showing that it did establish (Dreistadt and Flint, 1995; Pickett and Wall, 2003). The value of this predator in suppressing the target pest could not be measured because of the rapid and dramatic suppression of the whitefly by the parasitoid.

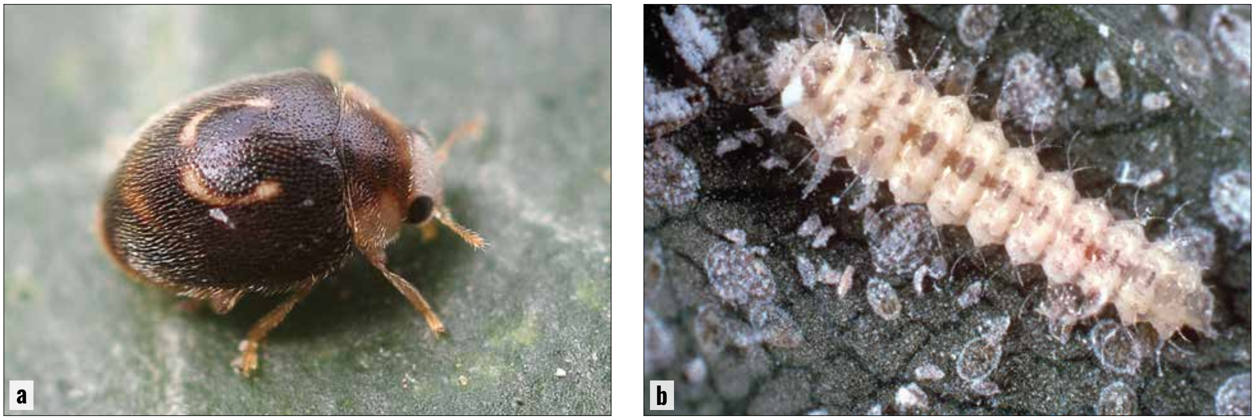

Figure 4. The lady beetle *Clitostethus arcuatus* (a) adult and (b) larva feeding on ash whitefly. (a: Gilles San Martin, Wikimedia CC BY-SA 2.0; b: UC Riverside)

HOW WELL DID IT WORK?

The ash whitefly project has been described as an example of classical biological control at its best, i.e., the reunion of an insect with its co-evolved, specialized natural enemy followed by the permanent suppression of a serious pest (Van Driesche et al., 2008; Heimpel and Mills, 2017). The establishment and impact of *E. inaron* was truly swift and dramatic. In research trials in southern California, parasitoids were released at two locations in May 1990 but not at control sites up to 14 km (8.4 mi) away. Parasitism levels increased almost immediately at the release sites, and whitefly densities decreased throughout the summer, while at the control sites whitefly populations increased. They increased through July after which the parasitoids naturally dispersed to both control plots. By October, whitefly densities were low at all four sites. One year, city streets were filled with clouds of whiteflies, and shade trees were completely coated in honeydew and nymphal whiteflies; the next or following year, they were gone (**Fig. 5a,b**). The public could see the impact of the program in their own backyards, and in this way, the project was similar to weed biocontrol efforts with highly visible changes in populations of a noxious weed, before and after establishment of a biocontrol agent. The impact of the ash whitefly program was statewide (Pickett et al., 1996). Results from life table studies by Gould et al. (1992a) in southern California indicated that *E. inaron* could suppress populations of ash whitefly by causing their average growth rates to drop to near replacement or lower. Their projections were accurate, as field studies verified (Pickett et al., 1996; Pickett

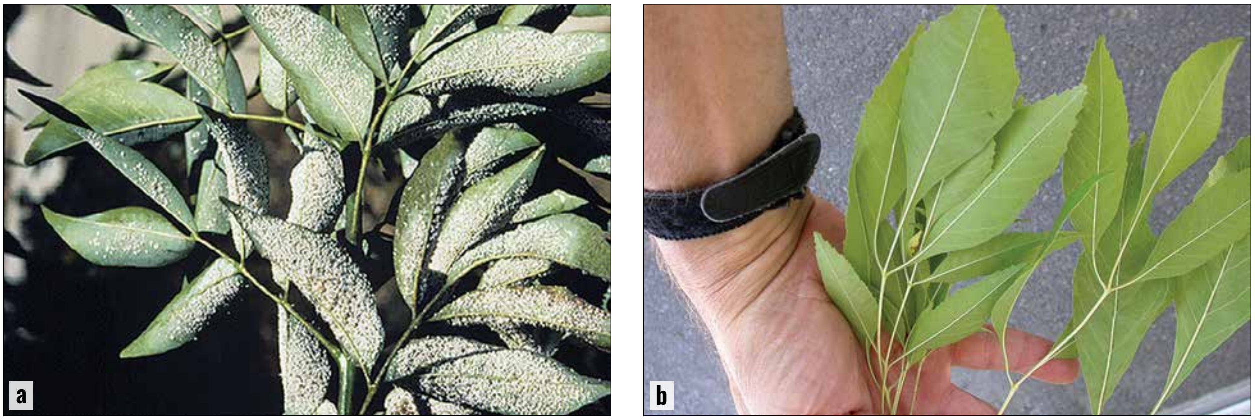

Figure 5. Ash whitefly (*Siphoninus phillyreae*) infestation (a) before and (b) after the introduction of *Encarsia inaron*, on ash. (C. Pickett, CDFA)

and Pitcairn, 1999; Pickett and Wall, 2003). The impact of this parasitoid caused populations of ash whitefly to rapidly decline throughout the entire state, and they have remained low ever since.

To measure the impact of *E. inaron* in northern California (as suggested by Van Driesche et al., 1991), Pickett and Pitcairn (1999) measured the population size of successive generations of ash whitefly. This was done by counting whitefly and parasitoid exit holes in late-instar whitefly nymphs once for each generation of ash whitefly at three locations in Davis, California. The emergence of adult parasitoids or whiteflies each leave unique holes in the cast skins of the late-instar nymphs (**Fig. 6a**). Generation one produced a total of 6,046 ash whitefly exit holes and 237 *E. inaron* (**Table 1**). By generation 6, no ash whitefly adults were produced, just 13 *E. inaron* adults, indicating a 99.8 % decline in the ash whitefly population over this time period (**Table 1, Fig. 6b**). Also, Pickett and Wall (2003) followed ash whitefly populations on 25 trees distributed amongst seven counties in northern California for ten years after *E. inaron* was released there. *Encarsia inaron* responded to changes in the ash whitefly population in a density-dependent manner over the entire period (**Fig. 6c**). Even minor increases in the whitefly population from one year to the next caused a rapid rebound in the level of parasitism, which kept the whitefly populations under control. Ash whitefly never returned to its pre-release average numbers of nine whiteflies (eggs + nymphs) per leaf, and never

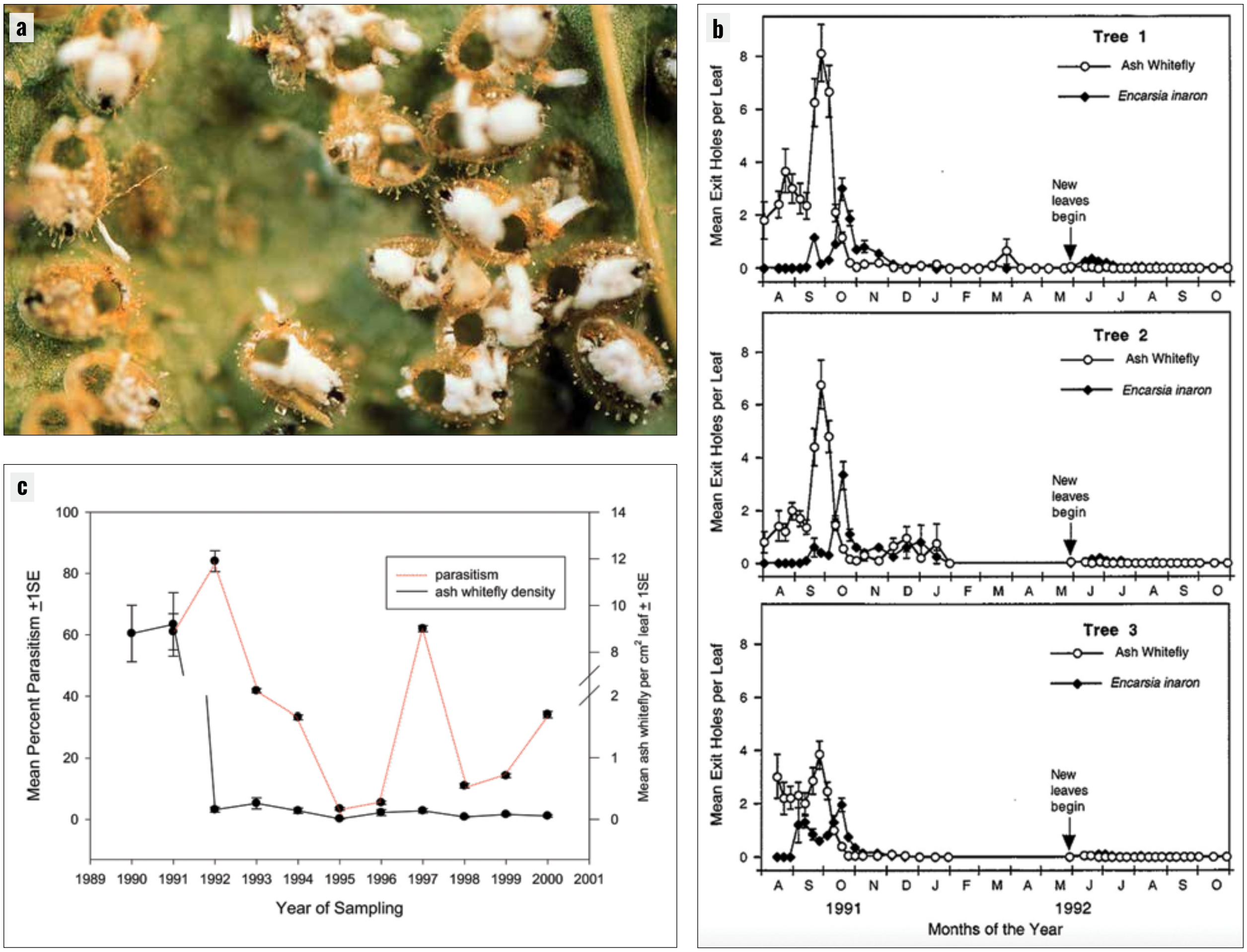

Figure 6. (a) Late-instar cast skins of ash whitefly (AWF, *Siphoninus phillyreae*) nymphs with exit holes caused by *Encarsia inaron*; (b) productivity of AWF and *E. inaron* adults from August 1991 to September 1992 on each of three sampled trees. Shown are mean number of exit holes per leaf (±1SE); open circles represent adult AWF exiting cast skins and black diamonds represent *E. inaron* exiting the same. A new set of leaves was tagged and sampled at the onset of new leaf development; (c) change in AWF numbers and parasitism by *E. inaron* in northern California from 1990 to 2000. (a: J. K. Clark; b: from Pickett and Pitcairn, 1999, reprinted with permission from Springer Nature; c: modified from Pickett and Wall, 2003)

Table 1. Production of adult *Encarsia inaron* and ash whitefly (*Siphoninus phillyreae*) for each ash whitefly generation (451 DD [day degrees] base 10°C [50°F] whitefly generation). Beginning of generation one is at first peak in production of ash whitefly adults. (modified from Picket and Pitcairn, 1999)

Generation	Calendar dates	Exit holes			N (number leaves)
		Total ash whitefly	Total *Encarsia*	*Encarsia*/ash whitefly	
1	8/23/91–9/27/91	6046	237	0.0392	1905
2	10/4/91–11/1/91	1866	1431	0.767	1172
3	11/22/91–4/10/92	132	190	1.44	903
4	4/24/92–5/29/92	5	0	0	373
5	6/12/92–7/3/92	16	192	12	1427
6	7/17/92–8/7/92	0	13	--	1423

exceeded 0.26 whitefly per leaf during the course of the post-release study, a 96% decrease. Although no effort was made to measure the impact of *C. arcuatus* in California, the predator also spread around the state and likely contributed to the maintenance of the low population of this whitefly. The beetles were found associated with ash whitefly infestations in northern California (Dreistadt and Flint, 1995; Pickett and Wall, 2003). *Clitostethus arcuatus* was never observed in southern California before its release.

There are no reports in the literature that *E. inaron* has attacked any whiteflies other than ash whitefly in North America since it was released. No host range studies were done before the release of this parasitoid because none were required for a field release permit from the federal or state governments at that time. *Encarsia inaron* has been reported emerging from at least five other whitefly species other than *S. phillyreae* from other parts of the world, but many of these species are agricultural pests (Gerling, 1990). *Encarsia inaron* was the only parasitoid that emerged from ash whitefly during our studies.

BENEFITS OF BIOLOGICAL CONTROL OF ASH WHITEFLY

The ash whitefly biological control project quickly established an effective biocontrol agent throughout the state of California. Forty-three of 58 California counties that had urban centers with high concentrations of ash whitefly hosts received parasitoids for distribution. Within two months of its releases, *E. inaron* were observed emerging from ash whiteflies (Pickett et al., 1996; Pickett and Wall, 2003). Within two years of the initial parasitoid releases, tree canopies and other surfaces were no longer covered in honeydew, and black sooty mold no longer coated plant foliage, which had previously reduced photosynthesis (**Fig. 4**). The risk to people from breathing fragments of whiteflies and their honeydew rapidly ceased, as did the honeydew deposits on sidewalks. The project also preserved the aesthetic value of urban trees and protected economically important agricultural crops. An economic analysis was conducted on the change in value of street trees, which was calculated as the difference between the trees' appraised value in the absence vs. presence of ash whitefly (Pickett et al., 1996; Jetter et al., 1997). Total benefits, based on the wholesale value of trees in 1990 for all regions assessed, was $324,171,888. The total cost of the biological control program was $1,224,352, which was the salary cost of collecting and importing *E. inaron* and the subsequent rearing and monitoring costs in carrying out the biocontrol program. Benefits of the project to urban trees at the wholesale level was $270 for every dollar spent. The most likely agricultural commodity to be damaged if ash whitefly had not been controlled was pomegranate, which in 1990 in California was worth $9,555,200 in sales. Pears, valued at $93,583,600, and apples at $111,360,600 also could have been affected (Pickett et al., 1996). Cities also benefited from a reduced need for tree removal or associated actions to remediate the effects of ash whitefly.

ACKNOWLEDGMENTS

The senior author is indebted to all the technical staff supporting the ash whitefly project in California during the 1990s. Dr. Chris Borkent assisted with the graphics for this book chapter. Dr. Michael Pitcairn and Ms. Robin Wall (co-workers and friends) shared in the discovery of classical biological control's power to stabilize populations of an invasive pest and promote harmony with our surroundings.

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Chapter 18

Biological Control of the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui*

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NON-TECHNICAL SUMMARY

The cycad aulacaspis scale, *Aulacaspis yasumatsui* (Hemiptera: Diaspididae), is a worldwide pest of cycads. In Florida, Hawaii, and elsewhere, cycads are used as urban landscape plants and exported for sale as potted plants. The scale has decimated the cycad production industry and reduced the use of the king sago palm, *Cycas revoluta*, and other cycads as ornamental plants. On western Pacific islands, the scale is destroying populations of native *Cycas micronesica* in natural habitats. The invasion of Guam by the cycad aulacaspis scale has led to the death of at least 90% of the island's *C. micronesica* trees. The parasitic wasp *Coccobius fulvus* (Hymenoptera: Aphelinidae) and the predatory beetle *Cybocephalus nipponicus* (Coleoptera: Cybocephalidae) were released in Florida and became established but did not provide satisfactory control of the pest. The parasitic wasp *Arrhenophagus chionaspidis* (Hymenoptera: Encyrtidae) was first detected attacking cycad aulacaspis scales in Florida in 2012, but its influence on pest populations has not been evaluated. In Hawaii, the lady beetle *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) seems to be a good biological control agent as it can be effective under a range of conditions. The parasitoid wasp *Aphytis lingnanensis* (Hymenoptera: Aphelinidae), or a cryptic species very similar to it, may have a significant effect on cycad aulacaspis scale populations in Hawaii, where infested plants seem to survive the presence of the scale insect when the parasitoid causes high levels of scale mortality. *Rhyzobius lophanthae* was introduced to Guam and is established but does not protect young cycads. *Arrhenophagus chionaspidis* occurs in Guam and appears to cause high rates of parasitism on the leaves. Attempts to introduce *C. fulvus* and the Hawaiian *Aphytis* species to Guam were unsuccessful. Exploration in Asia discovered a lady beetle, *Phaenochilus kashaya* (Coleoptera: Coccinellidae), which may be an effective biological control agent, but currently there are no plans to introduce this predator to western Pacific islands. Introductions of an *Aphytis* species from Hawaii and *C. nipponicus* from Florida may offer hope of saving the native *C. micronesica* in Micronesia.

Cave, R. D., A. Moore, and M. Wright. 2022. Biological control of the cycad aulacaspis scale, *Aulacaspis yasumatsui*, pp. 189–203. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

The cycad aulacaspis scale, *Aulacaspis yasumatsui* (Hemiptera: Diaspididae), was discovered infesting cycads in 1994 in the Montgomery Botanical Center in southern Florida, after it was unknowingly brought to the Center on infested plants from Southeast Asia (Tang et al., 2006), where it is native (Takagi, 1977). Infestations of the scale were particularly severe on *Cycas* spp. and *Stangeria eriopus* (a South African cycad in a monotypic genus). From this single infestation point, the scale spread throughout Florida and into other southeastern U.S. states and Texas. Hawaii became infested with the cycad aulacaspis scale by 1998, when the pest was detected on Oahu, and the scale has subsequently been found on the islands of Hawai'i and Kaua'i (Heu et al., 2003). The scale was found in Guam in 2003 on ornamental king sago palms (*Cycas revoluta*) (Terry and Marler, 2005). Within two years, the scale spread through most of Guam and invaded the native forests of *Cycas micronesica*, an indigenous tree unique to Micronesia (Moore et al., 2005a). The pest was discovered on Rota in 2007 (Calonje, 2008) and Palau in 2008 (Orapa and Cave, 2010). Cycad aulacaspis scale is now established in many tropical and subtropical regions and countries, including the West Indies, Mesoamerica, Indonesia, Singapore, Philippines, China, Vietnam, Micronesia, Africa, and southern Europe (Howard et al., 1999; Weissling et al., 1999; Hodgson and Martin, 2001; Moore et al., 2005a; Bográn et al., 2006; Germain and Hodges, 2007; Segarra-Carmona and Pérez-Padilla, 2007; Marler, 2012; Muniappan et al., 2012; Normark et al., 2017; Dimkpa et al., 2021; Macharia et al., 2021; Marler et al., 2021). The dispersal route responsible for this broad distribution is the international commercial movement of whole cycad plants, but private collectors may be another pathway for spread. The presence of cycad aulacaspis scales on the leaves is easily visible, but the insect can infest the coralloid roots (**Fig. 1a**), excised leaf bases on trunks, and cataphylls covered by tomentum (**Fig. 1b**), where they are difficult for phytosanitary inspectors to detect (Marler and Moore, 2010).

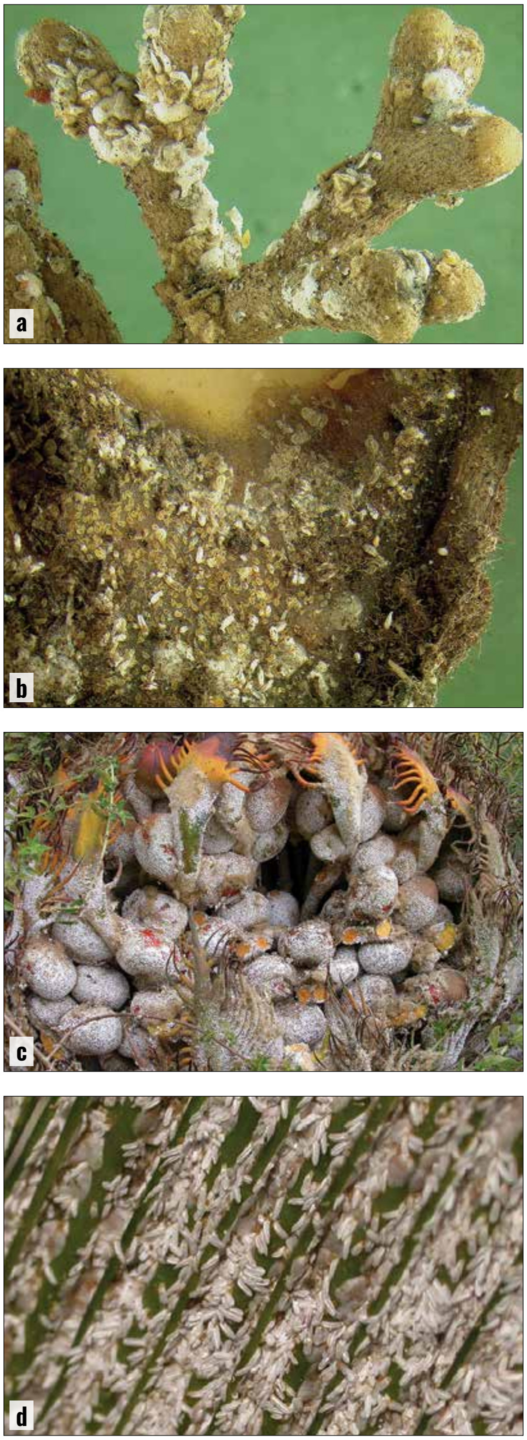

Figure 1. Cycad aulacaspis scale, *Aulacaspis yasumatsui*, on the (a) coralloid roots, (b) cataphyll, (c) seeds, and (d) leaves of *Cycas revoluta.* (a–d: R. D. Cave, University of Florida)

Nature of the Problem

The sessile adult female scale creates a round, white armor under which it feeds with piercing-sucking mouthparts and lays eggs (**Fig. 2**). From the eggs emerge tiny nymphs, called crawlers (**Fig. 2**), that have functional legs but initially do not feed. The crawlers walk to other plant parts or adjacent host plants or may

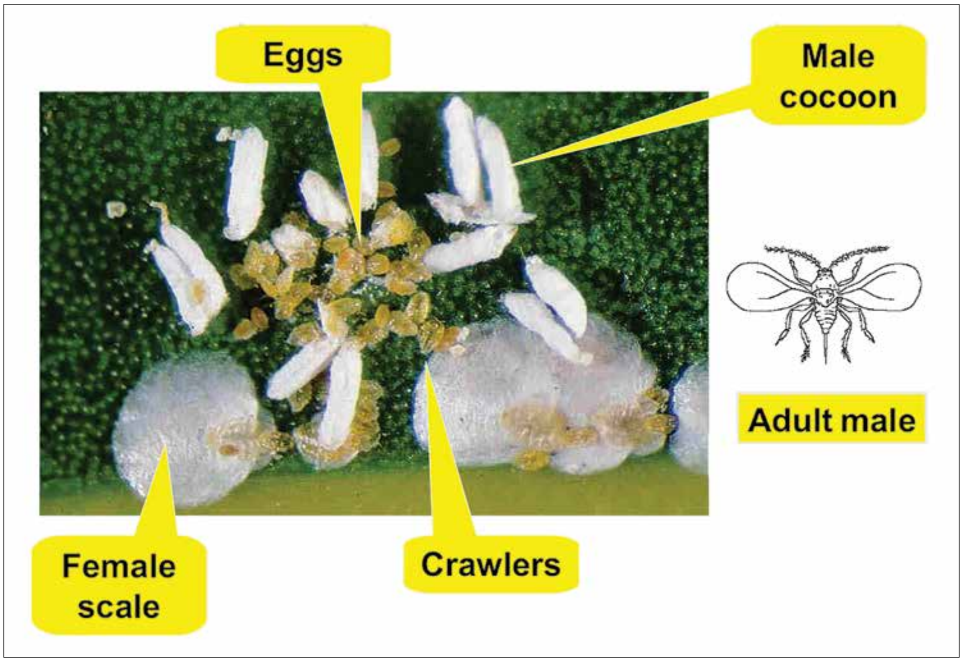

Figure 2. Life stages of the cycad aulacaspis scale, *Aulacaspis yasumatsui.* (Illustration by A. Moore, University of Guam)

be dispersed to other plants by the wind. When the crawler finds a suitable site on the plant, it settles, inserts its mouthparts, and molts to the next life stage, which lacks legs but has functional piercing-sucking mouthparts. During feeding, the sessile, older scales secrete substances that form the armor scale covering characteristic of the insect. The male 2nd-instar scale produces a white, three-ridged covering (**Fig. 2**) under which the insect molts three times and eventually emerges as a tiny adult with one pair of wings (**Fig. 2**) but no functional mouthparts. The 2nd-instar female secretes additional covering material but remains in place, where she molts to the wingless adult stage, mates with a mobile male, and then produces eggs to repeat the life cycle.

All plants in the family Cycadaceae and several species of Zamiaceae are hosts of the cycad aulacaspis scale (Marler et al., 2021). Uncontrolled, the scale forms dense populations on the trunk, roots, seeds (**Fig. 1c**), and leaves (**Fig. 1d**). Feeding by dense scale populations deprives the host of nutrients and weakens the plant, which may die of plant pathogen infections that healthy plants could tolerate or resist.

Based on an island-wide census of Guam's trees by the U.S. Forest Service in 2002, the year before the arrival of the cycad aulacaspis scale, the tree cycad *C. micronesica* was identified as the most abundant tree in Guam's forests (Donnegan et al., 2004). Within 12 years of the scale's invasion, *C. micronesica* was listed as threatened under the U.S. Endangered Species Act. Rapid decline of the *C. micronesica* population in Guam was documented by Marler and Krishnapillai (2020) (**Fig. 3**). Cycad stem counts declined to only 12.5% of the original number within the first three years of surveys (conducted from 2005 to 2008), and this decline continued, reaching 4% of the original count in succeeding years (2009–2020). In addition to high plant mortality, surviving cycads stopped reproducing in the research plots on Guam. The last seedling (0–10 cm [0–4 in] tall) was seen in 2006, and the last juvenile (10–100 cm [4–39 in] tall) was seen in 2014. In some areas of Guam, the mortality rate of 100-year-old *C. micronesica* trees has reached 100%.

Secondary effects of cycad aulacaspis scale on the health of *C. micronesica* are not obvious. Perhaps the most important secondary impact is much-reduced reproductive capability in plants recovering from scale infestation. Seeds from scale-infested plants are deficient in nonstructural carbohydrates, and germination rates are much lower, i.e., 43% of seeds from healthy plants germinate versus only 7% of seeds from infested plants (Marler and Cruz, 2019). In addition, Marler and Terry (2021) reported that mature male plants that survive the initial scale infestation have significantly smaller cones than healthy plants.

Marler (2013) reported on nondestructive stem-winching stress tests performed on *C. micronesica* trees to simulate the effects of typhoon-strength winds. Stems of plants that had not been infested by cycad aulacaspis scale were significantly stiffer than those that had been infested by the scale for either two or five years. Marler hypothesized that scale-infested plants would be more susceptible to stem failure during typhoons. Evidence supporting this hypothesis came two years later when Typhoon Dolphin passed over Guam on May 15, 2015. Marler et al. (2016) compared the level of damage from Typhoon Dolphin with that of a previous cyclone, Supertyphoon Paka, that damaged Guam's forests in 1997 when the *C. micronesica* population was healthy. Less than 2% of the healthy *C. micronesica* population exhibited wind snap damage during Supertyphoon Paka's peak winds of 298 km/h (185 mph). In contrast, Typhoon Dolphin's peak winds of only 170 km/h (106 mph) caused wind snap of 6% of Guam's unhealthy *C. micronesica* population after only 10 years of infestation by the cycad aulacaspis scale.

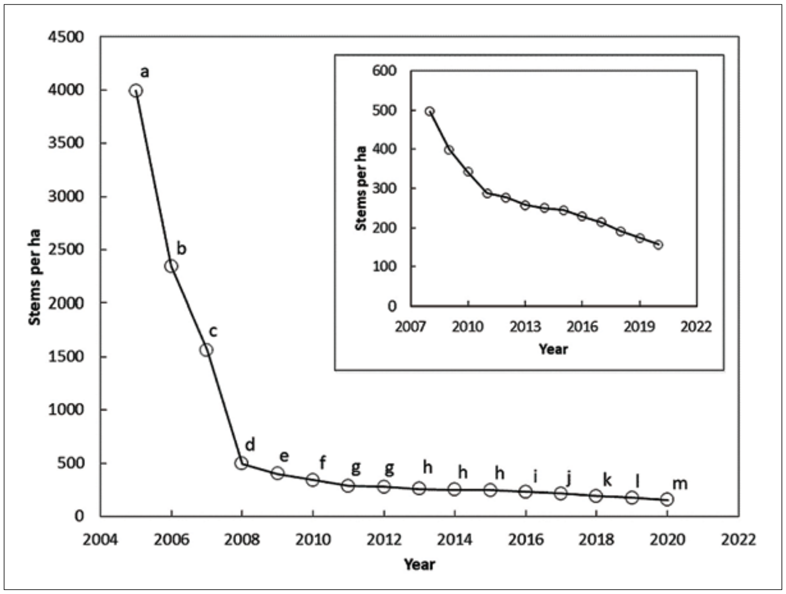

Figure 3. Number of *Cycas micronesica* stems per ha (all size categories) in 12 Guam habitats from 2005 to 2020. The inset shows results from 2008 to 2020 with a smaller vertical axis range. Ordinates of markers with the same letter are not significantly different. (from Marler and Krishnapillai, 2020, reprinted with permission)

Figure 4. *Cycas revoluta* with an intense infestation of the cycad aulacaspis scale, *Aulacaspis yasumatsui*. (R. D. Cave, University of Florida)

Cycads are highly desired by landscapers and homeowners as ornamental plants because they are long-lived, require low maintenance, and are resistant to most pests. Cycad aulacaspis scale infestations on ornamental cycads are unsightly (**Fig. 4**). *Cycas revoluta* (the king sago palm), the most popular ornamental cycad, is particularly susceptible to the scale. Worldwide, many ornamental king sago palms have died due to the cycad aulacaspis scale.

WHY CONTROL THIS INVASIVE SPECIES?

Populations of *C. micronesica* on several island groups (Guam, the Northern Mariana Islands, and other western Pacific islands) are now threatened by the cycad aulacaspis scale (**Fig. 5a,b**). Currently, unhealthy mature *C. micronesica* plants in Guam's forests are neither producing seeds (**Fig. 5d**) nor being replaced by juvenile plants (**Fig. 5c**). Without control of the invasive scale, this endemic plant, the most abundant tree in Guam's forests only two decades ago, is headed towards local extinction. Marler and Lawrence (2012) predicted the extinction of *C. micronesica* from western Guam by 2019, but small pockets of large trees still struggle to survive. There is an urgent need to control cycad aulacaspis scale so that some recovery can take place without further loss of biodiversity.

The disappearance of *C. micronesica* from Guam is likely to threaten the survival of other endangered organisms. The Mariana fruit bat, *Pteropus mariannus*, eats the fleshy, aromatic covering of *C. micronesica* seeds, which may be the bat's sole food source after a typhoon destroys less wind-tolerant fruit-bearing plants such as papaya (Haynes and Marler, 2005). In 2020, the U.S. Fish and Wildlife Service estimated that only 45 Mariana fruit bats remain in Guam in a single roost site on Andersen Air Force Base.

Figure 5. *Cycas micronesica* adversely affected by the cycad aulacaspis scale, *Aulacaspis yasumatsui*, in Guam: (a) mature tree at Tarague; (b) dead mother tree with living pup at Tarague; (c) dying young plant at Star Cave; (d) infested leaf petioles and seeds of mature tree on the campus of the University of Guam, Mangilao. (a–d: R. D. Cave, University of Florida)

A moth, *Anatrachyntis* sp. (Lepidoptera: Cosmopterigidae), is a probable pollinator of *C. micronesica* and possibly an obligate symbiont, i.e., it may not survive without the cycad. Larvae are numerous in male cones following pollen shedding, and they pupate in silken cocoons on the surfaces of cones (Marler and Muniappan, 2006). These authors hypothesized that the moth contributes to rapid degradation of the large and metabolically costly cone tissue after pollen dispersal. Terry et al. (2009) used sticky traps to sample insects and pollen in the vicinity of female *C. micronesica* cones. They observed that 30% of the pollen grains were associated with *Anatrachyntis* moths or moth scales and <5% with other insects; over 60% of the pollen was not associated with any insect, suggesting most of the pollen is dispersed by wind. Based on these observations, the authors hypothesized that *Anatrachyntis* sp. is an important pollinator of the tree.

Cycads are also economically valuable. The king sago palm is the most favored ornamental cycad species used in the international horticulture industry; nearly 110,000 plants were exported worldwide in 1993 (Whitelock, 2002). According to a report of the Convention on International Trade in Endangered Species, 99% of the 30 million cycads traded internationally from 1977 to 2001 originated from propagation nurseries (Anon., 2003a). Before 2003, 17 countries were involved in the international cycad trade (Anon., 2003b). Soon after the arrival of the cycad aulacaspis scale in Florida, exports of cycads from the state probably spread the scale internationally (Marler et al., 2021). All former cycad propagation nurseries in Florida have ceased production and export of the plants.

THE ECOLOGY OF THE PROBLEM

The cycad aulacaspis scale is a member of the armored scale family Diaspididae. Only two other species of *Aulacaspis* occur in North America, both of which are considered agricultural pests. *Aulacaspis rosae* is a pest of roses (Dekle, 1976), and *Aulacaspis tubercularis* is a pest of mangoes (Hodges and Hamon, 2016). Both species are adventive in North America and have a circumtropical distribution. Outside Thailand, the cycad aulacaspis scale is a non-native insect, so it is not a keystone species or a unique prey or host for any native predator or parasitoid.

Damage from the cycad aulacaspis scale in Guam, Rota, Palau, and other Micronesian islands threatens the ancient, once-extensive native forests of *C. micronesica* with destruction. The armored scale weakens cycad trees, reducing their resistance to tropical cyclone winds and delaying recovery after a storm (Marler and Lawrence, 2013). Dead cycad trees are not being replaced because the scale kills seeds and seedlings (Marler and Cruz, 2019). Native animals that depend on *C. micronesica* as food are adversely affected by the loss of plants (Haynes and Marler, 2005).

Chemical pest management of the cycad aulacaspis scale is expensive because applications of horticultural oils or synthetic pesticides must be made at frequent, regularly scheduled intervals (Howard et al., 1999; Emshousen et al., 2004; Hara et al., 2005; Bográn et al., 2006). Pesticide treatments are not always effective because there are physical refuges on the plant where scales remain inaccessible to treatments. Moreover, broad-spectrum pesticides are highly toxic to the parasitoids and predators of the cycad aulacaspis scale (Smith and Cave, 2006a). Pesticide applications to the cycad forests of Guam and other western Pacific islands would be economically unfeasible and harmful to native wildlife.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Biological Control Efforts in Florida

Classical biological control of the cycad aulacaspis scale in Florida began in 1998 when the parasitic wasp *Coccobius fulvus* (Hymenoptera: Aphelinidae) and the predatory beetle *Cybocephalus nipponicus* (Coleoptera: Cybocephalidae) (erroneously identified as *C. binotatus* [Smith and Cave, 2006b]), both from Thailand, were imported, released, and established in Miami-Dade County (Hodges et al., 2003). About 15,000 individuals of *C. fulvus* were released during February–April 2002 in 13 Florida counties and broadened the distribution of the wasp throughout southern Florida (Hodges et al., 2003). *Coccobius fulvus* individuals subsequently collected from northern Vietnam were released in Gainesville, Florida in 2007, and this cold-hardy race

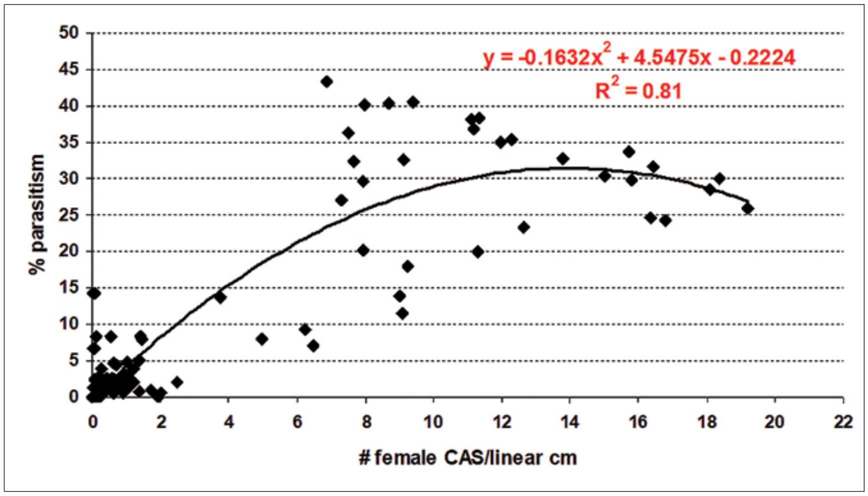

Figure 6. Rate of parasitism of female cycad aulacaspis scales (CAS), *Aulacaspis yasumatsui*, by *Coccobius fulvus* in relation to female scale density on *Cycas revoluta* in Florida. (R. D. Cave, University of Florida)

became established in northern Florida (R. Nguyen, pers. comm.). The wasp is now widespread throughout Florida, but its northern range is apparently defined by its intolerance of low winter temperatures. Rates of parasitism may be nearly 100% on individual leaflets, but overall parasitism by *C. fulvus* on a plant is usually between 10 and 40%, with greater parasitism occurring on leaves with high scale densities (**Fig. 6**). At extremely high densities, parasitism rates decrease because the scale population on a leaf is layered; scales on the plant surface are covered by other scales on top of scales, so are not accessible to female *C. fulvus*.

Larvae and adults of *C. nipponicus* are frequently observed in abundance on king sago palms infested with the cycad aulacaspis scale throughout Florida. The life history of the beetle was studied by Smith and Cave (2006b). Adults are long-lived and consume hundreds of scales during adulthood (**Table 1**). Females may lay over 300 eggs during their lifetime. The predator is widely distributed in Southeast Asia, where it occurs in very large numbers on scale-infested cycads in southern China and Vietnam (R. D. Cave, pers. obs.). *Cybocephalus nipponicus* was released in the northeastern United States to control euonymous scale (*Unaspis euonymi*), where it became established (Drea and Carlson, 1988; Alvarez and Van Driesche, 1998). According to label data on specimens in the Florida State Collection of Arthropods, *C. nipponicus* has been in Florida since at least 1990 (Smith and Cave, 2006c). The species also occurs in Texas and Hawaii. The wasp *Aphanogmus albicoxalis* (Hymenoptera: Ceraphronidae) parasitizes the prepupae and pupae of *C. nipponicus* (Evans et al., 2005). This parasitoid is native to Florida and may reduce the biological control capability of *C. nipponicus* there.

Table 1. Average longevity (days) and number of cycad aulacaspis scales (*Aulacaspis yasumatsui*) consumed per day and per life stage of *Cybocephalus nipponicus* (at 25°C [77°F], data from Smith and Cave, 2006), *Phaenochilus kashaya* (at 25°C, data from Manrique et al., 2012), and *Rhyzobius lophanthae* (at 24°C [75°F], data from Thorson, 2009). ND = no data.

Beetle Life Stage	*C. nipponicus*			*P. kashaya*			*R. lophanthae*		
	Longevity	Daily consumption	Total consumption	Longevity	Daily consumption	Total consumption	Longevity	Daily consumption	Total consumption
Instars I–III	ND	ND	ND	19	4–8	130	14	1–5	29
Instar IV	ND	ND	ND	12	21	246	6	5	58
Larva (total)	14	ND	ND	31	4–21	380	20	1–5	87
Adult female	110	4	440	158	29	915	104	3	281
Adult male	89	4	356	130	29	753	103	3	194

During exploration in Asia to find new natural enemies of the cycad aulacaspis scale, the lady beetle *Phaenochilus kashaya* (Coleoptera: Coccinellidae) (**Fig. 7**) was discovered in Thailand in 2007 (Cave et al., 2009a) and collected again in 2009. The beetle is not known to occur elsewhere (Giorgi and Vandenberg, 2012). Adults, larvae, and pupae were observed on *Cycas siamensis* in a forest near the Sakaerat Environmental Research Station in eastern Thailand. The cycads in the area had either very sparse infestations of the cycad aulacaspis scale or no scales at all (R. D. Cave, pers. obs.). The biology of this predator was studied in a quarantine facility by Manrique et al. (2012). Fourth instars eat about 3–5 times more scales/day than earlier instars at 25°C (77°F), and therefore consume more total scales than *C. nipponicus*. The consumption of scales by *P. kashaya* larvae is about 12 times more scales/day than by larvae of the predatory lady beetle *Rhyzobius lophanthae* (**Table 1**; Thorson, 2009). Adult females of *P. kashaya* can live up to six months at 25°C and lay eggs until shortly before death; males can live more than four months (**Table 1**). At 20°C (68°F), adult females and males live more than seven and five months, respectively. Because *P. kashaya* adults eat more scales per day and live considerably longer than *R. lophanthae* adults, *P. kashaya* can kill about 3.5 times more scales than *R. lophanthae* (**Table 1**). Female *P. kashaya* at 20°C each produce about

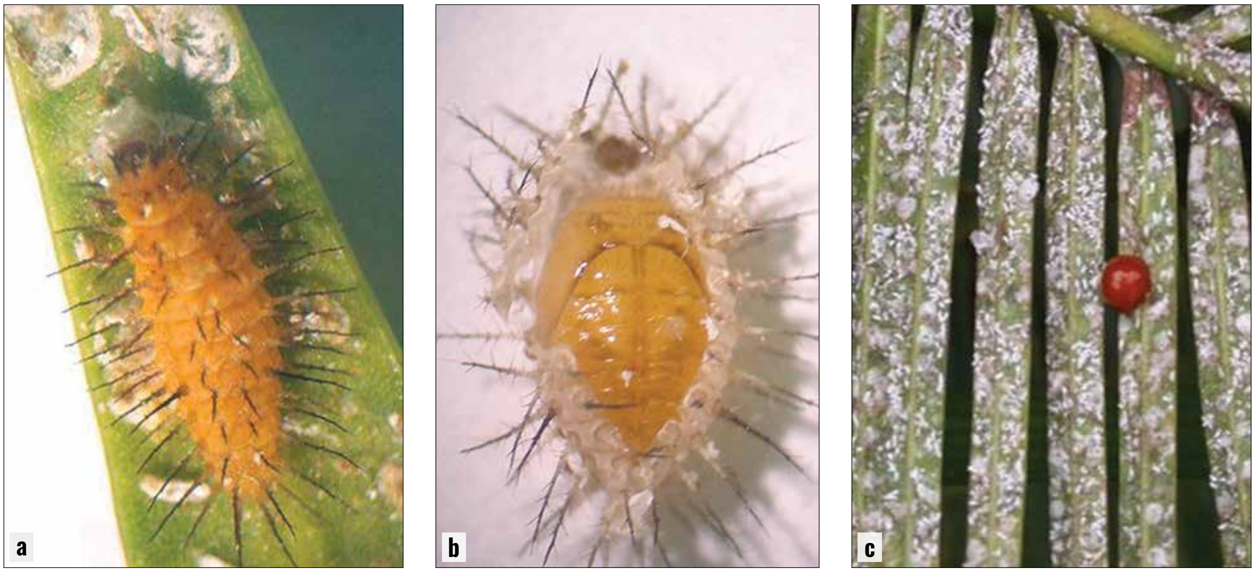

Figure 7. *Phaenochilus kashaya* larva, pupa, and adult. (a–c: R. D. Cave, University of Florida)

339 eggs during their adult life, while *R. lophanthae* adults feeding on cycad aulacaspis scales at 24°C (75°F) each lay only about 50 eggs (Thorson, 2009). Given these data, *P. kashaya* is undoubtedly a more voracious and prolific predator of cycad aulacaspis scales than *R. lophanthae* or *C. nipponicus*. A petition to release *P. kashaya* from quarantine in Florida was denied by USDA-APHIS-PPQ for multiple reasons. The natural enemy has not yet been released for biological control of the cycad aulacaspis scale anywhere.

The parasitoid *Arrhenophagus chionaspidis* (Hymenoptera: Encyrtidae) attacks cycad aulacaspis scales at rates ranging from 18 to 83% in China and Vietnam (R. D. Cave, unpublished data in report to APHIS PPQ). Material collected in Asia in 2007 was brought to quarantine in Florida for study and mass rearing. The parasitoid is very difficult to rear, and colonies of it could not be maintained. *Arrhenophagus chionaspidis* was discovered attacking cycad aulacaspis scale in Florida in 2012 (E. Rohrig, pers. comm.; R. D. Cave, pers. obs.). Ball and Stange (1979) reported the species attacking white peach scale (*Pseudaulacaspis pentagona*) in Florida. However, Bennett and Noyes (1989) subsequently stated that the *Arrhenophagus* species reported by Ball and Stange (1979) is *Arrhenophagus albitibiae*, a species described from Japan and known from Hong Kong and Sri Lanka. Therefore, it is uncertain if the *Arrhenophagus* species attacking cycad aulacaspis scale in Asia and Florida is *A. chionaspidis*, *A. albitibiae*, or an undescribed species. Molecular analysis might be able to resolve this conundrum. In Asia, the parasitoid was collected from plants with very heavy infestations of cycad aulacaspis scale. Therefore, *A. chionaspidis* does not appear to have potential as a biological control agent of cycad aulacaspis scale.

Aprostocetus purpureus (Hymenoptera: Eulophidae) was collected from female cycad aulacaspis scales in China and Vietnam in 2006 and brought to quarantine in Florida for study and mass rearing. The parasitoid was very difficult to rear, and colonies of it could not be maintained. This species has a wide distribution throughout India and Southeast Asia (Noyes, 2019). In Asia, it was collected from plants with very heavy infestations of cycad aulacaspis scale (R. D. Cave, pers. obs.). For these reasons, *A. purpureus* does not appear to have potential as a biological control agent of cycad aulacaspis scale.

Castillo et al. (2011) tested the entomopathogenic fungus *Cordyceps javanica* (given as *Isaria fumosorosea*) in the commercial product PFR97® (Certis USA, Columbia, MO) as a mortality agent of the cycad aulacaspis scale at 20°C and 30°C (86°F) in the laboratory. A concentration of 5.4×10^7 blastospores per ml (1.8×10^6/fl oz) of water applied to 1st instars resulted in mean infection rates of 73% at 30°C and 84% at 20°C eight days after application. The LT_{50} was lower at 30°C than at 20°C. These results indicate that *C.*

javanica may be a new biological control tool for suppressing infestations of cycads by the cycad aulacaspis scale, at least in horticultural settings.

Biological Control Efforts in Hawaii

No program of classical biological control against the cycad aulacaspis scale has been implemented in Hawaii. However, some natural enemies previously introduced to Hawaii have expanded their prey or host ranges to attack the scale. *Rhyzobius lophanthae*, introduced into Hawaii in 1894 for the control of scale insects (Funasaki et al., 1988), readily began preying upon cycad aulacaspis scale in Hawaii when the pest first arrived. It is considered the most promising agent for scale management (Hara et al., 2005), and it can be effective under a range of conditions from dry to mesic habitats (M. G. Wright, pers. obs.).

The parasitoid *Aphytis lingnanensis* (Hymenoptera: Aphelinidae) was introduced into Hawaii in 1964 as a biological control agent for Florida red scale, *Chrysomphalus aonidum* (Davis and Krauss, 1964). *Aphytis lingnanensis*, or a cryptic species very similar to it (G. A. Evans, pers. comm. to B. R. Kumashiro, 2009), appears to have a significant effect on cycad aulacaspis scale populations in Hawaii. Plants with quite severe infestations seem to survive the impacts of the scale insect when the parasitoid is present and causes high levels of scale mortality (M. G. Wright, pers. obs.). *Aphytis lingnanensis*, or a cryptic species very similar to it, was initially discovered as a parasitoid of the cycad aulacaspis scale in southern Texas (Flores and Carlson, 2009), but its influence on scale populations there was not evaluated.

In 2008, Bernarr R. Kumashiro of the Hawaii Department of Agriculture reared two other parasitoids from cycad leaves infested with the cycad aulacaspis scale and assumed they had emerged from this host rather than another armored scale species undetected on the leaves (B. R. Kumashiro pers. comm. to G. A. Evans, 2008). Gregory A. Evans of the USDA Systematic Entomology Laboratory identified one of these parasitoids as "*Pteroptrix leptocera* Huang [Hymenoptera: Aphelinidae] or a species very similar to it and new" (G. A. Evans pers. comm. to B. R. Kumashiro, 2009). Species of *Pteroptrix* are known as parasitoids of armored scales (Chen and Li, 2017) and one soft scale and a whitefly (Herting, 1972), but the precise hosts of *P. leptocera* are not known. Interestingly, Hui Ren of the Guangdong Entomological Institute reared *Pteroptrix chinensis*, a generalist parasitoid of scale insects (references in Noyes, 2019), from cycad aulacaspis scales in China (Cave, 2005). Evans identified the second parasitoid as *Plagiomerus* sp. (Hymenoptera: Encyrtidae). Species of *Plagiomerus* are known to parasitize several species of armored scales; *Plagiomerus aulacaspis* parasitizes *Aulacaspis citri* (Tan and Zhao, 1998). *Pteroptrix leptocera* and *Plagiomerus* sp. are probably self-introductions in Hawaii. Little is known of the effect of different environments and cycad species on the effectiveness of *Aphytis* sp., *Pteroptrix* sp., or *Plagiomerus* sp. as biological control agents of cycad aulacaspis scale.

Biological Control Efforts in Guam

In November 2004, about 100 adults of *R. lophanthae* were collected on Maui in Hawaii and imported to Guam (Moore et al., 2005b). This predatory beetle was chosen as the first biological control agent for introduction against the cycad aulacaspis scale in Guam because it was effective in Hawaii and relatively easy to introduce from there. Beetles were reared on scale-infested leaves cut from *C. micronesica*, collected from the laboratory rearing colony, and released in Guam National Wildlife Refuge at Ritidian Point starting in February 2005. Populations of the predator established readily. By July 2005, high densities of adults were observed on cycads at Urunao Beach, 1 km (0.6 mi) from Ritidian Point where one observer counted 57 beetles in one minute on scale-infested cycad leaves. Establishment and dispersion of *R. lophanthae* were monitored using yellow sticky traps between July 2005 and May 2006 (**Fig. 8**). Following establishment of *R. lophanthae* at Ritidian Point, 7,454 laboratory-reared and field-collected beetles were released on scale-infested cycads at 115 sites throughout Guam.

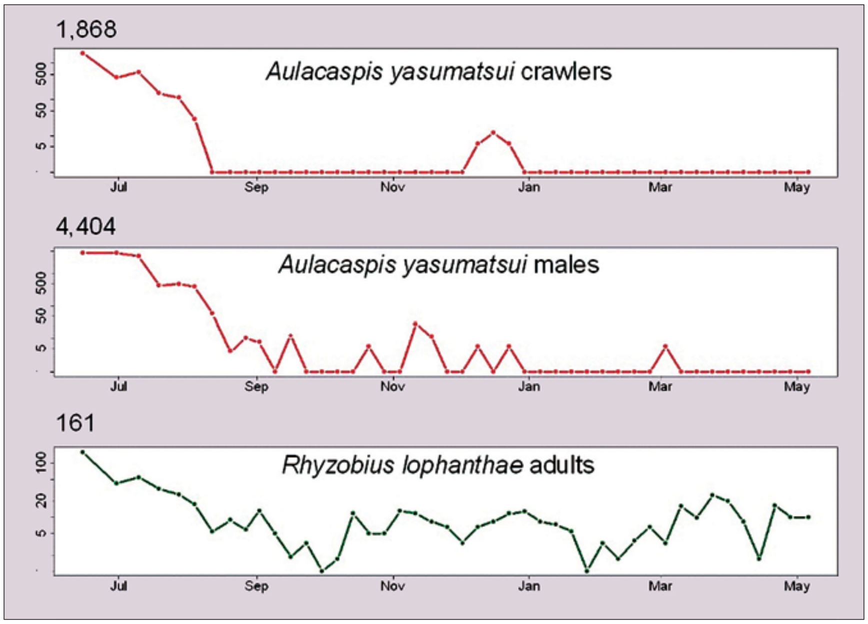

Figure 8. Insects trapped on yellow sticky cards at Ritidian Point, Guam following field release of *Rhyzobius lophanthae* in February 2005. X-axis runs from July 2005 through May 2006; Y-axis, in log scale, is the number of insects trapped per square meter per day. (from Moore et al., 2005b, reprinted with permission)

By 2010, *R. lophanthae* larvae or adults could be found on almost every infested cycad plant on Guam, and their predation on scales allowed mature cycads to survive. However, the *C. micronesica* population is not recovering because almost all seeds and seedlings are still being killed by the cycad aulacaspis scale and other causes (Marler and Terry, 2011). Marler et al. (2013) showed that predation rates by *R. lophanthae* are significantly lower on scales feeding close to the ground, and they suggested that this may partially account for the inability of the beetle to protect seedling plants. Although the causes of reduced scale predation near the ground are unknown, the authors suggested that a parasitoid might not have the same limitations because it would be much smaller than *R. lophanthae* and better able to reach scales within the cracks and crevices on *C. micronesica* and *C. revoluta* stems and roots. Unfortunately, no known parasitoid of the cycad aulacaspis scale attacks the scales settled on the coralloid roots in the soil.

In August 2005, R. Muniappan imported to Guam some 500 adults of the parasitoid *C. fulvus* that were sourced from a laboratory colony in Florida that had been established with parasitoids from China (Moore et al., 2005b). Of this shipment, 250 parasitoids were released in a cage with a potted scale-infested *C. revoluta* plant, and the other 250 parasitoids were released on scale-infested *C. micronesica* plants in the field at Marbo Cave on the northeastern coast of the island. This site was selected because *R. lophanthae* was not in the Marbo Cave area at that time because it had not been released there and had not yet dispersed into that area. Another 250 parasitoids received in September 2005 were released at the Marbo Cave site, as attempts to culture this species in the quarantine laboratory were not progressing satisfactorily. To determine if the parasitoid had established, a scale-infested frond of *C. micronesica* from the Marbo Cave area was collected four weeks after the release for examination under a binocular microscope. One parasitoid exit hole was observed on this frond. Four parasitoid exit holes were found when the same procedure was repeated in October 2005, but no exit holes were found on a sample examined in November 2005.

In a second attempt to introduce *C. fulvus*, G. V. P. Reddy imported adults from a laboratory colony in Florida, which had been established from wasps collected in Thailand. Some of these wasps were released on scale-infested cycads in Talofofo, Guam in 2008 (G. Reddy, pers. comm.). Attempts to establish a laboratory colony failed, and there was no evidence of establishment in the field.

In a third attempt to introduce *C. fulvus*, A. Moore imported specimens collected in Florida in September 2014 and October 2014. Half of both shipments were released at Ritidian Point, Guam. Attempts to establish a laboratory colony failed, and there was no evidence of establishment in the field. The reasons why establishment failed after the 2008 and 2014 releases are unknown. Possibilities are that too few individuals were released, parasitized scales were preyed on by *R. lophanthae*, and/or the individuals released were not sufficiently vigorous to find hosts.

In 2012, about 100 *A. lingnanensis* adults from Honolulu, Hawaii, were imported. These wasps were reared by University of Hawaii entomologist Leyla Kaufman from cycad aulacaspis scales infesting *C. revoluta* in a home garden. Upon arrival in Guam, the wasps were placed in a cage containing scale-infested *C. micronesica* leaves. All visible *R. lophanthae* adults and larvae were removed from these leaves, but there were undetected beetle eggs and 1st instars beneath scale covers to repopulate the leaves and eat all the scales before any adult wasps emerged. Thus, a laboratory colony was not established, and no field releases were made.

G. V. P. Reddy imported *A. chionaspidis* from a laboratory colony in Florida. Field releases in Guam were made during 2008. However, this parasitoid was already present in Guam via accidental self-introduction (G. V. P. Reddy, pers. comm., 2022). High proportions of the cycad aulacaspis scales at Ritidian Point were observed to be parasitized by *A. chionaspidis* in February 2013 (A. Moore, pers. obs.). The species identification was confirmed by John Noyes (Natural History Museum, London, UK). Surveys during 2017 and 2018 indicated that *A. chionaspidis* is the only parasitoid of cycad aulacaspis scale in Guam.

HOW WELL DID BIOLOGICAL CONTROL WORK?

Despite releases of *C. fulvus* parasitoids and *C. nipponicus* predators when the cycad aulacaspis scale was restricted to southeastern Florida, the scale quickly spread due to the movement of infested plants or natural dispersal by the wind. Although the natural enemies also dispersed throughout Florida, they did not provide adequate control (Wiese et al., 2005; Cave, 2006). Rates of parasitism by *C. fulvus* did not exceed 50% on leaves (**Fig. 6**), so parasitism was inadequate for effective control of the scale due to its rapid development and high fecundity (Cave et al., 2009b; Ravuiwasa et al., 2012). Although adults of *C. nipponicus* consume about four scales per day (Smith and Cave, 2006b) and the larvae are also predaceous, plants with the predator present were still heavily infested by the scale in Florida and Asia. No data have been collected to quantify the effect of *A. chionaspidis* on scale populations in Florida.

Because biological control of the cycad aulacaspis scale has been ineffective in Florida, nurseries in the state no longer grow king sago palms for retail businesses or export to out-of-state markets. Also, very few ornamental king sago palms remain in Florida's urban landscape; those that remain are infested by cycad aulacaspis scale, but they survive due to chemical applications by landscape managers and homeowners.

The introduction of *R. lophanthae* in Guam appears to have had a significant suppressive effect on cycad aulacaspis scale populations on large trees. Larvae and adults of the predator are found on almost every scale-infested cycad in Guam, preventing the scale from killing mature cycads. Recent observations of several trees revealed the presence of *R. lophanthae* and almost 100% predation of scales on leaves. Predation rates in the tree crowns and under bracts have not been measured. Some trees in isolated areas have dense cycad aulacaspis scale infestations on their leaves, megasporophylls, and seeds, and there is no indication of predation at these sites. Although intense predation occurs on mature trees, overall plant health often looks poor, and very few trees are producing seeds. Unfortunately, the *C. micronesica* population is not recovering because almost all seeds and seedlings are being killed by the scale. Predation by *R. lophanthae* does not protect either seeds or seedlings. Marler et al. (2013) showed that predation by *R. lophanthae* is significantly reduced close to the ground and suggested that this may partially account for the failure of the beetle to protect seedlings.

Parasitism by *A. chionaspidis* occurs across Guam. Some trees have high rates of parasitism, but others have no evidence of parasitism. Where the parasitoid is present, infested plants still have populations of female scales that are producing eggs. Attempts to introduce the parasitoids *C. fulvus* and *A. lingnanensis* were not successful.

BENEFITS OF BIOLOGICAL CONTROL OF CYCAD AULACASPIS SCALE

There probably is no hope of rebuilding the cycad production industry in Florida. No natural enemies of cycad aulacaspis scale have shown an ability to reduce scale populations to levels that can be practically and economically managed with chemical control. No biological control agents suppress the pest adequately.

Successful biological control of the cycad aulacaspis scale in the urban landscape, be it in Florida, Texas, Hawaii, or elsewhere, would benefit the environment where ornamental cycads have survived. Broad-spectrum pesticides applied as a soil drench and as foliar sprays, and horticultural oils applied to the leaves, are the primary means used by the landscape care industry and homeowners to protect cycads. However, chemical control can be expensive. For example, dinotefuran is a water-soluble systemic pesticide that can be applied either as a drench or twice a year to foliage for temporary control (Caldwell, 2005). A 1.4-kg (3-lb) container of Safari 20SG Systemic Insecticide with Dinotefuran costs about $422–468 (www.amazon.com; www.domyownpestcontrol.com). It is therefore not economical for homeowners to use it to treat just a few plants. On the other hand, applications of horticultural oils must be made at frequent, regular intervals (Howard et al., 1999; Emshousen et al., 2004; Hara et al., 2005; Bográn et al., 2006), and therefore their use is also costly. Some homeowners have applied pesticides not registered for use on cycads, such as flea and tick sprays (R. D. Cave, pers. obs.). If biological control agents cannot effectively control the cycad aulacaspis scale, then landscapers, homeowners, and botanical garden managers will continue to use costly pesticides that interfere with the resident natural enemies and contaminate soil and water.

Mature *C. micronesica* trees in Guam's forests are still succumbing to infestations by the cycad aulacaspis scale and are not being replaced by young plants. It is obvious that without a change, this endemic plant, the most abundant tree in Guam's forests only two decades ago, is headed towards local extinction. Restoration of Guam's forests to their pristine state is not possible with our current understanding of the scale's natural enemies. However, there is an urgent need to control cycad aulacaspis scale so that some cycad recovery can take place, without further loss of biodiversity.

Field release of the lady beetle *P. kashaya*, a voracious, oligophagous predator of armored scales, could contribute significantly to the biological control of the cycad aulacaspis scale in Guam and other western Pacific islands. Both the larval and adult stages consume the cycad aulacaspis scale, and the insect is easily reared in the laboratory. There is evidence in its homeland (Thailand) that it can suppress cycad aulacaspis scale populations to very low levels. If released in Guam, Rota, Tinian, and Palau, this beetle would likely reduce the abundance of cycad aulacaspis scale to levels that no longer threaten the survival of native *C. micronesica* forests on those islands. The actions of other natural enemies, such as *R. lophanthae* and *A. chionaspidis*, might also be enhanced. Although Agriculture and Agri-Food Canada, in its review of a petition to release *P. kashaya*, did not recommend release in the southeastern United States, the agency did recommend that *P. kashaya* be approved for release in Guam (P. G. Mason in litt. to R. Tichenor, 2014). However, USDA-APHIS-PPQ did not make any judgment on the latter recommendation. Currently, there are no plans to release *P. kashaya* in Guam because Guam lacks the professional capacity and funding to develop and implement a biological control program. However, an initiative to remedy this situation is underway. On the other hand, competition between *P. kashaya* and resident natural enemies (*R. lophanthae*, other lady beetles, and *A. chionaspidis*) in Guam must be considered.

New attempts to establish *C. fulvus* in Guam might lead to better biological control of the cycad aulacaspis scale, but studies are needed to investigate its searching behavior and ecology in the environment. Species of *Aphytis* that have been reared from cycad aulacaspis scales collected in China, Texas, and Hawaii need to be carefully examined taxonomically and biologically to determine their potential as biological control agents in Florida and Guam. Introduction of *C. nipponicus* may offer hope to save the native forests of *C. micronesica* in Micronesia.

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CHAPTER 19

Suppression of Mile-a-Minute Weed, *Persicaria perfoliata*, in the Eastern United States

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NON-TECHNICAL SUMMARY

Mile-a-minute weed, *Persicaria perfoliata* (Polygonaceae), was accidentally introduced into eastern North America with nursery stock in the 1930s. The vine spread slowly at first, but by the 1990s it was widely distributed in the Mid-Atlantic region, spreading rapidly, and causing considerable concern because of its ability to cover other vegetation, preventing forest regeneration and suppressing native plants. The biological control program against *P. perfoliata* began in 1996, and a permit for release of the host-specific mile-a-minute weevil, *Rhinoncomimus latipes* (Coleoptera: Curculionidae), was obtained eight years later, in 2004. The weevil shows all the characteristics of a desirable biological control agent, including a high reproductive rate, three to four overlapping generations per year in the Mid-Atlantic United States, extreme host specificity, excellent dispersal capability, and the ability to suppress the target weed. No harmful non-target effects occurred from the weevil's introduction, and its present and projected benefits are high. Although mile-a-minute weed is still present throughout the invaded area and can sometimes increase to noxious levels, the presence of the mile-a-minute weevil has reduced the weed's impact on native plants in many areas and, in areas where control of the weed is still needed, the weevil contributes substantially to integrated weed management.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Persicaria perfoliata (Polygonaceae) (**Fig. 1**), formerly *Polygonum perfoliatum*, is an annual vine that is widely distributed throughout Asia in its native range, including China, Korea, Japan, Indonesia, the Philippines, India, and parts of Russia (Liu et al., 2020). It was first introduced into eastern North America

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Hough-Goldstein, J. and E. Lake. 2022. Suppression of mile-a-minute weed, *Persicaria perfoliata*, in the eastern United States, pp. 204–211. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

in the 1930s as a contaminant of holly seed sent from Japan to a nursery near York, Pennsylvania (Moul, 1948). Before 1980, the plant's range was confined to five counties in Pennsylvania (Hill et al., 1981) and six counties in Maryland (Riefner and Windler, 1979). By the mid-1990s, however, it had spread further in those two states and was also reported in Virginia, Delaware, West Virginia, Ohio, New York, New Jersey, and the District of Columbia, with dramatic increases in the size of many populations (Oliver, 1996). Since then, it has spread north to New Hampshire, south to North Carolina, and west to Ohio, with isolated infestations found in Indiana and Iowa (EDDMapS, 2021). Additional range expansion in North America is likely to occur.

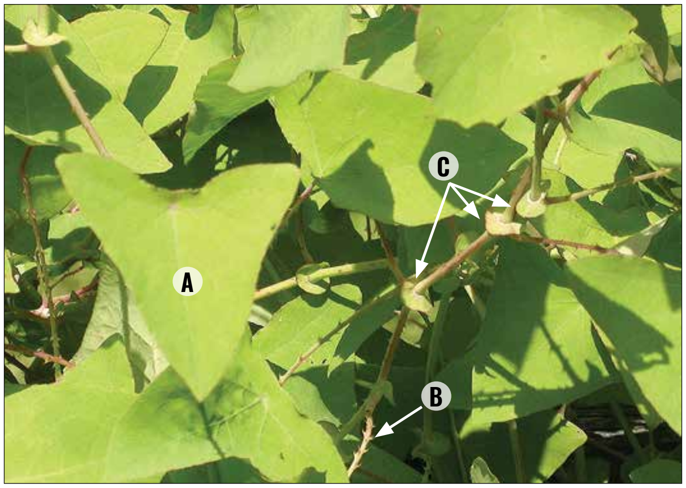

Figure 1. *Persicaria perfoliata*, mile-a-minute weed. Note triangular leaves (A), backward-projecting spines (B), and the flared sheath (ocreae) surrounding the stems (C). (J. Hough-Goldstein, University of Delaware)

Nature of the Problem

The vines of *P. perfoliata* (mile-a-minute weed or devil's tearthumb) can grow rapidly, overtopping other plants and producing masses of intertwining foliage (**Fig. 2**). Although the negative impacts of *P. perfoliata* in North America have long been recognized, there are no specific studies of its effects. Observed impacts include inhibition of reforestation and natural forest regeneration by smothering tree seedlings, interference with recreational use of natural areas, reduction in quality wildlife habitat, and likely negative effects on native flora (McCormick and Hartwig, 1995; Oliver, 1996; Wu et al., 2002). Mile-a-minute weed can quickly overrun utility rights-of-way where herbicides have been used to control unwanted woody vegetation. Isolated individual plants can produce thousands of seeds (called achenes) during the summer and fall (Hough-Goldstein et al., 2008), which can survive for up to six years in the seed bank (Hough-Goldstein et al., 2015). Many seeds are shed and germinate under the previous year's plants, leading to seedling densities averaging as high as 200–500 per 0.5 m^2 (37–93/ft^2) in the introduced range (Hough-Goldstein et al., 2009). Long-distance dispersal of seeds can occur by birds, deer, and water, in addition to accidental human-caused dispersal, for example through movement of nursery stock (Hough-Goldstein et al., 2015).

Figure 2. Landscape infested with mile-a-minute weed, *Persicaria perfoliata*. (J. Hough-Goldstein, University of Delaware)

WHY CONTROL THIS INVASIVE SPECIES?

The goal for suppression of mile-a-minute weed is to reduce its impact on native plants and allow for normal forest regeneration in disturbed areas. As an herbaceous annual, mile-a-minute plants die each fall, with new plants growing from seeds that germinate in the spring. A key aspect of long-term control is to deplete the existing seed bank and minimize additional seed production. Mile-a-minute plants have weak root systems, and the small seedlings are relatively easy to remove manually. Vines quickly grow over and through other vegetation, however, making mechanical control (through hand pulling or mowing) difficult to achieve without damaging desirable vegetation. The vining growth habit of this plant causes it to become draped over native or otherwise desirable plants, which makes control with broad-spectrum foliar herbicides difficult and potentially damaging to native plants. Pre-emergence herbicides kill new seedlings soon after germination, and these can be used effectively in early spring to control the weed without damaging most perennial plants; however, such treatments are incompatible with annual native plants, which may be killed (Templeton et al., 2020, but see Lake et al., 2014).

Given the ability of this invasive weed to spread and degrade native forest and edge plant biodiversity, and the difficulties in controlling the invader through either mechanical or chemical means, the classical biocontrol approach was selected to complement other management techniques. Biological control of mile-a-minute weed was intended to reduce the observed impacts of the plant on native plant communities and reduce herbicide use in areas such as rights-of-way, where the weed had to be controlled.

THE ECOLOGY OF THE PROBLEM

As with all weed biological control projects, whether the target is annual, biennial, or perennial, and regardless of the growth habit of the target, the goal is not to eradicate the target weed but to reduce its competitive ability relative to the surrounding vegetation. Insects can exert stress on plants in a variety of ways, through stem or root boring, defoliation, seed predation, or sucking on phloem or xylem. Thus, any sufficiently host-specific herbivore can potentially have a positive effect toward controlling an invasive weed. Our first step, then, was to find host-specific insects in the weed's area of origin in Asia.

Mile-a-minute weed is primarily a pest of natural ecosystems rather than commercial crops or rangelands, and therefore reduced competitive ability may be sufficient to allow the native plant community to increase. Long-term success, however, may require a combination of mechanical, cultural, biological, and chemical control techniques. Furthermore, because one successful colonization event can lead to a persistent seed bank (Turnbull et al., 2000), early detection and rapid response to new populations and management of the seed bank will be necessary.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Although mile-a-minute weed in the eastern United States is thought to have been introduced from Japan, the center of its native range is most likely China, and therefore this was where host-specific herbivores were sought when the project began (Ding et al., 2004). In China, *P. perfoliata* rarely attains the densities found in North America, suggesting that herbivores may be holding it to lower levels in its native range. However, it should be noted that *P. perfoliata* is widely used in traditional Chinese folk medicine (Liu et al., 2020), and, therefore, collection by people may also affect its population in Asia.

A collaborative project was initiated in 1996 between the USDA Forest Service and the Chinese Academy of Agricultural Sciences Institute of Biological Control to survey for and screen potential biological control agents in China for release against mile-a-minute weed in the United States (Ding et al., 2004). One hundred and eleven insect species were found feeding on mile-a-minute weed in China, including leaf-feeders, stem-borers, and fruit- and seed-feeders. Both generalists and specialists were present, and they caused extensive damage to plants. In a similar survey conducted in the United States in the 1980s, Wheeler and Mengel (1984) found 45 insect species feeding on mile-a-minute weed. Most of these insects were sap or leaf-feeders, all were generalists, and together they caused minimal damage to the plant, except for Japanese beetles, *Popillia japonica* (Coleoptera: Scarabaeidae), which can cause extensive defoliation.

Although eleven of the insect species collected by Ding et al. (2004) were considered potentially important because of the damage they caused, their likely narrow host range, or both, only one, *Rhinoncomimus latipes* (Coleoptera: Curculionidae), was ultimately determined to be host-specific enough to introduce into North America. This small weevil was tested on more than 50 plant species from 17 families in China from 1999 to 2002 (Ding et al., unpublished data). Weevils were sent to the USDA-ARS quarantine facility in Newark, Delaware where they were further tested on 28 species, mostly in the family Polygonaceae and including representatives of different tribes and sections within the family (Price et al., 2003; Colpetzer et al., 2004a). *Rhinoncomimus latipes* did not feed at all on any plant species outside the Polygonaceae. The adult weevils fed slightly on a few species in the family Polygonaceae, but they laid no eggs, and their larvae did not survive on any host except mile-a-minute weed. Based on these results, the weevil was approved for release by the USDA-APHIS in 2004. Host-specificity was later confirmed in an open field experiment, where weevils abandoned all potential non-target host species other than mile-a-minute weed even when the preferred plant host was removed (Frye et al., 2010).

Rhinoncomimus latipes, the mile-a-minute weevil (**Fig. 3**), overwinters in the adult stage in leaf litter or soil, emerging in the spring soon after mile-a-minute seedlings develop. The weevil lays eggs on leaves, stems, or buds of mile-a-minute weed plants. Eggs hatch in 3–5 days, and newly emerged larvae bore into the stems, where they feed internally. Once mature, the larvae leave the stem and pupate in the soil. Adult weevils emerge about a week later and seek mile-a-minute plants for mating and egg-laying, initiating a new generation (Price et al., 2003; Colpetzer et al., 2004b). Three to four generations can develop each year in the Mid-Atlantic region (Lake et al., 2011; Hough-Goldstein et al., 2016).

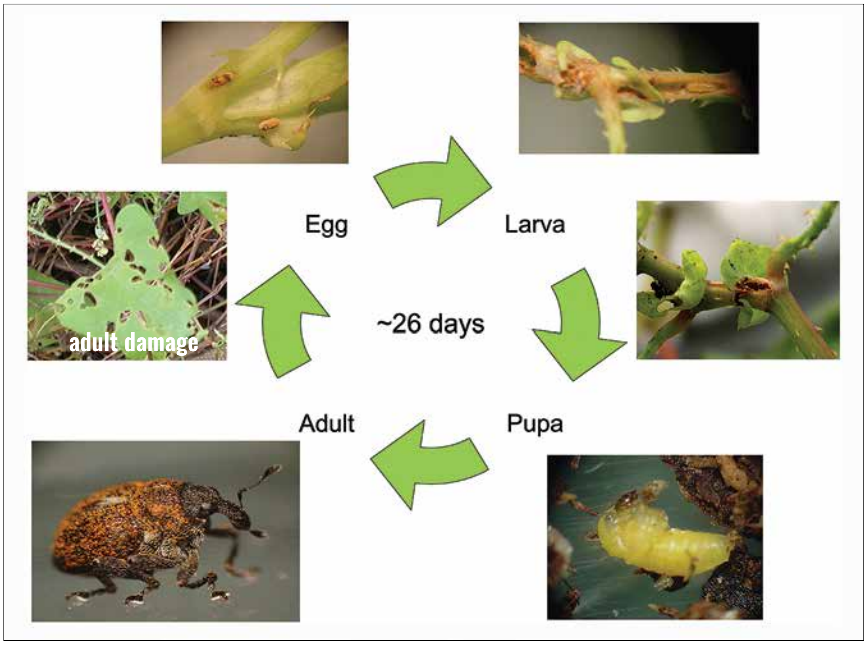

Figure 3. *Rhinoncomimus latipes* life cycle. (E. Lake, Mt. Cuba Center)

Mass rearing techniques were developed by the New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory (Hough-Goldstein et al., 2015). Between 2004 and 2020, the efforts of this laboratory plus production by smaller rearing facilities resulted in the release of more than 800,000 weevils in eleven states. Weevils established at nearly every release site (Hough-Goldstein et al., 2009, 2015; C. Detweiler, pers. comm.).

HOW WELL DID BIOLOGICAL CONTROL WORK?

Initial weevil releases were monitored using counts made inside standardized fixed quadrats (ten 1 x 0.5 m [3.3 x 1.6 ft] quadrats, approximately 10 m [33 ft] apart, at each site) at eight locations, with each release site paired with a non-release control site. Within 2–3 years, at three sites mile-a-minute weed densities dropped to <25% of weed levels before release of the weevils (**Fig. 4**), with no change in mile-a-minute weed at the non-release sites (Hough-Goldstein et al., 2009). At a fourth location, there was a similar level of reduction, but the noon-release control site was rapidly invaded by dispersing weevils, and therefore was not available for comparison. The other four locations were on islands in rivers, and mile-a-minute populations were reduced at both release and non-release sites, apparently due to environmental conditions such as late frost and extreme drought (Hough-Goldstein et al., 2009). Initial studies indicated weevils dispersed at a rate of 1.5–2.9 m (4.9–9.5 ft) per week after release in dense patches of mile-a-minute weed (Lake et al., 2011), while sampling on a broader scale showed weevils dispersed an average of 4.3 km (2.7 mi) per year (Hough-Goldstein et al., 2009).

In field cage experiments, weevils caused substantial plant mortality, delayed seed production, and reduced seed weight and viability (Hough-Goldstein et al., 2008; Smith and Hough-Goldstein, 2014). Surviving plants in field cages also showed loss of apical dominance, with damaged plants producing large numbers of lateral branches with small terminals and "stacked" nodes, close together on stems (Hough-Goldstein et al., 2008). This can be part of a compensatory response, in which a plant may produce more seeds (at the ends of the larger number of terminals). However, for a light-adapted vine such as *P. perfoliata*, numerous short terminals may not allow the plant to achieve needed sun exposure when in competition with other plants.

A replicated open-field study where weevils were released in the center of dense mile-a-minute populations showed significant reductions in mile-a-minute weed populations and higher weevil populations from 2005 to 2008 (Lake et al., 2011). In 2009, however, substantial spring rainfall and

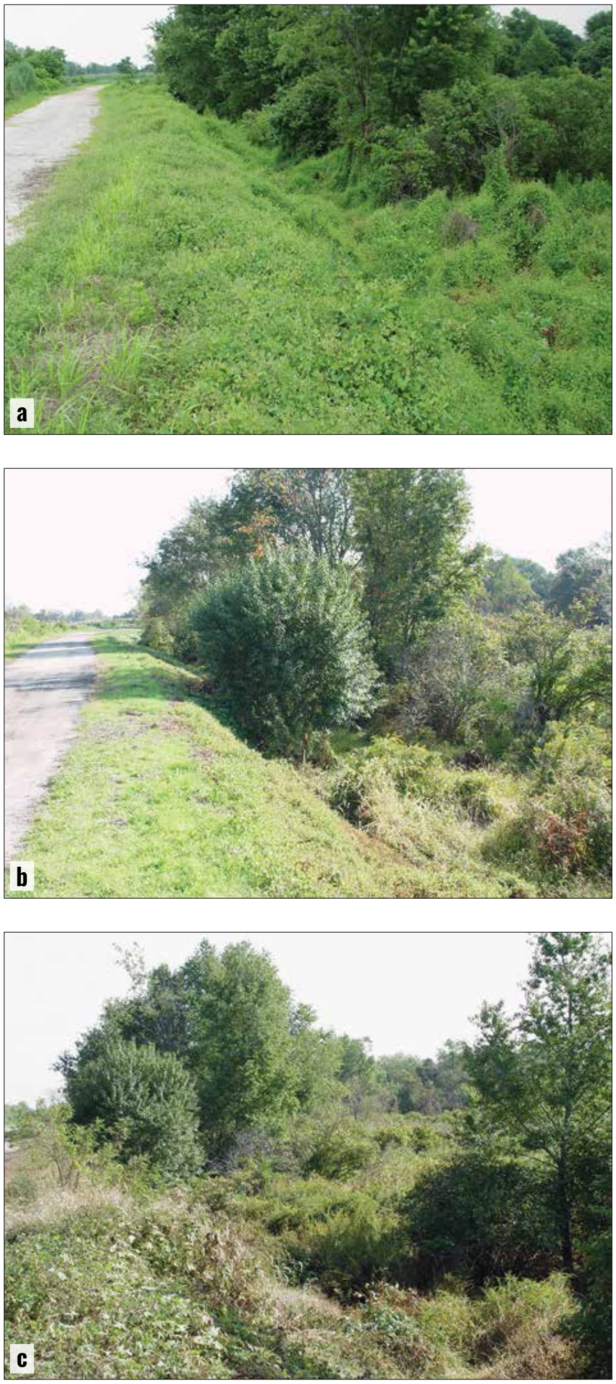

Figure 4. Mile-a-minute weed, *Persicaria perfoliata*, (a) at the Floodgate Road, New Jersey release site in July 2004, (b) October 2006, and (c) October 2007 after *Rhinoncomimus latipes* feeding. Note the *Prunus* sp. bush in the foreground (middle) was not visible before weevil release in 2004 (a), because it was covered by mile-a-minute weed. The *Prunus* sp. grew once mile-a-minute was reduced. (a–c: Mark A. Mayer, New Jersey Department of Agriculture)

relatively cool temperatures likely slowed the development of the weevils while allowing rapid growth of mile-a-minute weed, resulting in a higher mile-a-minute weed population and a reduced number of the weevils. In 2010, however, weevil density again increased, and mile-a-minute weed cover declined, likely due to the opposite climate conditions (low rainfall and high temperatures) (Hough-Goldstein et al., 2016).

Laboratory and greenhouse experiments confirmed that, where water is limited, mile-a-minute weed produces fewer seeds with lower viability and plant biomass is reduced compared to plants that are well-watered (Berg et al., 2015). Both mile-a-minute weed and its associated weevil perform better in sun than in shade. Weevils are attracted to sunny areas and develop best on sun-grown plants (Hough-Goldstein and LaCoss, 2012; Smith and Hough-Goldstein, 2013). Mile-a-minute weed can persist at shady sites, near locations where it is suppressed by weevils in sunny spots, but plants in the shade are less vigorous and produce fewer seeds.

The amount of time required for development of mile-a-minute weevils at different temperatures was determined and used to develop a model for weevil population growth (Hough-Goldstein et al., 2016). As with all insects, mile-a-minute weevils develop more quickly at warmer temperatures, and because this weevil has the potential for multiple generations each year, much larger weevil populations can develop during a warm year compared to one with a cool spring and summer.

When abiotic conditions are not favorable for the weevil, additional use of herbicides and/or mechanical controls may be advisable to reduce the likelihood of a large pulse of seeds being added to the seed bank and extending the timeline for management efforts. Furthermore, increased applications of pre-emergent herbicides may be necessary the following spring (Hough-Goldstein et al., 2015, 2016).

Another important factor affecting weevil impacts on mile-a-minute weed populations is the composition of the surrounding plant community. Mile-a-minute weed populations can increase explosively if seeds are present in the soil and the surrounding plant community is disturbed, for example where trees have fallen naturally or been harvested, where power line rights-of-ways have been mowed or treated with herbicide, or where a site has been cleared in preparation for future construction. Mile-a-minute weed, however, is less likely to invade a well-established plant community, where other vegetation can outcompete it for light resources, particularly if the weevil is simultaneously attacking the weed. If the surrounding plant community consists primarily of other invasive, non-native plants, it may be beneficial to replant with desirable native plants at the same time that weevils are released (Cutting and Hough-Goldstein, 2013; Lake et al., 2014). Integrating the weevil, herbicide applications, and plantings of competitive native vegetation can, at least temporarily, limit mile-a-minute weed populations, prevent the invasive species treadmill, and increase the abundance of native plants (Lake et al., 2014). Due to different site histories, conditions, and plant communities, adaptive management may be needed over the long-term.

BENEFITS OF BIOLOGICAL CONTROL OF MILE-A-MINUTE WEED

Although mile-a-minute weed is still present throughout the invaded area and can sometimes increase to noxious levels, the presence of the mile-a-minute weevil has made this much less likely, and biocontrol contributes substantially to integrated weed management where control of the weed is still needed (Hough-Goldstein et al., 2015).

Mile-a-minute weed is still expanding its range, with recent populations developing in northern Ohio, Indiana, Iowa, and New Hampshire (EDDMapS, 2021). Long-range spread probably occurs with seeds in pots or in soil around the roots of nursery stock. An important component of control of this weed is education and prevention, including efforts to increase public awareness of potential mechanisms of spread (e.g., Rathfon, 2016). Mile-a-minute weed can rapidly form large infestations after establishing in a new area where the biocontrol weevil is not present (Hough-Goldstein et al., 2015). However, in small weed infestations, if seedlings are removed and plants are prevented from going to seed for several consecutive years, eradication may be possible (Miller et al., 2018; Templeton et al., 2020). Early detection and rapid response to new patches of mile-a-minute weed, both within and outside the generally infested area, may prevent the development of a seed bank and the need for long-term management. Efforts should be made to release the weevil along the invasion front of mile-a-minute weed, where the weed has established populations that can no longer be eradicated.

Mile-a-minute weed will continue to expand its range, assisted by long-distance dispersal events by animals consuming the seed and accidental spread by humans. It is critical to continue to manage new populations of the weed both at the invasion front as well as in regions already invaded. States where new mile-a-minute weed populations are found should carefully monitor infestations and make new weevil releases unless new mile-a-minute weed patches can be eradicated. Weevils can be obtained from the New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory or collected and moved from sites where they are abundant.

ACKNOWLEDGMENTS

We would like to thank the many land managers, conservation organizations, state parks, and private landowners in Delaware and southeastern Pennsylvania for their assistance and cooperation by 'donating their mile-a-minute patches to science' while we implemented and studied the impacts of biological control. Skilled and dedicated insect rearers at the Phillip Alampi Beneficial Insect Laboratory in New Jersey were key in developing the program, as were collaborators throughout the Northeast who assisted with the release and monitoring of the mile-a-minute weevil. We also thank the undergraduates, graduate students, and technicians who assisted with mile-a-minute research at the University of Delaware. Finally, we would like to thank Richard Reardon, USDA Forest Service, Forest Health Technology Enterprise Team (FHTET, retired), for financial support.

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CHAPTER 20

Biological Control Success Against Winter Moth in the Northeastern United States

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NON-TECHNICAL SUMMARY

Winter moth, *Operophtera brumata* (Lepidoptera: Geometridae), is native to Europe. It invaded the northeastern United States in the late 1990s, where it caused widespread defoliation of forests and shade trees. In Massachusetts, the defoliated area ranged from 2,266 to 36,360 ha (5,600–89,848 acres) per year between 2003 and 2015. In 2005, we initiated a biological control program against winter moth in Massachusetts, using the specialist tachinid parasitoid *Cyzenis albicans*. We chose this species because it had been introduced (along with the generalist ichneumonid parasitoid *Agrypon flaveolatum*) and successfully controlled winter moth in Nova Scotia in the 1950s and on Vancouver Island, Canada in the 1970s. Because *A. flaveolatum* potentially posed some risk to non-target insects, we introduced only the specialist *C. albicans*. Each year for 14 years, we collected several thousand *C. albicans* in British Columbia and released the adults the following year at widely-spaced sites in the northeastern United States. As of 2020, we had established *C. albicans* at 41 of 44 sites from southeastern Connecticut to coastal Maine. Beginning in 2005, we made yearly estimates of winter moth density. By 2016, winter moth density had declined from 100–500 pupae/m^2 to 0–10 pupae/m^2 (9–46 pupae/ft^2 to 0–1 pupae/ft^2) at these sites, an average reduction of 98%. This drop in winter moth density was coincident with the onset of 10–40% parasitism by *C. albicans*. At a site in Wellesley, Massachusetts, this decline occurred in 2012, and winter moth densities have remained low there through the present (2022). Defoliation by winter moth in Massachusetts has been undetectable by aerial survey since 2016. Studies of predation on winter moth pupae at these and other sites revealed that predation rates became density-dependent only after *C. albicans* had reduced pupal densities to a level that is now comparable to the density reported at a site in England where winter moth is not an outbreak species.

Elkinton, J. S., G. H. Boettner, and H. J. Broadley. 2022. Biological control success against winter moth in the northeastern United States, pp. 212–223. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

In the late 1990s, defoliation by a green inchworm was first detected near Boston, in the northeastern United States. At first it was assumed to be a native inchworm, such as fall cankerworm, *Alsophila pometaria*. However, a flight of male moths in late December of 2002 suggested it might be the European species known as winter moth, *Operophtera brumata* (Lepidoptera: Geometridae). It is called winter moth because it flies in early winter. Our taxonomic collaborators confirmed this identification in 2003 (Elkinton et al., 2010, 2015). There had been three previous invasions of winter moth in North America, one to Nova Scotia in the 1930s, a second to Oregon in the 1950s, and a third to Vancouver Island in British Columbia in the 1970s; none of these invasive populations spread much beyond the region of their initial invasion. Andersen et al. (2021) showed that all four introductions to North America originated independently from Europe.

In 2005, we initiated a regional survey of winter moth in the northeastern United States and adjacent parts of Canada using pheromone-baited traps (Elkinton et al., 2010) (**Fig. 1**). With the help of various collaborators, we deployed traps from Long Island (near New York City), New York and southeastern Connecticut, northward to Maine, New Brunswick, and Nova Scotia, Canada. Unfortunately, winter moth shares the same pheromone with a native North American species known as Bruce spanworm, *Operophtera bruceata*. This species is ubiquitous across northern North America, even though it almost always remains

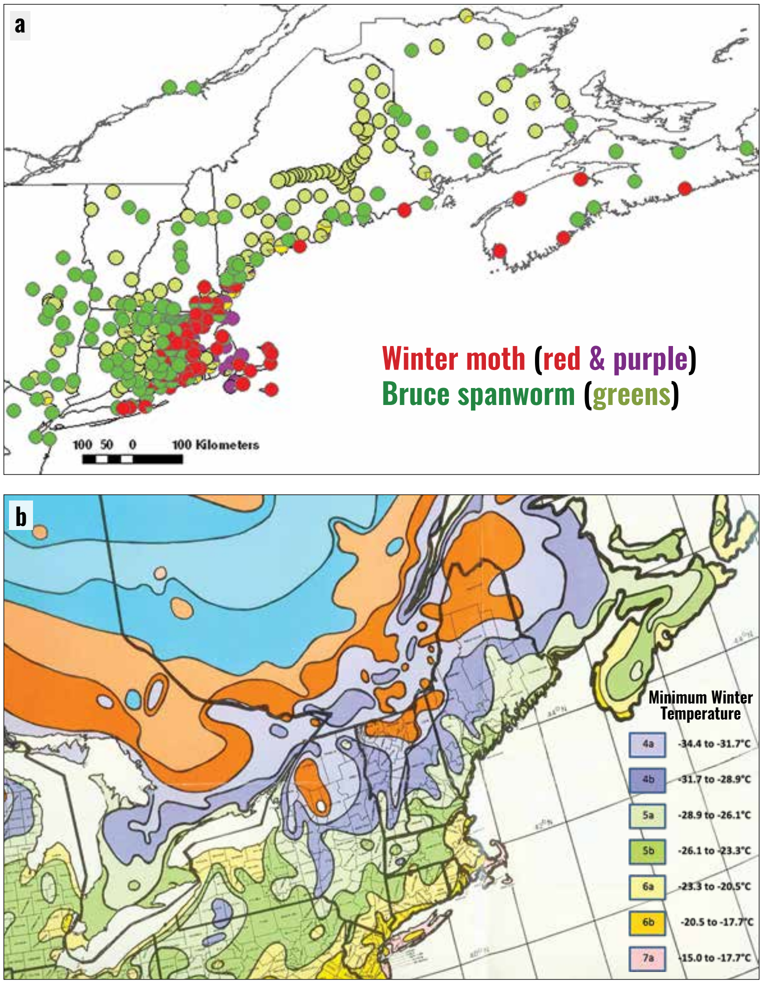

Figure 1. (a) Distribution of winter moth (*Operophtera brumata*) and Bruce spanworm (*Operophtera bruceata*) catches in pheromone-baited traps in northeastern North America, 2005–2007. Most moths were Identified via DNA , red for winter moth, dark green for Bruce spanworm. Others were identified by dissection of genitalia, purple for winter moths, light green for Bruce spanworm. A few moths had intermediate genitalia (yellow). Each circle represents a pie diagram indicating the proportion of 10 moths in each category, in each trap. (b) Plant cold hardiness zone map for northeastern North America. (a: modified from Elkinton et al., 2010; b: modified from Cathey, 1990 and Elkinton et al., 2010)

at low density. Thus, all the traps we deployed filled up with little brown moths, some of which were winter moths and some Bruce spanworm. The two species are not easy to tell apart as wing patterns are not reliable. Instead, we examined the male genitalia, which have a slightly different shape between the two species (Eidt et al., 1966). Unfortunately, we encountered many with genitalia whose shapes were intermediate in form, suggesting that hybridization was occurring between the two species. This conjecture was later confirmed by DNA analysis. For a more reliable distinction between the two species, we turned to extraction and sequencing of the CO1 "barcoding gene", a mitochondrial gene that has become a central tool for insect identification (Hebert et al., 2003).

Identification of moths from survey traps (**Fig. 1a**) revealed a maritime distribution for winter moth that closely matched the USDA Plant Hardiness Zone Map (Cathey, 1990), which is based on minimum winter temperatures (**Fig. 1b**). This pattern of distribution possibly explains why winter moth remained confined to Nova Scotia for many decades after its invasion there in the 1950s. Presumably, winter moth was unable to penetrate the much colder regions immediately to the west in New Brunswick (**Fig. 1b**). This plant hardiness zone map also suggested that the warmer temperatures that may have allowed the winter moth outbreak to occur in Massachusetts also occur elsewhere in the eastern United States. Thus, we suspected that we were on the verge of a major defoliator invasion analogous to that of *Lymantria dispar* (formerly known as gypsy moth). Over the decade following 2003, winter moth became a major defoliator all over eastern Massachusetts, including Cape Cod and Martha's Vineyard, as well as in Rhode Island (Elkinton et al., 2014) (**Fig. 2**). At the same time, winter moth was spreading west across Massachusetts, as documented by pheromone-baited traps deployed along an east-west highway. Between 2005 and 2011, winter moth spread 50 km (31 mi) into the central part of the state (Elkinton et al., 2014). During this same period, however, virtually no defoliation occurred in central Massachusetts, at least as recorded by aerial surveys (**Fig. 2**). In 2012, defoliation by winter moth occurred for the first time along the coast of Maine, a phenomenon that was likely linked to the warming temperatures in the Gulf of Maine (Elkinton et al., 2015).

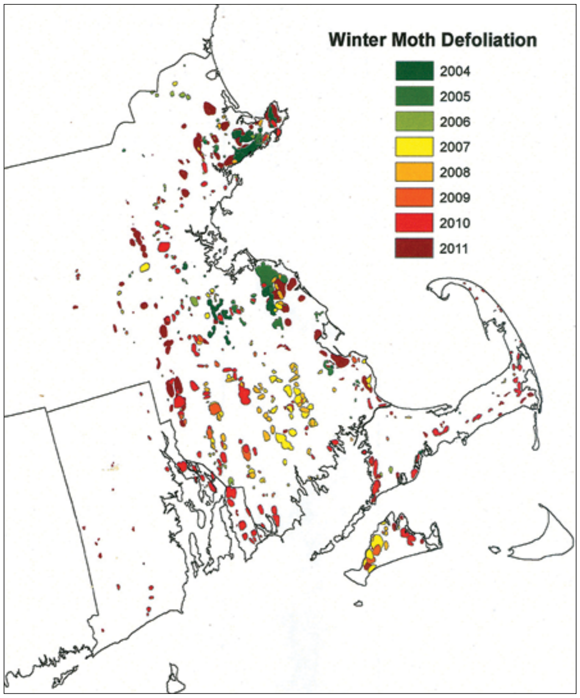

Figure 2. Spread of defoliation by winter moth (*Operophtera brumata*) across eastern Massachusetts from 2004–2011, compiled from annual aerial surveys. (modified from Elkinton et al., 2014)

NATURE OF THE PROBLEM

Winter moth is extremely polyphagous, feeding on a wide range of host plants, including oaks (*Quercus*), maples (*Acer*), birches (*Betula*), and many others (Wint, 1983). It is a potential orchard pest, and larvae develop well on apple (*Malus*). In Massachusetts and Rhode Island, winter moth was especially damaging to blueberry (*Vaccinium*) crops. Larvae feed inside the buds, where they are inaccessible to most pesticides, destroying the developing berries before the buds open. Many blueberry growers in Massachusetts lost whole crops during the winter moth outbreak, amounting to thousands of dollars of losses each. Winter moth is also a pest of cranberries (*Vaccinium*; A. Averill, pers. comm.). Winter moth eggs typically hatch at or before bud-break of the host plant, and the larvae (**Fig. 3a**) bore into the expanding buds so that much of the damage occurs before leaf expansion. Although there may be many larvae per bud in outbreak populations, defoliation of oak and maple rarely reached 100% (in Massachusetts and Rhode Island at least), presumably because the larvae finish feeding and pupate in late May before defoliation is complete. Larvae then drop to the ground where they pupate (**Fig. 3b**) inside earthen cocoons near the soil surface beneath the tree. Pupae remain in the soil until November and December, when the adults (**Fig. 3c,d**) emerge. The female, as in many inchworm (geometrid) species, has no wings (**Fig. 3d**). Instead of flying, she spends all of her energy on egg production. Females emit pheromone to attract the flying males. After mating, females climb the trunk of the host tree, laying about 250 eggs singly on the tree bark.

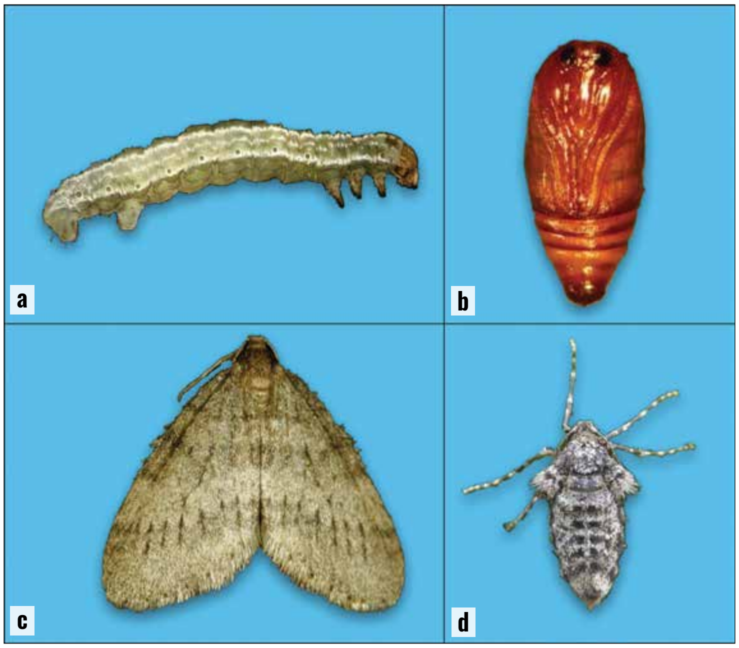

Figure 3. Winter moth (*Operophtera brumata*) life stages: (a) larva; (b) pupa; (c) adult male; (d) adult female. (a–d: D. Wagner; reprinted from Elkinton et al., 2018)

The typical damage caused by winter moth is partial defoliation of the leaves, which can cause lasting damage to the tree. Simmons et al. (2014) showed that defoliation by winter moth caused significant decline in growth in red oak (*Quercus rubra*) in Massachusetts, as measured by growth increments seen in cores of tree stems. Embree (1967) reported that repeated defoliation by winter moth resulted in as much as 40% tree mortality in red oak stands in Nova Scotia.

Unlike other defoliators, such as *L. dispar* (formerly gypsy moth) or forest tent caterpillar (*Malacosoma disstria*), whose outbreaks are terminated by disease epizootics, winter moth outbreaks suffer no such epizootics, even though the moth does have a viral disease (Graham et al., 2004; Burand et al., 2011). Consequently, winter moth defoliation persisted year after year in Nova Scotia in the 1950s (Embree, 1965; Elkinton et al., 2015) and, later, in Massachusetts (Elkinton et al., 2021). As a result, many forest and shade trees started to die after several consecutive years of defoliation.

WHY CONTROL THIS INVASIVE SPECIES?

By 2010, winter moth was causing widespread defoliation of the dominant deciduous forest and shade trees across eastern Massachusetts and Rhode Island (**Fig. 2**). It was causing complete losses of blueberry crops and was a serious pest of apples and cranberries—all important, traditional crops in southern New England. The defoliation continued year after year, and many valuable shade trees were dying across the region. Town managers and homeowners in eastern Massachusetts were spending millions of dollars each year on pesticides to protect shade trees or to remove and replace dying or dead trees. A successful biological control effort, such as that previously achieved in Nova Scotia and in the Vancouver region of British Columbia, would permanently solve these problems and save these annual costs.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Biological control efforts against winter moth in North America began in Nova Scotia in the 1950s, where winter moth had invaded in the 1930s (Hawboldt and Cuming, 1950). Natural enemies for release in Nova Scotia were collected in France and Germany (Roland and Embree, 1995) with the help of the Commonwealth Institute of Biological Control. A total of 63 parasitoid species known to attack winter moth in Europe were considered for release in Nova Scotia (Wylie, 1960). The most abundant species collected were the tachinids *Lypha dubia* and *Cyzenis albicans* and the ichneumonid *Agrypon flaveolatum*.

Between 1954 and 1957, releases of more than 1,000 *C. albicans* were made at one location (Oak Hill) in Nova Scotia (Graham, 1958). In addition, 250 *A. flaveolatum* were released in 1956. Releases of both parasitoids continued elsewhere in Nova Scotia until 1965. Before 1963, the parasitoids were collected in Europe; thereafter, they were collected at sites in Nova Scotia where they had become abundant. Two other species of tachinids, including *L. dubia*, and two other ichneumonid species were released (Graham, 1958; Roland and Embree, 1995), but these species were not recovered.

Parasitism by both *C. albicans* and *A. flaveolatum* increased at the Oak Hill site in Nova Scotia beginning in 1959 and reached a peak of about 40%, by both species together, in 1962, whereupon winter moth densities declined to undetectable levels (Embree, 1965) and have remained at low density ever since. Subsequent work showed that low densities of winter moth persisted in Nova Scotia apple orchards over the following decades (McPhee et al., 1988), but that parasitism by *C. albicans* and *A. flaveolatum* declined to around 10%. Predators of winter moth pupae in the soil are now thought to prevent low-density populations from increasing (Pearsall and Walde, 1994).

A repetition of this winter moth biological control program (based on the same two parasitoids) was later carried out on Vancouver Island in western Canada after winter moth invaded the area in the 1970s (Gillespie et al., 1978). Winter moth populations declined, mainly due to *C. albicans*, within several years of the first parasitoid releases (Embree and Otvos, 1984). The rapid success of this project, compared to the one in Nova Scotia or the subsequent one in the northeastern United States, was likely due to the larger number of *C. albicans* flies that were released into a winter moth population that covered a much smaller geographical area than was the case in the other two invaded areas (Elkinton et al., 2021).

In Massachusetts, we decided to release only *C. albicans* (**Fig. 4**) and not *A. flaveolatum*, because the latter species is thought to attack the larvae of several non-target moth species. Furthermore, preliminary DNA evidence suggests that *A. flaveolatum* may be a group of unseparated cryptic species needing further taxonomic work. In contrast, Embree and Sisojevic (1965) showed that *C. albicans* was a specialist attacking only winter moth.

To collect *C. albicans*, we traveled to locations where the fly was established and collected late-instar winter moth larvae, some of which had larval *C. albicans* immature stages inside them. In 2004, we attempted to collect *C. albicans* in Nova Scotia, but the densities of winter moth were too low to do so. In subsequent years, we traveled to Vancouver Island, where winter moth densities had remained sufficiently high to enable us to collect many thousands of winter moth larvae over several weeks before larvae in the field pupated. We reared the collected larvae to their pupal stage and then dissected the winter moth pupae in mid-summer to determine the proportion that were parasitized by *C. albicans*. From parasitized winter moth pupae, we

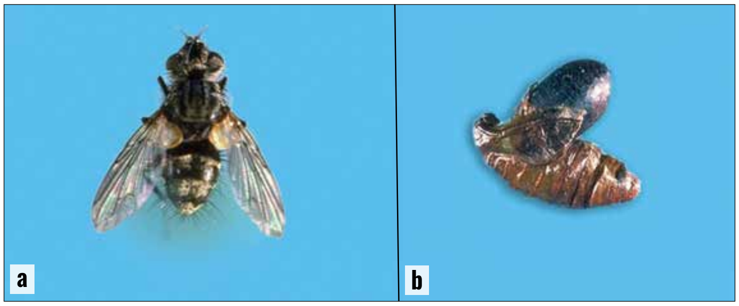

Figure 4. *Cyzenis albicans* (a) adult and (b) puparium inside winter moth pupa. (a,b: Nicholas Condor; reprinted from Elkinton et al., 2018)

obtained *C. albicans* puparia by rearing. These puparia were then held over the summer months in Petri dishes in growth chambers at 12°C (54°F) in summer and fall, and then dropped to 4°C (39°F) in winter. In April, fly puparia were moved to room temperature to induce adult fly emergence. Once flies had emerged, they were allowed to mate, and we then took the flies and released them at new release sites each year (Elkinton et al., 2021). At each site, our goal was to release 1,500–2,000 flies. In 2014, we started to recover large numbers of *C. albicans* near an early release site in Wellesley, Massachusetts, and so we switched our *C. albicans* collection efforts to that location to support more releases elsewhere.

In 2016, we altered our release strategy. Instead of releasing adult flies in the spring, in November we placed *C. albicans* puparia in peat moss inside screen cages and partially buried the cages in the soil at the intended release site. This allowed flies to emerge in spring as dictated by local temperatures. When flies had emerged inside cages, we opened the cage to allow them to escape. This release method resulted in higher rates of *C. albicans* establishment (Elkinton et al., 2021).

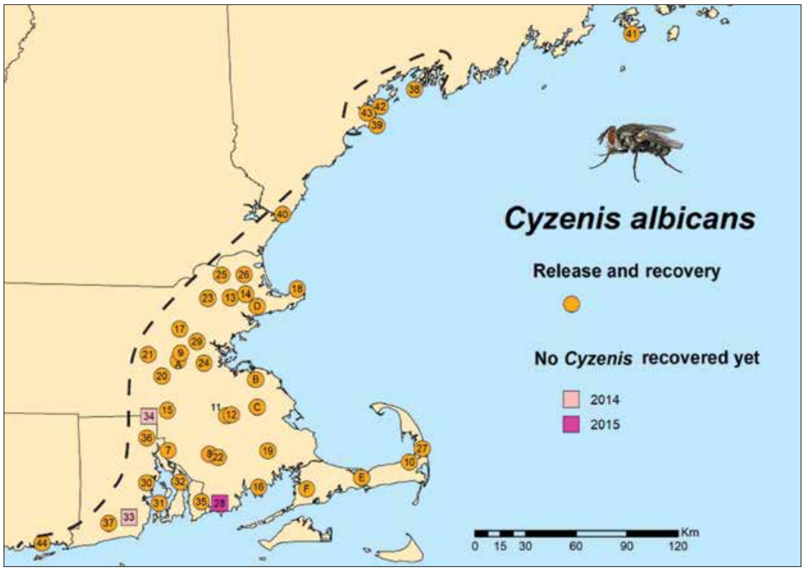

Figure 5. *Cyzenis albicans* release and recovery locations in New England, 2005–2018. Dotted line delineates the boundary of the area with defoliating populations of winter moth, numbers refer to site IDs. As of 2020, we had not yet recovered *C. albicans* from several sites where releases had been made in 2014 or 2015, as indicated by purple squares. (reprinted from Elkinton et al., 2021 with permission from Wiley)

HOW WELL DID IT WORK?

By 2021, we had introduced >80,000 *C. albicans* flies, distributed across 44 sites in eastern Massachusetts, Rhode Island, Connecticut, and Maine, and establishment of the fly had been confirmed at 41 of them (**Fig. 5**) (Elkinton et al., 2021). Similar to events in Nova Scotia in the 1950s (Embree, 1965), we found that in Massachusetts, 3–6 years were required after release before we recovered *C. albicans* at our release sites. Because there is only one generation per year of both the fly and the winter moth, several years were needed for the 1,500–2,000 flies released per site to catch up with the millions of winter moth larvae at a site and become detectable in field-collected larvae.

We made our first recovery of *C. albicans* at the Wellesley, Massachusetts site in 2010, following a 2008 first release there (**Fig. 6a**). By 2016, at each of our six long-term study sites (**Fig. 6**), winter moth densities had declined from 100–500 pupae/m^2 to 0–10 pupae/m^2 (9–46 pupae/ft^2 to 0–1 pupae/ft^2), and these low densities persisted in subsequent years. This decline coincided with the year when parasitism reached 10–40%.

From 2003 to 2018, personnel from the Massachusetts Department of Conservation and Recreation conducted annual aerial surveys in June, when winter moth defoliation had reached peak levels. Surveyors sketched the location of defoliation onto topographic maps, and this information was digitized as GIS polygons whose areas were then summed (**Fig. 7**). Ground survey crews checked each defoliated site to verify that winter

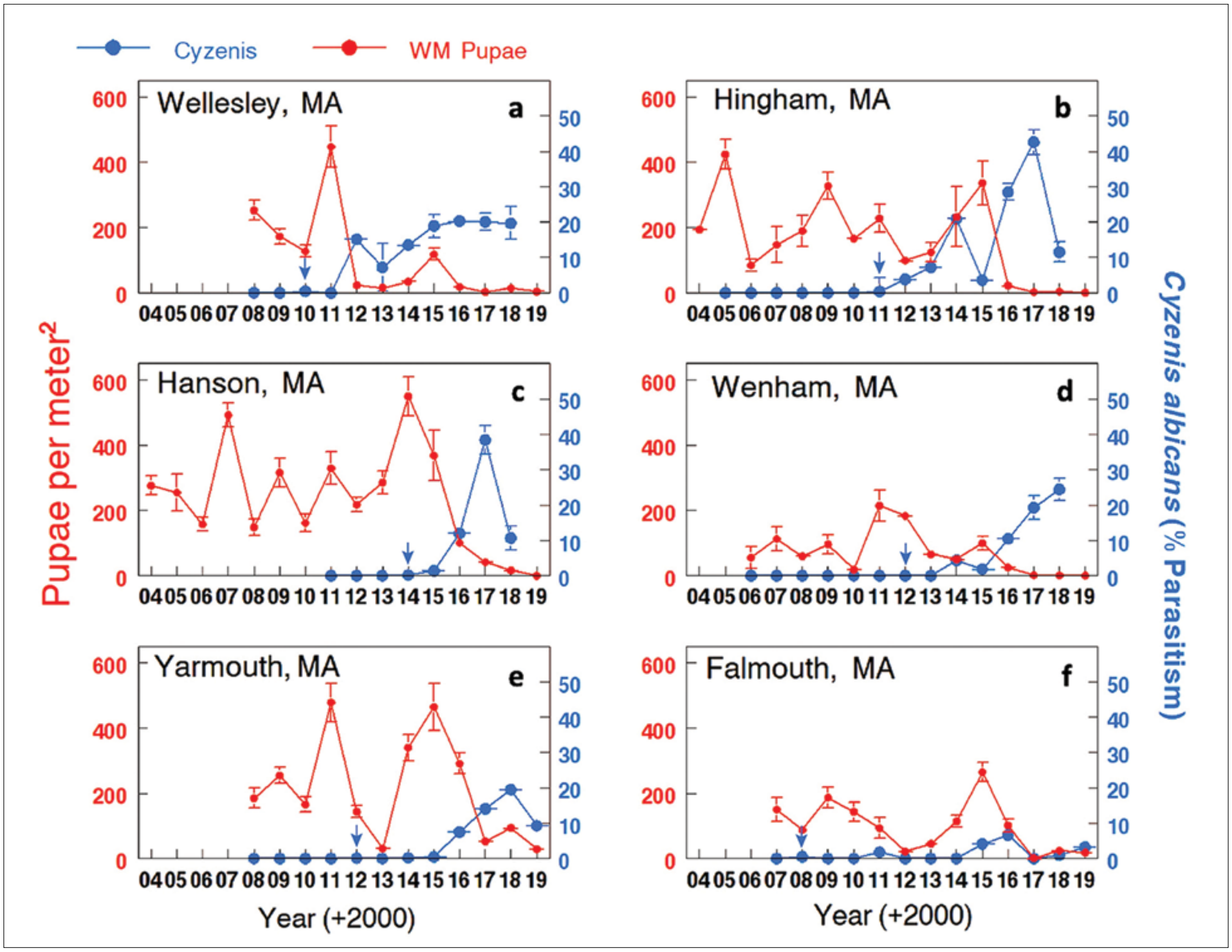

Figure 6. Yearly density of winter moth (*Operophtera brumata*) pupae (red) and percent parasitism by *Cyzenis albicans* (blue) following release at six widely spaced release sites in Massachusetts. Blue arrows indicate year of first recovery of *C. albicans*. (reprinted from Elkinton et al., 2021 with permission from Wiley)

moth was the cause of the defoliation. Annual defoliation by winter moth in Massachusetts ranged from 2,266 to 36,360 ha (5,600–89,848 acres) between 2003 and 2015, but defoliation declined to undetectable levels from 2016 to 2018 (**Fig. 7**). The decline coincided with the widespread occurrence of parasitism by *C. albicans*.

We conclude that releases of *C. albicans* converted winter moth into a non-pest in eastern Massachusetts and Rhode Island. We expect that these low densities will persist indefinitely as they have in Nova Scotia and in the Vancouver region of Canada.

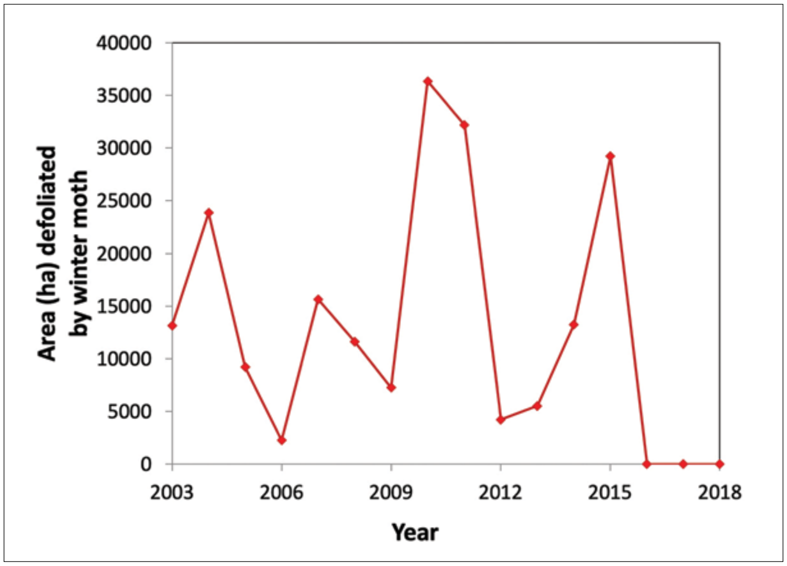

Figure 7. Area (ha) defoliated by winter moth (*Operophtera brumata*) in Massachusetts from 2003–2018 based on aerial survey followed by confirmation by ground surveyors. (reprinted from Elkinton et al., 2021 with permission from Wiley)

THE ECOLOGY OF THE BIOCONTROL PROGRAM

Winter moth is one of the world's most widely studied insects in terms of population ecology. Classic ecological studies (Varley and Gradwell, 1960, 1968; Varley et al., 1973) helped introduce the analysis of life tables to insect ecology and promoted the use of key-factor analysis to identify the main determinants of population density change. These authors studied winter moth in an oak forest near Oxford, England, and they concluded that its population was regulated at low density by predation on pupae in the soil or leaf litter beneath the study trees. Mortality during the egg and larval stages, which they called "overwintering mortality," accounted for most of the year-to-year variation in winter moth density. Although this mortality was the key factor responsible for population density change between years, it was not density dependent, and thus did not serve to regulate winter moth densities. They believed that overwintering mortality was dominated by starvation and dispersal due to asynchrony of winter moth larval hatch with budburst. Subsequent research suggested that pupal predation was mainly caused by staphylinid and carabid beetles (Frank, 1967). Mortality caused by the tachinid *C. albicans* was not a major factor in these low-density populations.

On Vancouver Island, British Columbia, Roland (1994) studied factors governing the dynamics of invasive winter moth following *C. albicans* establishment. He presented evidence that *C. albicans* reduces winter moth densities to a level at which pupal predation caused density-dependent mortality and regulated low-density populations, much as it does in England (Varley et al., 1973) (**Fig. 8a**). Research by Pearsall and Walde (1994) in Nova Scotia also supports this view. They showed that mortality in low-density populations in apple orchards was dominated by pupal predation and that parasitism by *C. albicans* varied between 4 and 28%, far lower than the initial values in 1960–1962 reported by Embree (1966). Studies in England by Raymond et al. (2002), however, indicate that in some habitats, predation by pupal predators declines with higher-density populations of winter moth, potentially allowing winter moth to escape into an outbreak phase (**Fig. 8b**). This result implies that above some threshold density of winter moth, predators in the soil become saturated and cannot keep up with, or regulate, winter moth densities.

In Massachusetts, we studied predation on winter moth pupae at sites across Massachusetts, including those illustrated in **Fig. 6**, to see if these earlier findings applied to winter moth populations in New England (Broadley, 2018; Broadley et al., in press). The soil-dwelling predator community at these sites consisted of many species of predatory beetles and two species of shrews. With predator exclusion experiments that involved placing pupae in wire-mesh cages of different mesh sizes, we demonstrated that many of these predators were feeding on winter moth pupae. Pupal predation was found to increase with winter moth density, but only after *C. albicans* was established at the study sites (**Fig. 8c**). The role of *C. albicans*, therefore, appears to be to stabilize winter moth pupal populations at a low enough density for pupal predators to regulate winter moth populations, as Roland (1994) had demonstrated on Vancouver Island. Indeed, the

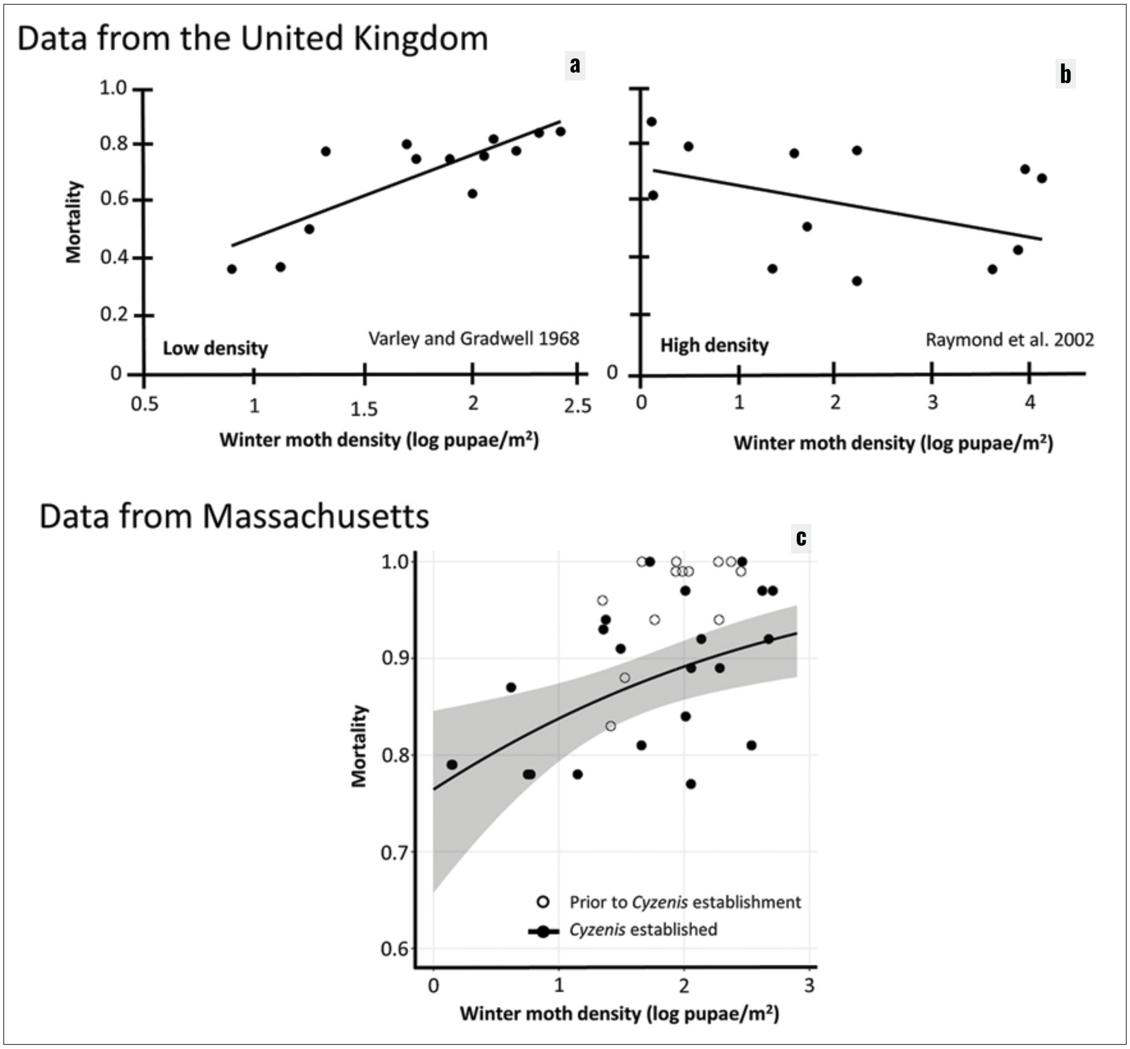

Figure 8. Yearly proportion of winter moth (*Operophtera brumata*) pupae eaten by predators at (a) a site near Oxford England; (b) on a moor in Scotland; and (c) at sites in eastern Massachusetts. (a: modified from Varley et al., 1973; b: modified from Raymond et al., 2002; c: modified from Broadley, 2018; Broadley et al., in press)

densities of winter moth pupae at our sites in Massachusetts are now comparable to those reported by Varley et al. (1973) in England (**Fig. 8a**). Before the establishment of *C. albicans*, pupal densities were higher, and there was no evidence of density-dependent pupal predation, presumably because some of the important predators were approaching saturation (Broadley et al., in press). But the very high densities (10,000 pupae/m^2) reported by Raymond et al. (2002) at outbreak sites in England were never observed in Massachusetts (**Fig. 8c**). Broadley et al. (in press) noted that mortality to winter moth from soil predators in Massachusetts decreased following the establishment of *C. albicans*, in contrast to the data from Nova Scotia and British Columbia, as presented by Roland (1994), where mortality increased following establishment of *C. albicans*. A likely explanation for this difference is that the predator community at the sites in Nova Scotia and British Columbia may be less robust than at the sites in Massachusetts, so that the predators were well above the saturation threshold at outbreak densities, as in **Fig. 8b**, whereas at our sites in Massachusetts at winter moth's highest densities, predation rates were at peak values close to, but not above the saturation threshold (**Fig. 8c**).

Cyzenis albicans achieves its highest rates of parasitism at high winter moth densities because *C. albicans* is attracted to partially defoliated leaves (Roland et al., 1995) and lays tiny eggs along the margins of damaged leaves. Parasitism of winter moth larvae occurs when they consume those eggs. When defoliation levels decline, it is more difficult for *C. albicans* to find suitable leaves on which to lay its eggs. This relationship explains why parasitism by *C. albicans* declined in Nova Scotia following the decline of winter moth populations to low density after 1962. The same is likely to happen in eastern Massachusetts and Rhode Island. Nevertheless, *C. albicans* will remain available to respond to any increases in winter moth density with increasing parasitism rates. In this way, *C. albicans* may resemble other high-density specialists in other population systems, such as the LdMNPV virus of *L. dispar* (formerly gypsy moth) that reliably causes the collapse of high-density populations but causes minimal mortality in low-density populations (Elkinton and Liebhold, 1990).

This research illustrates why it is important to understand the impact of *C. albicans* in the context of all the other causes of mortality occurring in the winter moth system. It is vital that we try to quantify and explain the other factors influencing winter moth densities, in addition to *C. albicans*. This is only sometimes accomplished in other biological control projects.

BENEFITS OF BIOLOGICAL CONTROL OF WINTER MOTH

Winter moth, once a major forest and shade-tree defoliator in southeastern New England, is now a non-pest and is likely to remain so permanently. This biological control project has saved municipalities and homeowners in this region millions of dollars through avoidance of frequent pesticide application costs and has likely had conservation benefits for non-target species likely to be affected by such pesticide applications. The blueberry growers in the region no longer face losses of their crops, and winter moth no longer has the potential to become a major new invasive defoliator across larger portions of the eastern United States.

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CHAPTER 21

Biological Control of Ambermarked Birch Leafminer

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NON-TECHNICAL SUMMARY

The ambermarked birch leafminer (AMBLM), *Profenusa thomsoni* (Hymenoptera: Tenthredinidae), is a leaf-mining sawfly native to Eurasia, ranging from the United Kingdom to Turkey and Japan and invasive in North America. This leafminer was accidentally introduced into the eastern United States in about 1923 and subsequently spread to midwestern states and Canadian provinces. Reaching Alaska around 1996, infestations peaked at over 140,000 acres (56,600 ha) and spanned from Haines to Fairbanks. The most severe damage was found throughout the Anchorage area and other urban centers of the state such as Fairbanks and Soldotna. The damage caused by AMBLM is mostly aesthetic, but recent analyses show that it appears to also have slowed the growth rate of Alaska white birch trees, *Betula neoalaskana*, in the area. As a result, it is suspected that the loss of leaf area to leaf mining by AMBLM can affect tree growth, given the short growing season in Alaska.

To control the spread and damage from AMBLM, a biological control project was started in 2003, and the parasitoid wasp *Lathrolestes thomsoni* (Hymenoptera: Ichneumonidae) was selected for release. Parasitized leafminer larvae were collected from the provinces of Northwest Territories and Alberta in Canada and moved to Alaska where adult parasitoids were reared for release. From 2004 to 2008, 3636 adults of *L. thomsoni* were released in Alaska white birch stands in Anchorage, Soldotna, and Fairbanks, Alaska. As of 2011 when the control program ended, this parasitoid had established at most release sites.

Coinciding with the release and establishment of *L. thomsoni* in Alaska in 2006, permanent research plots were established to measure the density of AMBLM and determine the effectiveness of the biological control program. The percentage of leaves mined was determined annually at 20 research sites from 2006 to 2011 to monitor changes in pest density. By 2011, the percentage of leaves mined had declined from over 70% in 2006 to just 19%. Post-project sampling done in 2019 showed that the percentage of leaves mined had decreased further to around 8%.

Two additional ichneumonid parasitoids, adventive or perhaps native, were also discovered attacking AMBLM during the biological control project. These parasitoids, *Lathrolestes soperi* and *Aptesis segnis*, were found to have caused significant levels of mortality to AMBLM populations (27% and 14% mortality, respectively) in 2011. A follow-up survey in 2019 compared the relative abundance of the two *Lathrolestes*

Soper, A. L. and J. C. Andersen. 2022. Biological control of ambermarked birch leafminer, pp. 224–231. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

species that parasitize larvae in leaf mines. That study found that the combined parasitism by the two *Lathrolestes* species was 70%, of which two thirds was caused by the introduced agent *L. thomsoni*. While *A. segnis*, a soil-dwelling ectoparasitoid, was not resampled in 2019, it also likely contributed additional mortality to the pest population.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Figure 1. Ambermarked birch leafminer, *Profenusa thomsoni*, female ovipositing on a white birch leaf. (US Forest Service)

The ambermarked birch leafminer (AMBLM; **Fig. 1**), *Profenusa thomsoni* (Hymenoptera: Tenthredinidae), invaded North America at the beginning of the 20th century and was first reported in the eastern United States in 1923 (Ross, 1951; MacQuarrie, 2008) where it never became a high-density pest. In eastern North America, this sawfly occurs in the United States from New England to the Great Lake States and in Canada from the Maritimes to Manitoba. In western North America, this leafminer was first reported in Alberta, which it invaded before 1970 (Digweed, 1995) and where it reached high densities in the early 1990s (Digweed, 1995). From Alberta, the leafminer spread north and west in Canada. By 1991, AMBLM was first detected in Alaska in the town of Haines; however, the pest was not correctly identified until 1996 when many birch trees showed symptoms of damage in the Anchorage bowl region (Snyder et al., 2007). Surveys for this pest's damage in Alaska were done from 2004 to 2006 (Snyder et al., 2007) and showed that AMBLM was widespread in southcentral Alaska, as well as in the Fairbanks area. Aerial flight surveys in 2003 estimated that AMBLM damage affected over 32,000 acres (12,140 ha) in the Anchorage bowl and extended into the Matanuska-Susitna Valley (Wittwer, 2004; Snyder et al., 2007). Surveys conducted through 2006 found that AMBLM was present in >20% of the surveyed area. Due the geographic isolation in Alaska and limited transportation corridors, it was initially thought that there would be some protection from the spread of invasive species. However, the spread of AMBLM in Alaska suggested that there was a strong association of human population centers and major travel routes with the establishment of the leafminer (Snyder et al., 2007). Moreover, the speed at which the pest moved throughout the state, and the concentration of damage in the Anchorage bowl, made this a pest of primary concern for the USDA Forest Service (**Fig. 2**).

Nature of the Problem

The ambermarked birch leafminer is a serious defoliator of several species of birch (*Betula* spp.) and is a species of primary concern in the urban areas of Alaska where birch trees are dominant. The immediate impact of leaf mining of birch trees is aesthetic. Presently, there have been no studies on the long-term effect of leaf mining on the health and growth of birch, but in the northern latitude of Alaska where the growing season is short, it is likely to slow the growth of the tree (MacQuarrie, 2008; Van Driesche et al., unpub. data.). Additionally, it is suspected that severe defoliation could increase tree susceptibility to secondary infections (Hoch et al., 2000; Snyder et al., 2007).

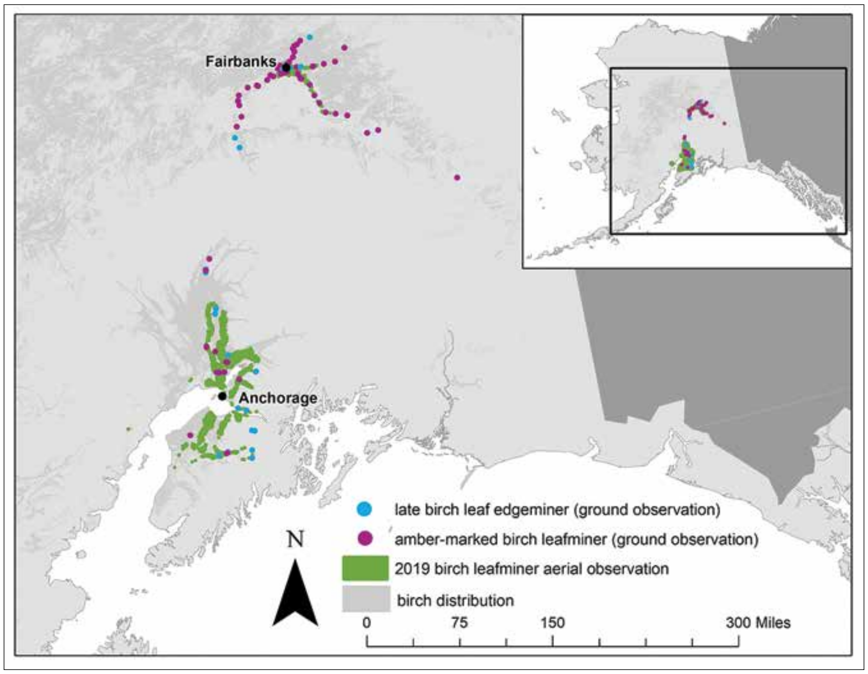

Figure 2. Map depicting the area and extent of ambermarked birch leafminer, *Profenusa thomsoni*, presence and defoliation in Alaska. (US Forst Service)

WHY CONTROL THIS INVASIVE SPECIES?

The invasion of AMBLM led to widespread browning (**Fig. 3**), due to leaf mines (areas of damage caused by larval feeding between the upper and lower epidermis of their host leaf) that occupied much of the leaf of Alaska white birch in urban and forested areas of the Anchorage bowl and parts of the interior. This mining, while primarily aesthetic, was suggested to have led to an increase in pesticide use, particularly in the Anchorage area, but records of changes in use levels are not available. While the initial goal of the biological control program was to reduce the use of pesticides and improve the trees' appearance, it was speculated that due to the short growing season in the Anchorage bowl, any type of leaf damage could result in reduced plant growth rates and overall health. Physiological impacts of the leafminer on birch trees were unknown when the biological control program was initiated. However, recently Van Driesche et al. (unpub. data) used tree-ring data to examine changes over time in the growth of Alaska white birch trees in the Anchorage Bowl from 1984 to the present. This dataset includes pre-introduction growth rates (from 1984 to 1995), peak outbreak growth rates (from 1996 to 2007), and post-biological control

Figure 3. Alaska white birch tree leaves showing leaf mining damage from ambermarked birch leafminer, *Profenusa thomsoni*, larvae. (US Forest Service)

growth rates (2008–present). Based on their analyses of Alaska white birch growth rings, Van Driesche et al. (unpub. data) found that growth rates before the outbreak of AMBLM averaged around 2.1 mm (0.08 in) per year. However, after the outbreak of AMBLM in the region, growth dropped significantly to around 1.8 mm (0.07 in) per year. Their data also showed that after the achievement of biological control of AMBLM in the region, growth rates continued to decrease before stabilizing at around 1.3 mm (0.05 in) per year (95% CI: 1.21 to 1.52). Whether this continued reduction is the result of the emergence of a second pest in this system, the late birch leaf edgeminer, *Heterarthrus nemoratus* (Hymenoptera: Tenthredinidae), or is indicative of the inability of birch species in high-latitude locations to rebound after damage from AMBLM is unknown. However, in a similar high-latitude setting, birch trees that suffered defoliation in northern Fennoscandia caused by the feeding of two moths, *Epirrita autumnata* and *Operophtera brumata* (both Lepidoptera: Geometridae), failed to fully recover even one hundred years after their initial defoliation events (Vindstad et al., 2019). In that region, climate change (Jepsen et al., 2008), as well biotic factors such as tree age (Young et al., 2014), may be interacting to compound the effects of tree stress in northern latitudes. Taken together, the results from Van Driesche et al. (unpub. data) and the results from northern Fennoscandia suggest that moderate amounts of leaf damage, as might be expected from a leafminer, could result in profound and cascading effects for birch tree growth and fitness in northern latitudes.

THE ECOLOGY OF THE PROBLEM

Life history information and life tables for Alaska populations of AMBLM were developed by MacQuarrie et al. (2008). In Alaska, AMBLM has one generation per year, the population is parthenogenetic, and no males are known (Benson, 1959; MacQuarrie, 2008). Adults emerge from late-June to August and deposit their eggs singly on the central midrib of birch leaves (Martin, 1960; Digweed, 2006). Many eggs can be laid per leaf by one or several females when population densities are high. In late July and August, larvae feed on the inner tissues of the leaf, and leaves turn brown and cease to photosynthesize effectively. In mid-to-late-August, mature larvae drop to the soil, where they form earthen cells and overwinter as prepupae or pupae (Digweed, 2006). The adult sawflies emerge the following summer from June through August.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The classical biological control program against AMBLM was started in Alaska in 2003 through the combined efforts of the USDA Forest Service, the Canadian Forest Service, and the University of Alberta, with the goal of introducing a highly specialized parasitoid known at the time as *Lathrolestes luteolator* (Hymenoptera: Ichneumonidae), but which is now correctly recognized as *L. thomsoni* (**Fig. 4**). This parasitoid had been observed attacking AMBLM populations in Edmonton, Alberta, where it was associated with the collapse of the leafminer population there (Digweed et al., 2003; MacQuarrie, 2008). Subsequently, additional leafminer populations from Edmonton and Edson in Alberta, and Hay River and Fort Smith in the Northwest

Figure 4. The introduced biological control agent *Lathrolestes thomsoni* inspecting ambermarked birch leafminer, *Profenusa thomsoni*, mines before parasitizing the larvae. (A. Soper, Cal Poly)

Territories, were used as sources to collect parasitized leafminers to then rear parasitoids for release in Alaska. *Lathrolestes thomsoni* parasitoids were collected as either immature stages inside parasitized host larvae in the year preceding their release or as adults in July and August of the year of release. Individuals collected were then either reared to the adult parasitoid stage before release or were released directly if adult parasitoids had been field-collected.

Most of the adult parasitoids released in Alaska were obtained by rearing parasitized leafminers, using techniques modified from Fuester et al. (1984) and described in detail by MacQuarrie (2008). Infested leaves were collected from urban birch trees (located in city parks or landscaped yards) in western Canada between late-July and early-September and then placed in tubs of soil for host pupae to overwinter. Leafminers in tubs were either overwintered in Alberta, and then taken to Alaska in the spring, or were transported in fall to Alaska and overwintered there. In spring, cages were checked daily for insect emergence from mid-June until parasitoid emergence ended, typically around mid-August. The first releases of this parasitoid in Alaska were made in 2004 and 2005 by Chris MacQuarrie, then a graduate student at the University of Edmonton, Alberta. In 2006, the University of Massachusetts Amherst joined the biological control program and continued the project until the end of 2011.

In August 2007, Andrew Bennett, an ichneumonid Research Taxonomist with the Canadian National Collection of Insects, determined that the parasitoid being released in Alaska was not *L. luteolator* as previously believed and that past publications had applied that name incorrectly to specimens from Canada. In 2009, Alexey Reshchikov of the University of St. Petersburg revised the Nearctic members of the genus *Lathrolestes* and described the parasitoid being released as a new species. Based on his results, it was determined that there were no records of the European species *L. luteolator* from North America, and the parasitoid being released for biological control of AMBLM in Alaska was then described as *L. thomsoni* to reflect its host association (Reshchikov et al., 2010).

During studies evaluating the establishment of *L. thomsoni* in Alaska, two other ichneumonid parasitoids of unknown origin were found attacking AMBLM. The first new parasitoid was an unnamed endoparasitoid of AMBLM larvae in leaf mines, which was later described as *Lathrolestes soperi* (Reshchikov et al., 2010). The second parasitoid was first noticed in 2007 among insects caught in emergence cones placed over soil under birch trees infested with AMBLM. Andrew Bennett of the Canadian National Collection identified that species as *Aptesis segnis*, but little was then known about this species apart from inferences made from notes on specimens in museum collections and old agricultural reports (Parrott and Fulton, 1915). More recently, Soper and Van Driesche (2019) compiled a synthesis of its life history and its role in the life cycle of AMBLM in Alaska. The species seems to be broadly distributed across Canada, having been collected from Saskatchewan, Alberta, and Quebec (Krombein et al., 1979).

During the beginning phases of the biological control project, the presence of these two additional parasitoid species led to some concern that they would hinder the establishment of the introduced biological control agent. As a result, a DNA analysis was developed to identify the species of *Lathrolestes* larvae or eggs found in parasitized hosts from leaf mines. These analyses showed that *L. soperi* contributed significant mortality to AMBLM larvae, with parasitism by *L. soperi* increasing from 8% in 2006 to over 27% in 2011 at non-release sites (Soper and Van Driesche, 2019). In 2019, total parasitism from both species of *Lathrolestes* was 70%, and the introduced species *L. thomsoni* was responsible for more than two thirds (71%) of total parasitism, with the remainder (29% of total) being from *L. soperi* (Andersen et al., 2021).

HOW WELL DID BIOLOGICACL CONTROL WORK?

In univoltine pests and parasitoid systems, population adjustments often require several years to reach new equilibria after agent introduction (Huffaker et al., 1976). Formal funding for the project ended in 2011, but some further post-release monitoring eight years later proved important in gaining a final understanding of the outcomes in the system (Andersen et al., 2021). In 2019, fifteen years after the first releases of *L. thomsoni*, a survey found that AMBLM had been reduced to non-pest status across most study sites in the Anchorage Bowl (Andersen et al., 2021). Results presented by Soper et al. (2015) and Andersen et al. (2021) showed that the percent of leaves mined by AMBLM decreased from ~ 70% in 2006 to ~ 8% in 2019, and that concurrent with this decline in the level of leaf mining by AMBLM in the Anchorage bowl, the proportion of AMBLM that were parasitized increased from ~10% in 2006 to ~70% in 2019 (**Fig. 5**). Surveys of long-term study sites established by Soper et al. (2015) beginning in 2006, found high levels of parasitism by *Lathrolestes* species at most of the 11 study sites: nine sites had parasitism rates ≥67%, and two sites had parasitism ≥95% (Andersen et al., 2021). DNA analyses showed that both *Lathrolestes* species have persisted or increased, with *L. soperi* present at most sites at levels similar to those observed in 2011, while *L. thomsoni* contributed high levels of additional, new parasitism (Andersen et al., 2021).

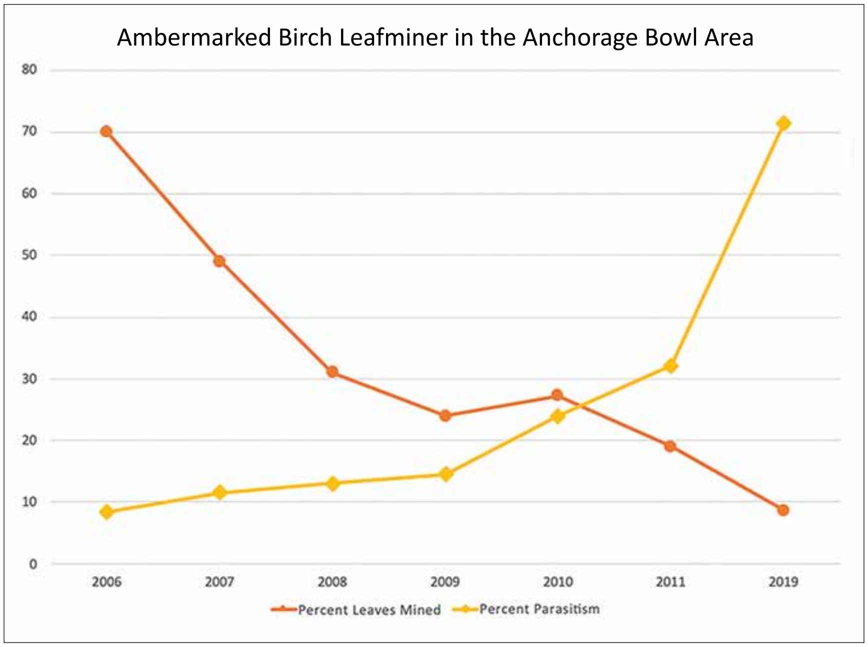

Figure 5. The change in the percent parasitism of ambermarked birch leafminer (*Profenusa thomsoni*) (gold) and the corresponding decrease in the percent of leaves with ambermarked birch leafmines (orange) from 2006 to 2019. (modified from Andersen et al., 2021)

While the efforts to suppress AMBLM were highly successful, a second invasive European tenthredinid leafminer, *H. nemoratus*, has subsequently reached the Anchorage bowl area and greatly increased in density (Van Driesche et al., unpub. data). This species was first noted in Alaska in 2003 (Snyder et al., 2007). Lundquist et al. (2012) reported 36% of the leaves were mined by *H. nemoratus* (a number comparable to AMBLM at the time). As AMBLM's density declined, the density of leaves mined by *H. nemoratus* increased, ultimately accounting for >90% of all leafmines in 2021 (Van Driesche et al., unpub. data). Following the suppression of AMBLM through biological control, *H. nemoratus* has become the dominant leafminer attacking Alaska white birch in the Anchorage Bowl.

BENEFITS OF BIOLOGICAL CONTROL OF AMBLM

Classical biological control of AMBLM met its goal of reducing the pest's density. This likely had the effect of reducing pesticides applied for its control in urban areas, but no records exist that quantify this result. Tree growth rates of Alaska white birch declined during the period of highest AMBLM density. However, based on Van Driesche et al. (upub. data), suppression of AMBLM did not result in the return of birch growth rates to pre-invasion levels. While retrospective correlations of tree growth and observed events do not prove causality, it appears that the most likely cause of the decline of birch growth was indeed leaf mining and the failure of growth to rebound after suppression of AMBLM may have been due to the invasion of a second leaf mining species, *H. nemoratus*, concurrent with the suppression of AMBLM. Alternatively, it could be indicative of the sensitivity of birch species to defoliation in northern latitudes. Further work is required to understand stressors for birch trees in Alaska, and a logical next step would be to bring *H. nemoratus* under biological control, possibly using agents that were studied and introduced into the northeastern United States in the 1930s (Dowden, 1941).

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CHAPTER 22

Progress Toward Successful Biological Control of the Invasive Emerald Ash Borer in the United States

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NON-TECHNICAL SUMMARY

Emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), was first detected infesting ash (*Fraxinus* spp.) trees in southeastern Michigan and nearby Ontario in 2002. Shortly after the discovery of EAB in North America, researchers began foreign exploration for natural enemies of this destructive beetle in northeastern Asia, where it is native. This activity led to development of a classical biological control program in 2003. In 2007, North American regulatory agencies approved the first environmental releases of three EAB hymenopteran parasitoid species from China to EAB-infested forests in southeastern Michigan: *Oobius agrili* (Encyrtidae), *Tetrastichus planipennisi* (Eulophidae), and *Spathius agrili* (Braconidae). *Oobius agrili* parasitizes EAB eggs, whereas the two other species parasitize EAB larvae. In 2015, a fourth EAB parasitoid, *Spathius galinae* (Braconidae) from the Russian Far East, was approved for release in northern states. To date, one or more of these introduced parasitoid species has been released in over 350 counties in 30 EAB-infested states and Washington, D.C. in the United States, and four provinces of Canada. Recent studies in Michigan and several northeastern states, where parasitoids were released five or more years ago, indicate that two larval parasitoids (*T. planipennisi* and *S. galinae*) have established co-existing populations in EAB infesting different size-class ash trees; there they complement each other by partitioning host resources, and they play a significant role in suppressing EAB populations. The egg parasitoid *O. agrili* has also established and spread in EAB-infested forests. It is still too early to determine if biological control will result in significant improvement in ash recovery and regeneration. However, the results from long-term field studies in Michigan and several states in the northeastern United States reveal that ash saplings and pole-size ash trees now experience low EAB densities, which are regulated by established populations of the three introduced biocontrol agents. Researchers and land managers are hopeful that surviving North American ash trees will be protected from EAB and ultimately become overstory trees after successful

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areawide suppression of EAB populations by these introduced natural enemies. Monitoring over the next 10–20 years is required to evaluate survival of the different ash tree species impacted by EAB.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), was first identified as the sole factor causing ash (*Fraxinus* spp., family Oleaceae) tree mortality in southeastern Michigan and nearby Ontario in 2002 (**Fig. 1**). During the next 20 years, the spread and establishment of EAB has been confirmed in 35 states and Washington, D.C. in the United States and five Canadian provinces (CIFA, 2022; Emerald Ash Borer Information, 2022). Dendrochronological examination of infested (dead) ash trees in the epicenter of the EAB invasion in Michigan and genetic analyses of beetles collected from different geographic regions of North America and Asia strongly suggest that this beetle arrived in southeastern Michigan in the early to mid-1990s, probably in solid wood-packaging materials and dunnage used in cargo ships originating in northern China (Bray et al., 2011; Keever et al., 2013; Siegert et al., 2014). After EAB's accidental introduction, it presumably established and spread throughout the forests of southeastern Michigan and nearby Ontario where ash trees were once abundant (Haack et al., 2002, 2015; Poland and McCullough, 2006; Pugh et al., 2011; Herms and McCullough, 2014). Emerald ash borer is a strong flier and can disperse more than 10 miles (16 km) per year; however, its long-range spread occurs primarily through movement of firewood and other ash products that contain live stages of EAB (Haack et al., 2010; Prasad et al., 2010; Taylor et al., 2010; Kashian and Witter, 2011; Haack and Petrice, 2021).

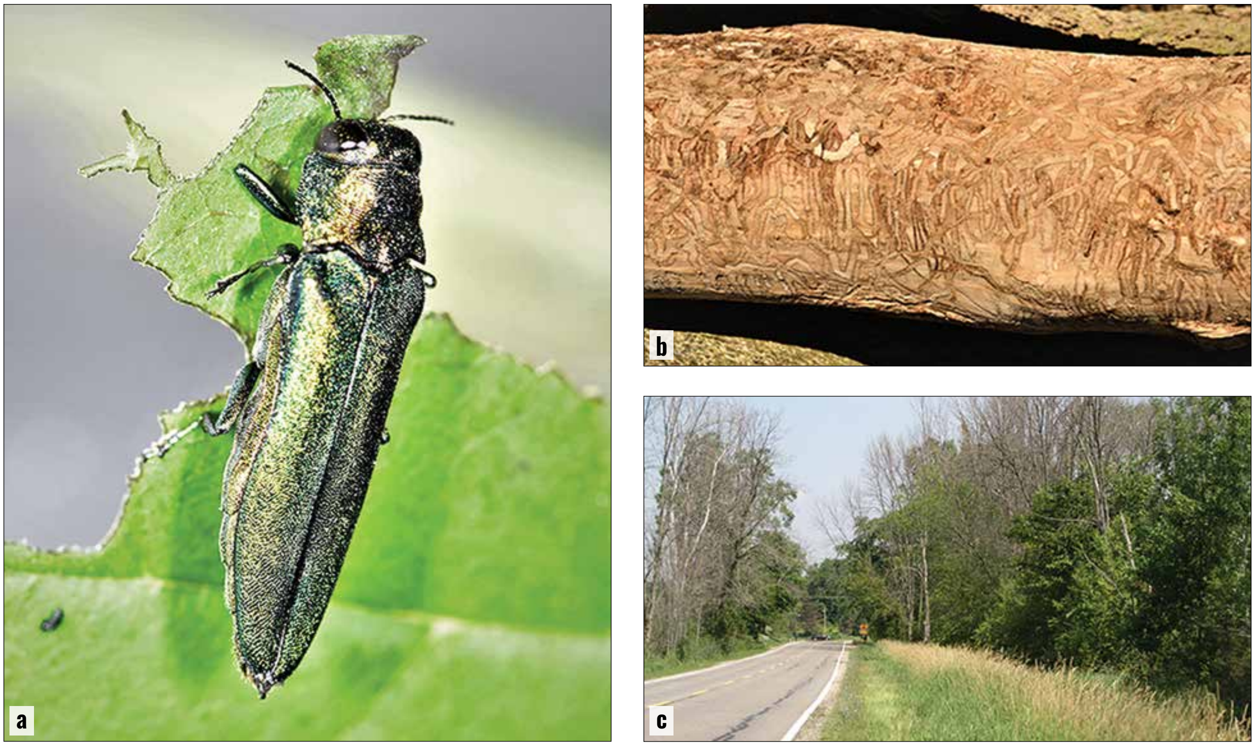

Figure 1. Emerald ash borer (*Agrilus planipennis*, EAB) and its damage to North American ash trees: (a) EAB adult beetle feeding on an ash leaf; (b) feeding galleries of EAB larvae in the phloem and cambium (under the bark) of an ash tree; and (c) widespread and extensive mortality of ash trees caused by EAB ca. 5 years after its initial detection in East Lansing, Michigan. (2009) (J. Duan, USDA-ARS)

Ash species native to North America are highly susceptible to EAB, and even healthy North American trees are quickly killed by EAB (Rebek et al., 2008; Rigsby et al., 2015; Villari et al., 2016). After North American ash trees were introduced to China for reforestation and widely planted in plantations and as landscape trees, their high susceptibility to EAB became increasingly evident, starting in the 1990s (Yu et al., 1992; Liu et al., 2003, 2007; Wei et al., 2004, 2007; Zhao et al., 2005; Wang et al., 2010; Duan et al., 2012a, Straw et al., 2013; Haack et al., 2015; Dang et al., 2021). In the invaded region of North America, EAB populations dispersed quickly in both urban and natural areas due to the abundance of native ash trees in forests, the widespread planting of susceptible North American ash species for landscaping, and movement of EAB-infested materials through human activities (see review in Herms and McCullough, 2014).

Nature of the Problem

Although susceptibility to EAB varies among ash species (e.g., Rebek et al., 2008; Tanis and McCullough, 2012, 2015), all ash species native to North America are known to be susceptible to mortality from EAB, including the most common species—green (*Fraxinus pennsylvanica*), white (*Fraxinus americana*), and black (*Fraxinus nigra*)—as well as the less widespread blue (*Fraxinus quadrangulata*) and pumpkin ash (*Fraxinus profunda*). The potential economic costs associated with the EAB invasion were estimated to be $1 billion per year from 2009 to 2019 (Kovacs et al., 2010), and the ecological impacts on North American forests are already severe and widespread. Emerald ash borer killed 99% of healthy ash trees in some forests infested early in the EAB invasion, and it has the potential to functionally extirpate ash trees from the continent (Klooster et al., 2014; Herms and McCullough, 2014). The loss of ash diversity and abundance in natural forests in earlier invaded regions (e.g., Midwestern and Mid-Atlantic states) has damaged biodiversity and ecosystem processes such as nutrient cycling (Gandhi et al., 2010, 2014; Pugh et al., 2011; Kashian and Witter, 2011; Ulyshen et al., 2011, 2012; Flower et al., 2013; Stephens et al., 2013; Jennings et al., 2017; Morin et al., 2017; Engelken et al., 2020; Jacobsen, 2020).

More recently, EAB was observed attacking the white fringetree (*Chionanthus virginicus*, a species in the same family as ash that is native in the southeastern United States) when it was planted as an ornamental near heavily infested ash trees in Ohio and nearby states (Cipollini, 2015; Peterson and Cipollini, 2020). Because EAB was not confirmed to attack host plants other than ash (Anulewicz et al., 2008), its successful development in *C. virginicus* represents a host range expansion and suggests other woody plants in Oleaceae planted in North America may be susceptible to EAB attack (e.g., forsythia, lilac, privet). In a recent laboratory study, EAB completed development on European olive tree, *Olea europaea* (Oleaceae), suggesting EAB may become an economic pest of olive crops in the United States (Cipollini et al., 2017).

WHY CONTROL THIS INVASIVE SPECIES?

Initial efforts to control EAB focused on containment and eradication of newly detected EAB populations by destroying every ash tree within a 0.5-mile (0.8-km) radius of an EAB-infested ash, as well as restricting the movements of ash materials (e.g., nursery trees and firewood) from the quarantined areas to non-infested areas (Federal Register, 2003; Cappaert et al., 2005; Sawyer, 2007; Taylor et al., 2010). Eradication efforts were abandoned by 2009 because EAB populations in many infested areas (such as Michigan, Ohio, and Maryland) were already too high and too widespread, and EAB-detection methods were inadequate to locate new infestations (GAO, 2006; Poland and McCullough, 2006; Herms and McCullough, 2014). Subsequently, efforts shifted toward slowing the spread of EAB to new areas through regulatory restrictions on the movement of EAB-infested wood or plant materials, insecticide treatment of susceptible trees (either artificially girdled or naturally stressed) as trap trees (e.g., Mercader et al., 2015; Sadof et al., 2021), and classical biological control via the introduction of natural enemies originating in EAB's native range. By 2021, federal quarantine rules restricting the movement of this pest and infested plant materials were discontinued in the United States (Federal Register, 2020), although some state and regional restrictions remain. Thus, protection of North American ash tree species against EAB now relies on sustainable management strategies. Because it is neither environmentally safe nor economically feasible to protect all ash trees in natural forests against EAB using insecticides, classical biological control is currently the only sustainable option available (Bauer et al., 2014, 2015; Duan et al., 2018; USDA-APHIS, 2020; USDA-APHIS/ARS/FS, 2021).

Evidence from field observations in the Russian Far East and northeastern China strongly suggests that several specialized egg and larval parasitoids have protected the more susceptible North American ash species in parts of EAB's native range (Liu et al., 2003, 2007; Duan et al., 2012a; J. Duan, unpub. data). This protection against EAB by specialized natural enemies may occur at two different phases of EAB attack. First, saplings and young susceptible North American ash trees in Asia might be initially colonized by EAB at only low levels because there are few available beetles migrating from resistant Asian ash species. Second, the relatively higher abundance of natural enemies specialized to attack EAB in the pest's native range, notably several species of egg and larval parasitoids, may allow parasitoid populations to increase more rapidly via numerical and functional responses and attack incipient infestations of EAB on susceptible ash species. Both processes may suppress EAB to low densities by slowing its population growth rate.

THE ECOLOGY OF THE PROBLEM

EAB is a beetle that feeds on phloem and neighboring tissues (sapwood and cambium), hereafter referred to only by 'phloem', and it specializes on ash (*Fraxinus* spp.) trees (Yu, 1992; Haack et al., 2002). It has a relatively long lifecycle, requiring one or two years per generation, and a female beetle can lay over 100 eggs (Rutledge and Keena, 2012; Haack et al., 2015; Duan et al., 2020a; Petrice et al., 2021a). Adult beetles normally emerge from infested ash trees from late spring through early summer and feed on ash foliage for about two weeks before mating and oviposition (Rutledge and Keena, 2012; Jennings et al., 2014). Feeding on ash foliage by adult beetles rarely causes significant damage to ash trees. However, tunnelling by the larval stages (1st–4th instars) in the phloem effectively girdles host trees, severely disrupting water and nutrient movement in the trunk, branches, and stems (>0.4 in or 1 cm in diameter).

Generally, infested mature ash trees die 3–5 years following initial EAB attack, even with only moderate larval densities (Knight et al., 2013). In ash-dominant forests, the EAB invasion process can be described as having three phases: the cusp, crest, and core (Burr and McCullough, 2014). The initial infestation, or cusp phase, occurs during the first few years at newly infested sites, while EAB populations slowly build, followed by the crest phase when EAB numbers increase rapidly, causing widespread ash mortality. The core phase follows and lasts 5–10 years after the initial infestation, by which time most larger ash trees have died and the EAB population has collapsed (Burr et al., 2018).

In North America, it is not feasible to attempt to immediately protect susceptible overstory ash trees against EAB in a newly invaded area through the introduction and establishment of specialized natural enemies from Asia because methods to detect early EAB infestations (cusp phase of invasion) are lacking. Moreover, the EAB biocontrol agents are expensive and difficult to rear, and the release a few thousand parasitoid wasps against hundreds of thousands of EAB beetles will not control the invasive EAB populations in time to protect the large ash trees. Susceptible North American ash species are abundant and widespread, and invasive EAB populations grow rapidly in the newly invaded forests (crest phase), making it difficult to suppress EAB outbreaks before mature ash trees suffer substantial mortality. In the post-EAB invaded (core phase) hardwood forests of North America, ash trees are typically scarcer than in the pre-invaded forests, and EAB populations are greatly reduced. Establishment of the introduced EAB parasitoids in the post-invaded forests may effectively protect younger surviving ash trees, saplings, and seedlings by moderating the frequency and amplitude of future EAB outbreaks, as in EAB's native Northeastern Asian range. Subsequently, ash density and size are expected to increase, resulting in a partial return to pre-invasion conditions. Tree regrowth is a slow process, which could allow adequate time for populations of the introduced parasitoids to expand to levels needed to suppress EAB populations below a tolerance threshold for *Fraxinus* spp.

PROJECT HISTORY THROUGH BIOCONTROL AGENT ESTABLISHMENT

Foreign Exploration for Natural Enemies

Shortly after EAB was detected in Michigan, the United States Department of Agriculture (USDA) initiated research to develop a classical biocontrol program for EAB. Exploration started in 2003 for EAB natural enemies in regions of northeastern Asia where it is native, including the Chinese provinces of Hebei, Heilongjiang, Jilin, Liaoning, and Shandong, and the cities of Beijing and Tianjin (Liu et al., 2003). Three major hymenopteran natural enemies of EAB were discovered: the egg parasitoid *Oobius agrili* (Encyrtidae) and two larval parasitoids, *Tetrastichus planipennisi* (Eulophidae) and *Spathius agrili* (Braconidae) (Liu et al., 2003, 2007; Zhang et al., 2005; Yang et al., 2005, 2006). Since then, several other minor species were found in China, including a larval-pupal parasitoid, *Sclerodermus pupariae* (Bethylidae) (Wang et al., 2010; Yang et al., 2012; Wang et al., 2016). Foreign exploration for EAB natural enemies in both Japan and Mongolia was unsuccessful due to the lack of observable EAB populations. However, exploration in the Russian Far East in EAB-infested North American ash planted as landscape trees as well as artificially stressed Asian ash in natural forests resulted in the discovery of three hymenopteran EAB natural enemies: the egg parasitoid *Oobius primorskyensis* (Encyrtidae) and the larval parasitoids *Spathius galinae* and *Atanycolus nigriventris* (Braconidae) (Belokobylskij et al., 2012; Duan et al., 2012a, 2019a; Yao et al., 2016). In addition, foreign exploration in South Korea identified low densities of EAB populations infesting Asian ash trees, and three natural enemy species were found: *S. galinae*, *Tetrastichus telon* (Eulophidae), and a parasitic beetle *Teneroides maculicollis* (Cleridae) (Williams et al., 2010; Gould et al., 2015).

Selection of Potential Agents and Regulatory Permission for Release

The selection of Asian EAB natural enemies for introduction to North America was based on field surveys of natural enemies and their impacts on EAB populations in Asia, combined with generally accepted characteristics of potentially successful biological control agents (e.g., Doutt and DeBach, 1964; Kimberling, 2004). Attributes of candidate natural enemies include but are not limited to (1) high degree of host specificity, (2) high searching ability, (3) high reproductive rate, (4) good spatial and temporal synchrony with the host, and (5) adaptability to a wide range of ecological conditions. For example, *S. pupariae* did not meet several of these criteria as a potential EAB biological control agent because (1) species in this genus are known to sting humans (Gordh and Moczar, 1990), (2) its hosts include a broad range of wood-boring insects (Tang et al., 2012), and (3) the parasitism of EAB in China is low (Yang et al., 2012). Another braconid species, *A. nigriventris*, from the Russian Far East seemed promising, but scientists were unable to successfully rear it in the laboratory. The remaining two egg parasitoids (*O. agrili* and *O. primorskyensis*) and three larval parasitoids (*T. planipennisi*, *S. agrili*, and *S. galinae*) were all considered promising candidates as biological control agents and were imported into quarantine in the United States for further testing shortly after their discovery (for reviews see Bauer et al., 2014, 2015; Duan et al., 2018). The safety and host specificity of Asian parasitoids were assessed for environmental release in North America using data collected from field surveys of other wood-boring insects in China and the Russian Far East and laboratory testing of Asian and North American wood-boring insects. Host specificity of these parasitoids was found to be highly constrained by the close phylogenetic relatedness of potential nontarget hosts to EAB (Federal Register, 2007, 2015; Yang et al., 2008; Bauer et al., 2008, 2014; Duan et al., 2015a). Field data from China and the Russian Far East showed that these parasitoids do not attack other wood-boring insects in ash, such as bark beetles (Curculionidae: Scolytinae) or longhorned beetles (Cerambycidae) (Yang et al., 2008; Duan et al., 2015a). Host specificity studies in the laboratory found that EAB was the only host of *T. planipennisi*, whereas the other four potential biocontrol agents (*O. agrili*, *O. primorskyensis*, *S. agrili*, and *S. galinae*) attacked some other Asian or North American *Agrilus* species (**Table 1**). In contrast to the high rates of parasitism by these parasitoids in EAB, rates in non-target species were consistently much lower, even under laboratory conditions, which promotes maximum parasitism (Federal Register, 2007, 2015; Yang et al., 2008; Bauer et al., 2014; Duan et al., 2015a, 2019a). Based on both laboratory and field host-range studies, the predicted non-target impact from introduction of these Asian parasitoids for EAB biocontrol was low levels of parasitism of some North American *Agrilus* species.

Table 1. Number of non-target insect taxa tested with EAB parasitoids from China and the Russian Far East petitioned to the North American Plant Protection Organization for first environmental releases in North America as EAB biocontrol agents. (modified from Duan et al., 2018)

Parasitoid Species	Orders tested	Families tested	Species tested	*Agrilus* spp. tested	*Agrilus* spp. attacked
Oobius agrili [1]	2	6	18	6	3
Tetrastichus planipennisi [1]	3	6	14	5	0
Spathius agrili [1]	2	6	18	9	5
Spathius galinae [2]	3	6	15	6	1
Oobius primorskyensis [3]	3	6	30	10	7

[1] Data compiled from Federal Register, 2007; Yang et al., 2008; Bauer et al., 2014
[2] Data compiled from Federal Register, 2015; Duan et al., 2015a
[3] Data compiled from Duan et al., 2019a

Release and Establishment of EAB Natural Enemies in North America

In 2007, after completion of extensive field and laboratory studies, the three EAB parasitoid species from China (*O. agrili*, *T. planipennisi*, and *S. agrili* [**Fig. 2a–f**]) were approved for release in the United States (Federal Register, 2007; Bauer et al., 2008, 2014, 2015). In Canada, releases of *T. planipennisi* were approved in 2013 and *O. agrili* in 2015 (Duan et al., 2018). *Spathius galinae* (**Fig. 2g,h**) from the Russian Far East was subsequently approved for release in the United States in 2015 (Federal Register, 2015) and in Canada in 2017 (CFIA, 2018). However, *O. primorskyensis* was not approved for release in the United States, and its release will not be reconsidered until the petition is revised and resubmitted with additional justification and research (Gould and Duan, 2018; J. Duan and J. Gould, unpub. data).

In the summer and fall of 2007, adults of *O. agrili*, *T. planipennisi*, and *S. agrili* were released in small numbers in EAB-infested ash stands in two counties in southern Michigan (Bauer et al., 2014, 2015; Gould et al., 2015; Duan et al., 2018). Field studies in the spring of 2008 revealed successful reproduction

Figure 2. (next page) *Oobius agrili*, a solitary and parthenogenic egg parasitoid of emerald ash borer EAB, (a) adult female wasp laying an egg inside an EAB egg and (b) EAB eggs parasitized by *O. agrili*. *Tetrastichus planipennisi*, a gregarious eulophid larval endoparasitoid of EAB, (c) adult wasp drilling through ash bark to oviposit in an EAB larva and (d) a parasitized EAB larva being consumed internally by a brood of *T. planipennisi* larvae. *Spathius agrili*, a gregarious braconid larval ectoparasitoid of EAB, (e) adult female wasp drilling through ash bark to oviposit on an EAB larva and (f) a brood of parasitoid larvae pupating in their cocoons after consuming and developing on an EAB larva in its gallery under the bark of an ash tree. *Spathius galinae*, a gregarious braconid larval ectoparasitoid of EAB, (g) adult female wasp drilling through ash bark to oviposit on an EAB larva and (h) a brood of parasitoid larvae, pupating in their cocoons, after consuming and developing on an EAB larva in its gallery under the bark of an ash tree. (a, c-h: J. Duan, USDA-ARS; b: L. Bauer, US Forest Service)

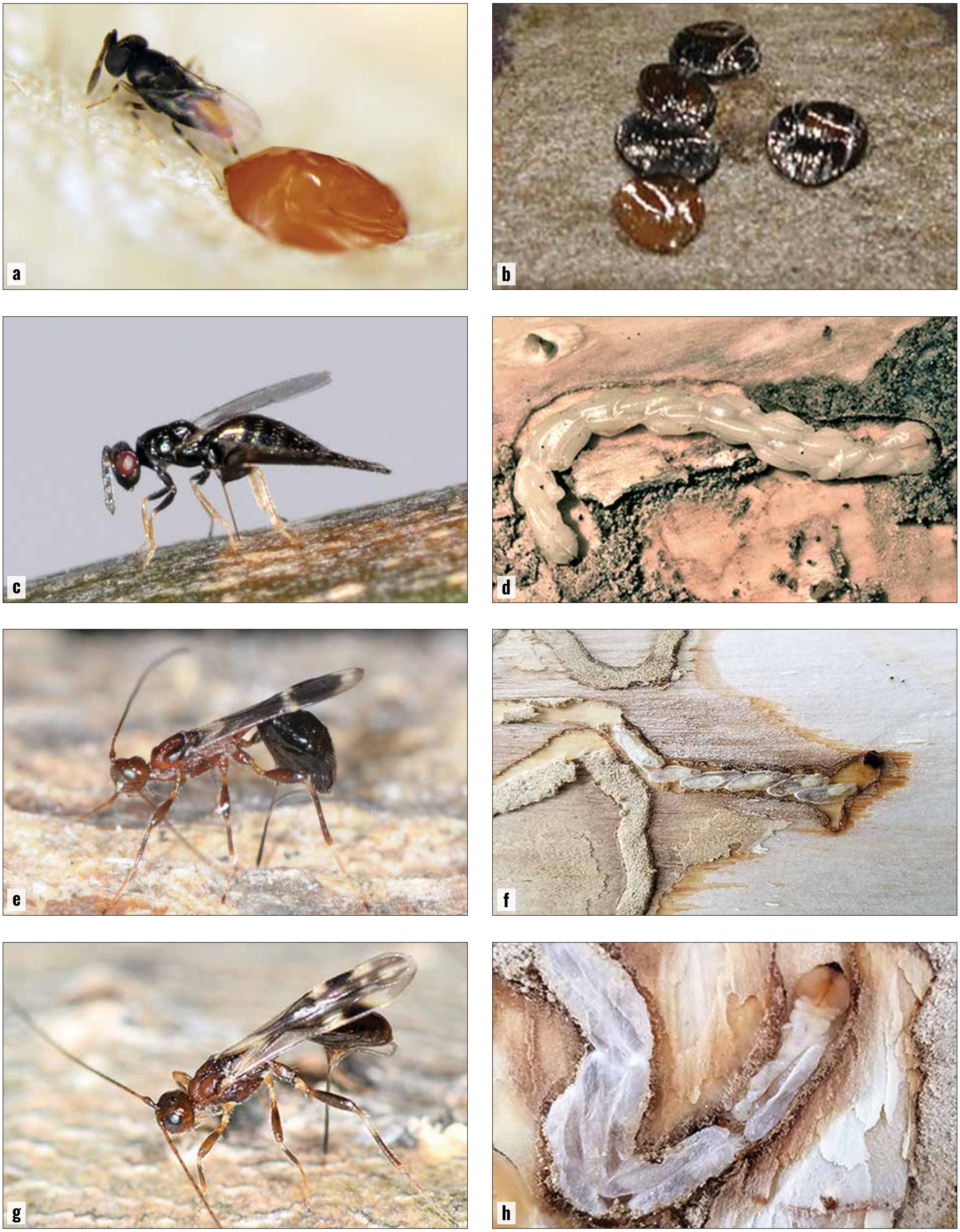

Figure 2. Emerald ash borer parasitoid species approved for release in the United States (explanation of images given on previous page).

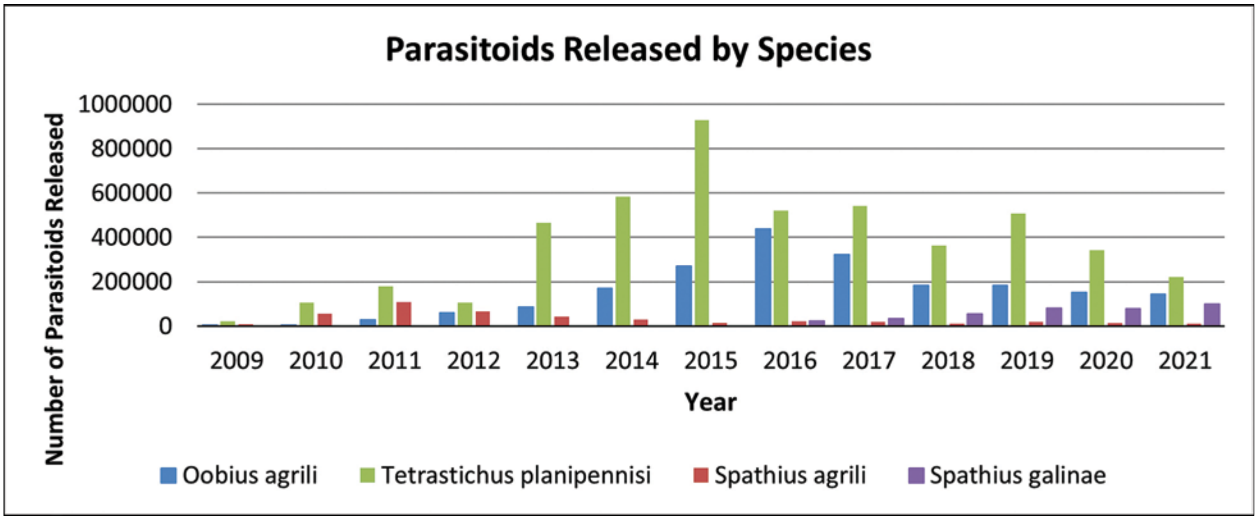

Figure 3. Total number of the Asian parasitoids released for biocontrol of emerald ash borer in the United States from 2009 to 2021. (B. Slager and J. Gould unpub. data, USDA-APHIS Brighton Biocontrol rearing facility)

and overwintering of all three parasitoids species in Michigan, and additional releases were made at EAB-infested forests in Ohio and Indiana, as well as additional locations in Michigan (Bauer et al., 2014, 2015; Duan et al., 2018). In 2009, USDA constructed a rearing facility in Brighton, Michigan for mass-rearing the three EAB parasitoid species, which greatly increased production and field releases of these biocontrol agents (**Fig. 3**). As rearing methods progressively improved, parasitoid production of the three Chinese species, and later *S. galinae* from Russia, increased in the years that followed. However, production of *S. agrili* was purposely reduced because its release in northern states was discontinued by 2013 due to lack of establishment (Duan et al., 2018; Jones et al., 2020). As of the fall of 2021, at least one parasitoid species has been released in more than 350 counties in 30 of the 35 EAB-infested states and Washington D.C. in the United States and in four of the five EAB-infested provinces in Canada (**Fig. 4**) (MapBiocontrol, 2021).

To assess establishment of introduced EAB biocontrol agents, various recovery and sampling methods have been developed and tested, such as tree-cutting and rearing parasitoids from bolts, field-debarking to look for parasitized larvae, outer bark sampling for recovery of EAB eggs and egg parasitism, use of sentinel host larvae or eggs, and deployment of yellow pan traps (e.g., Duan et al., 2012b, 2012c, 2013; Jennings et al., 2018; Petrice et al., 2021b; Rutledge et al., 2021; USDA–APHIS/ARS/FS, 2021; Quinn et al., 2022a,b). Although all three EAB biocontrol agents from China were recovered from EAB larvae or eggs one year after their first release in several northern states, only *O. agrili* and *T. planipennisi* have been consistently recovered for two or more years after final release. These two species are now considered widely established and spreading naturally beyond their initial release sites. More than a decade since its first release in 2007 in Michigan, establishment of *S. agrili* remains unconfirmed in the northern United States and most of the Mid-Atlantic region. However, its parasitism of EAB larvae has been found sporadically at some sites south of the 40th parallel, where this species continues to be released (e.g., Hooie et al., 2015; Aker et al., 2022). Recent field work on *S. galinae*, which was released between 2015 and 2021 in Michigan, Maryland, Colorado, Connecticut, Illinois, Massachusetts, New York, Rhode Island, and Tennessee in the United States, has found it to be establishing and spreading in nearly all release areas and beyond (Duan et al., 2019b, 2020; Aker et al., 2022; J. Gould, pers. obs.).

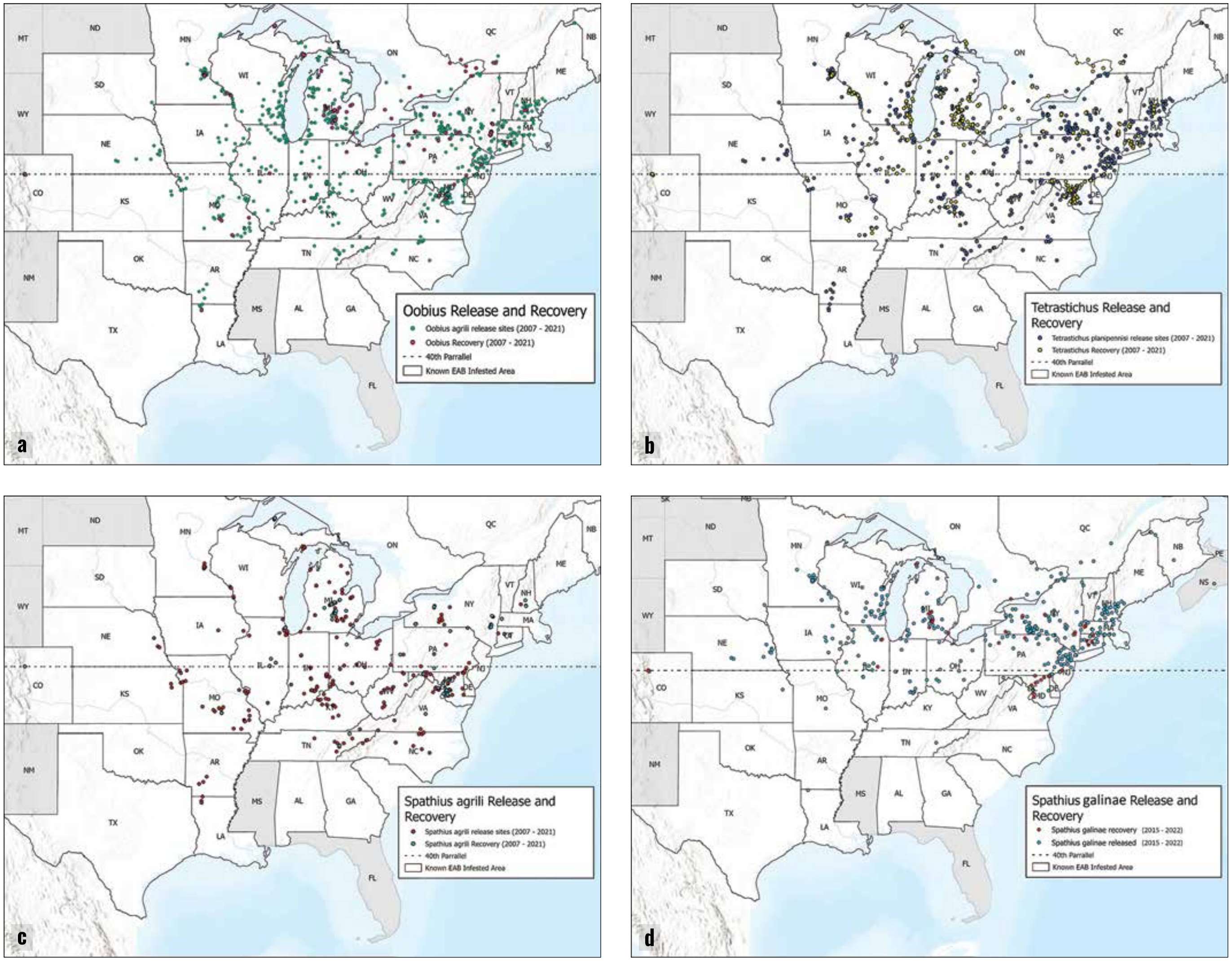

Figure 4. Maps showing known regions of North America invaded by emerald ash borer (EAB), and the release and recovery sites for biocontrol agents introduced from Northeast Asia into North America from 2007 to 2021 for classical biological control of EAB, as reported on the EAB biocontrol geospatial database (mapbiocontrol.org 2021): (a) *Oobius agrili*; (b) *Tetrastichus planipennisi*; (c) *Spathius agrili*; and (d) *Spathius galinae*. In the United States, releases of *O. agrili*, *T. planipennisi*, and *S. agrili* began in 2007, and releases of *S. galinae* began in 2015, thus there are fewer release and recovery sites for *S. galinae* than for the other three biocontrol agents. In Canada, *T. planipennisi* releases began in 2013, *O. agrili* in 2015, and *S. galinae* in 2017. Note: some release and recovery sites are not visible due to the close geographic proximity of many sites at the scale of these maps, especially in states with many release and recovery sites.

HOW WELL DID BIOLOGICAL CONTROL WORK?

To evaluate establishment, spread, and impacts of the biocontrol agents on EAB population dynamics and ash tree health, six long-term study sites were established in southern Michigan between 2007 and 2010, and each was comprised of paired release and non-release control plots. At each release plot, 1,000–3,000 females of *O. agrili*, *S. agrili*, and *T. planipennisi* were released. In subsequent years, infested ash trees were sampled to estimate EAB egg and larval parasitism and other causes of larval mortality (Duan et al., 2013; Abell et al., 2014). During the first five years after release of the three Chinese parasitoids at these Michigan sites (2008–2011), average EAB egg parasitism by *O. agrili* was 1 to 4%, but by 2014 it increased to ~28% in release and control plots (Duan et al., 2012b, 2013, 2015b). Spread of *O. agrili* from the release plots to the control plots, however, was slow (Abell et al., 2014). Overall, the impact of *O. agrili* in suppressing EAB population growth, as well as the natural spread of this biocontrol agent, has yet to be determined because sampling EAB eggs from ash bark layers and crevices is labor-intensive and difficult to standardize (Duan et al., 2011; Abell et al., 2014; Jennings et al., 2018; Petrice et al., 2021b). Moreover, parasitism of EAB eggs by *O. agrili* is patchy, and therefore, relatively more intensive sampling is needed to recover it and quantify its impact on EAB population dynamics (Petrice et al., 2021b).

For *T. planipennisi*, larval parasitism ranged from 1 to 6% from 2008 to 2011, but by 2014, it increased to ~ 30% in both the release and control plots (Duan et al., 2012b, 2013, 2015b). Moreover, the results from recent studies found that *T. planipennisi* can spread rapidly across EAB-infested forests surrounding release sites (e.g., Jones et al., 2019; Rutledge et al., 2021; Quinn et al., 2022a). Life table analyses, including seven years of data from the six Michigan study sites, revealed that *T. planipennisi* also contributed significantly to the reduction of net EAB population growth rates in the aftermath of the EAB invasion outbreak within approximately four years of its initial release (Duan et al., 2014, 2015b). Although local generalist natural enemies, such as *Atanycolus* spp. and woodpeckers, played a significant role in reducing invasive EAB populations during its outbreak phase, the introduced specialist *T. planipennisi* has since become the dominant mortality factor of EAB larvae in the aftermath of the EAB invasion in Michigan (**Fig. 5**).

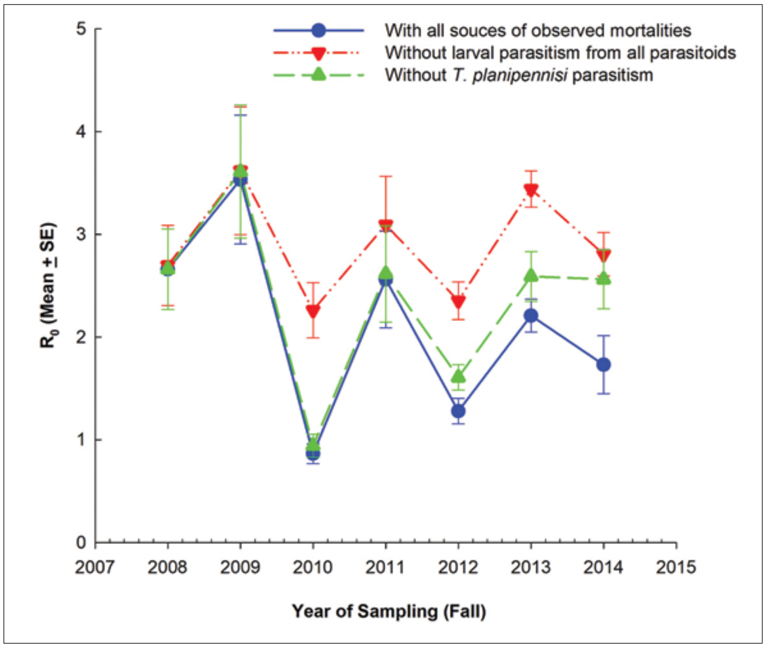

Figure 5. Net population growth rates (R_0) of emerald ash borers infesting young ash trees (DBH = 7 – 21 cm) across six different study sites in southern Lower Michigan, where the larval parasitoid *T. planipennisi* was released between 2007 and 2010. Blue solid line represents RO estimated using life table analysis by including all sources of the observed larval mortalities. Red-dotted line represents R_0 estimated using the same lifetable analysis after excluding larval parasitism from all larval parasitoids including both the native North American parasitoids (primarily *Atanycolus* spp.) and the introduced larval parasitoid *T. planipennisi.* Green-dashed line represents R_0 estimated using the same lifetable analysis after excluding only *T. planipennisi* from the life table, assuming mortality rates from other factors would not change due to increases in EAB densities. (modified from Duan et al., 2015)

Results from field surveys in China, the Russian Far East, and the United States showed that EAB larval parasitism by *T. planipennisi* is concentrated in smaller-diameter ash trees (Liu et al., 2007; Abell et al., 2012; Duan et al., 2012a; Jennings et al., 2016). A recent study of randomly sampled ash saplings (2–6 cm or 0.8–2.4 in DBH [Diameter at Breast Height]) at the six study sites in Michigan found that *T. planipennisi* was the dominant mortality factor, causing 36–85% parasitism of older EAB larvae (3rd and 4th instar), thereby reducing EAB damage in young ash (Duan et al., 2017a). Studies in regenerating ash in eastern and western New York showed similar results, with avian predators (primarily woodpeckers) and *T. planipennisi* reducing EAB population growth to zero at six sites in western New York (Gould et al., 2022). As suspected, the ability of *T. planipennisi* to parasitize EAB in larger ash trees, which have thick bark (>3.2 mm or 0.125 in thick), is limited by its short ovipositor that ranges from 1.9 to 2.6 mm (0.07–0.1 in) long (Abell et al., 2012; Duan et al., 2020b). Consequently, *T. planipennisi* does not attack EAB larvae feeding under thick bark on the lower boles of larger ash trees (>12 cm or 4.7 in DBH) (Abell et al., 2012).

To achieve parasitism of EAB larvae attacking larger-diameter overstory ash trees, and perhaps to reduce widespread ash mortality, EAB biocontrol efforts are now focused on introducing and establishing species of *Spathius,* which have longer ovipositors in EAB-infested regions of North America, presumably with *S. agrili* in the southern states and *S. galinae* in the north (Jones et al., 2020; Aker et al., 2022). However, the lack of consistent recovery of *S. agrili* from many previous release sites in Michigan (Duan et al., 2013, 2015b), New York, Maryland (Jennings et al., 2016), Tennessee (Hooie et al., 2015), and Kentucky (Davison and Rieske, 2016) suggests that this species may not be well suited for EAB biocontrol in the eastern deciduous forests of North America.

Releases of *S. galinae* began in the summer of 2015 after its approval for field release in Michigan and several northeastern and Mid-Atlantic states including Connecticut, Massachusetts, New York, and Maryland. The ovipositor of *S. galinae* averages 4–6 mm (0.16–0.24 in), more than twice the length of *T. planipennisi*'s ovipositor. Consequently, *S. galinae* can attack EAB larvae feeding in ash trees up to 30 cm (12 in) DBH (Duan et al., 2012a; Murphy et al., 2017). Recent field studies from both Michigan and the northeastern states showed that *S. galinae* has established self-sustaining populations in areas where *T. planipennis* was previously released (Duan et al., 2021a, b). Further lifetable analyses showed that *S. galinae* alone contributed a 31–57% reduction of invasive EAB populations during the outbreak (crest) phase (Duan et al., 2021b). Alongside the local generalist natural enemies (e.g., avian predators and some native parasitoids), *T. planipennisi* and *S. galinae* are now the dominate parasitoid species, reducing average densities of live EAB larvae to a low density (<7 live larvae per m^2 [<0.65 live larvae/ft^2] of tree phloem) between 2015 and 2020. This EAB larval density is expected to provide an opportunity for ash recovery and regeneration in the aftermath of the EAB invasion (**Fig. 6**).

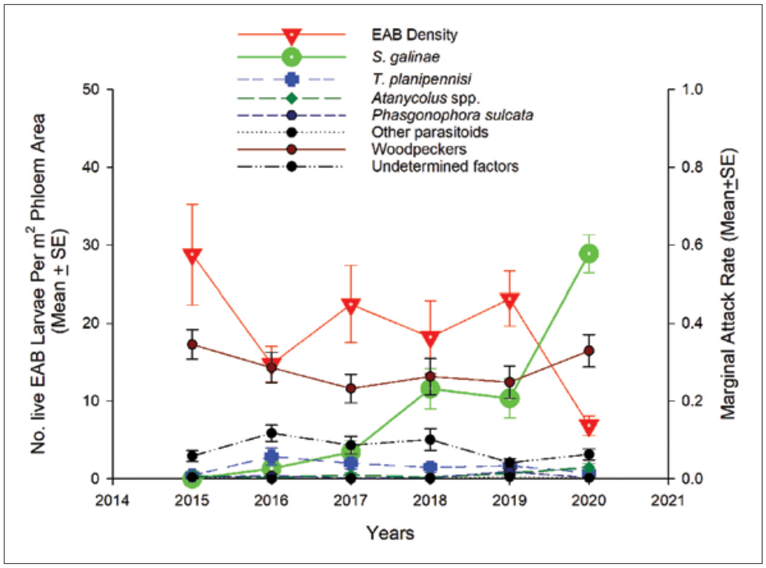

Figure 6. Mean (± SE) EAB (*Agrilus planipennis*) density and mean (± SE) marginal attack rates by different groups of parasitoids, woodpeckers and other avian predators, and undetermined factors observed across the five different study sites in northeastern United States (Connecticut, Massachusetts, and New York) where the three introduced biocontrol agents *(Spathius galinae, Tetrastichus planipennisi,* and *Oobius agrili*) were released from 2015 to 2017. Note: Parasitism by the egg parasitoid (*O. agrili*) is not presented in this study. (modified from Duan et al., 2021b)

BENEFITS OF BIOLOGICAL CONTROL OF EAB

In the eastern and midwestern United States and Canada, EAB's invasion caused 95–100% mortality of the overstory ash trees in many invaded areas within a relatively short period (5–10 years) following its first local detection (e.g., Kashian and Witter, 2011; Klooster et al., 2014). The classical biological control program against EAB in the United States is showing considerable potential for this pest's sustainable management by reducing population densities to a sufficiently low level to allow recovery of ash saplings and young trees, allowing them to fill in forest gaps, successfully reproduce, and reach the canopy over time. Recovery and regeneration of ash trees due to the success in EAB biocontrol could eventually lead to restoration of ash-dominated hardwood forest ecosystems, as well as provide opportunities for recovery of the ash nursery and timber industries. In addition, benefits of ash recovery resulting from EAB biocontrol are likely to accrue without additional costs because the established biocontrol agents are self-sustaining and may effectively maintain EAB population densities at low levels and moderate outbreak amplitude and frequency in the aftermath forests.

Figure 7. Healthy green ash (*Fraxinus pennsylvanica*) trees (left and center) observed in 2018 recovering from initial EAB (*Agrilus planipennis*) invasion, at one of the emerald ash borer biocontrol study sites in southern Michigan, established between 2007 and 2010, where earlier EAB attack killed many trees (right). (J. Duan, USDA-ARS)

In southern Michigan, where the establishment and spread of *T. planipennisi* and *O. agrili* have been confirmed since they were first introduced in 2007, densities of ash were higher in forests closer to sites where more parasitoids were released (Margulies et al., 2017). In these EAB biocontrol sites, researchers also found that healthy ash saplings (4–16 per 100 m^2 [0.4–1.5/100 ft^2) and pole-size young trees (2–9 per 100 m^2 [0.2–0.8/100 ft^2]) have persisted (**Fig. 7**) despite formerly high EAB densities that had resulted in loss of most of the original overstory ash trees by 2010 (Duan et al., 2017ab; J. Duan and T. Petrice, unpub. data). With the most recent establishment of *S. galinae*, it is very likely that the parasitoids released by the EAB biological control program will provide significant services by enhancing the survival, recovery, and growth of large-diameter ash trees, thereby promoting forest recovery in North America (Duan et al., 2017ab; Margulies et al., 2017; Kashian et al., 2018; Duan et al., 2020b). However, recovery of mature North American ash trees, following successful suppression of EAB with biological control, will take time due to the long lifespan of *Fraxinus* spp. The nature of tree regrowth and regeneration will likely take more than a decade to develop an overstory ash canopy (Kashian and Witter, 2011; Kashian, 2016).

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GROUP 4
PROTECTING RANGELAND, GRASSLAND, AND NATURAL AREAS

Dalmatian toadflax, *Linaria dalmatica*, is an invasive weed threatening rangeland and natural areas in North America. The introduction of biological control agents has successfully controlled this species in many parts of its invaded range. (Travis McMahon, MIA Consulting)

Chapter 23

Parasitoids and Pathogens Used Against Imported Fire Ants in the Southern United States

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NON-TECHNICAL SUMMARY

The red imported fire ant (*Solenopsis invicta*, Hymenoptera: Formicidae), black imported fire ant (*Solenopsis richteri*), and their hybrid, collectively called imported fire ants, or fire ants, are invasive ants that have become ubiquitous throughout the southern United States. They are aggressive ants that inflict painful stings, which in some cases cause anaphylaxis. Their large, ecologically dominant populations can result in damage to crops, livestock, wildlife, and infrastructure (e.g., electrical equipment) with total estimated costs of over $8 billion annually for damage and control. Both the red and black species were established in the United States by the late 1930s. An imported fire ant quarantine (covering >365 million acres [148 million ha]) was instituted in the United States to slow the transport of materials with fire ants to non-infested areas. Fire ant stings are a major problem for people, livestock, and pets, with control measures often requiring repeated use of insecticides. Fire ants can thrive in a wide array of habitats, including unmanaged and/or inaccessible landscapes, which serve as reservoirs for infestation of inhabited areas. Biological control is arguably the only viable regional strategy to suppress established populations of invasive ants. Six species of parasitoid flies and two pathogens (a microsporidium and a virus) have been released (1997–2014) and established for the biological control of fire ants in the United States. The flies attack worker ants and inhibit their foraging, while the pathogens cause the debilitation of queens and workers. Dramatic declines in fire ant populations have not occurred, but there is evidence for gradual long-term declines since biocontrol agents were established. Also, the biocontrol agents appear to slow the reinfestation of areas temporarily cleared of fire ants via insecticide treatments. These agents represent the first concerted efforts to utilize classical and augmentative biological control for the long-term suppression of invasive fire ants. Knowledge gained from these efforts will improve the biological control of fire ants and facilitate projects against other invasive ants.

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HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Red imported fire ants (*Solenopsis invicta*, Hymenoptera: Formicidae), black imported fire ant (*Solenopsis richteri*), and their hybrid, are often collectively referred to as simply fire ants or imported fire ants. These ants are aggressive, stinging ants accidentally imported from South America. They currently infest over 365 million acres (148 million ha) in the United States and are established in 16 southern states and territories (Davis et al., 2001; APHIS, 2021). The black and the red imported fire ants are believed to have been accidentally introduced to the Mobile, Alabama area around 1918 and 1933, respectively (Lofgren, 1986; Tschinkel, 2006). A reproductively viable hybrid of these species occurs in the United States across a broad zone from northwestern Georgia (Gardner et al., 2008), northern Alabama (Graham et al., 2005), and southern Tennessee (Oliver et al., 2009; Valles et al., 2021) westward to north and central Mississippi (Streett et al., 2006), a region lying between the ranges of *S. richteri* mostly to the north and *S. invicta* to the south (Vander Meer and Lofgren, 1988). The red imported fire ant (*S. invicta*) is the predominant species in the United States, and since the late 1990s it has also invaded Australia, mainland China, Taiwan, South Korea, Japan, Mexico, and parts of the West Indies (Wetterer, 2013; Lyu and Lee, 2017; Murakami, 2018). The extensive spread of fire ants is attributed to the transport of cargo that harbors fire ants (for example, nursery stock, sod, soil, and construction equipment) and natural dispersal of queens during mating flights (Culpepper, 1953; Markin et al., 1971; Callcott and Collins, 1996; Ascunce et al., 2011; APHIS, 2015).

Nature of the Problem

Figure 1. Red imported fire ant, *Solenopsis invicta*, queen with tending worker ants atop brood (larvae and pupae). (S. Porter, USDA-ARS)

Fire ants are social insects that live in colonies with a sterile female worker caste and a reproductive caste of queens and males. There are two genetic forms of fire ants: monogyne colonies with only a single reproducing queen (**Fig. 1**) and polygyne colonies with numerous (2–400+) egg-laying queens per colony (Glancey et al., 1975; Vargo and Fletcher, 1989). The number of queens per colony is a key factor affecting the number of colonies, or nests, per acre. Monogyne fire ants are territorial, so nests (**Fig. 2a**) are further apart resulting in fewer nests per acre (e.g., 70 nests/acre [28 nests/ha]). In contrast, polygyne colonies are less combative with interconnected sister colonies, and thus nests can be more closely spaced (**Fig. 2b**), which results in much higher nest densities (e.g., 220 nests/acre [89 nests/ha]) (Porter et al., 1992; Macom and Porter, 1996). Mature colonies of monogyne fire ants can contain as many as 230,000 workers per nest (Tschinkel, 1988). Polygyne populations often have fewer workers per nest, but higher nest densities, resulting in 2–3 times more ants per acre than for monogyne populations (e.g., 86 million vs. 44 million per acre [35 million vs. 18 million per ha] [Macom and Porter, 1996]). Once either form of this invasive ant establishes, high reproductive rates and aggressive behavior facilitate the growth of extremely large populations and the ant's expansion into diverse habitats across agricultural, urban, and natural ecosystems. Thus, this aggressive, stinging ant can be prevalent almost everywhere people live, work, and visit!

Figure 2. (a) Large fire ant nest or mound; (b) numerous fire ant nests or mounds in an infested field. (a: S. Porter, USDA-ARS; b: USDA-APHIS)

WHY CONTROL INVASIVE FIRE ANTS?

Figure 3. Numerous pustules from stings by a swarm of fire ants. (S. D. Porter and D. Wojcik, USDA-ARS)

The economic impact of fire ants in the United States has been estimated at more than $8 billion annually (adjusted to 2021 dollars) in direct damage and associated control costs (Lard et al., 2006). These losses include damage to crops, injuries to livestock, damage to electrical infrastructure (such as short-circuiting transformers), and control costs of households and business for pest control in buildings and landscapes. The aggressive, stinging behavior of fire ants is painful, and stings may result in anaphylaxis or secondary infections (**Fig. 3**). Thus, fire ants are considered a public health pest (US EPA, 2002), and they have been the cause of multimillion-dollar lawsuits (deShazo et al., 2004; Oi, 2008). Another major impact is the reduction by fire ants of the local faunal biodiversity, which occurs because of fire ants' large, dominant populations (Porter and Savignano, 1990; Carroll and Hoffman, 2000; Gotelli and Arnett, 2000; Wojcik et al., 2001; Morrison, 2002; Allen et al., 2004; LeBrun et al., 2012).

The fire ant is especially difficult to control because it can infest many, varied habitats, including small spaces that provide harborage for nests. If there is food and water accessible to foraging workers and a suitable microclimate for the queens and rearing of brood, colonies can grow. Unlike many crop pests, fire ant proliferation is not tied to a specific plant host or growth stage, but rather it can infest many different types of landscapes. Because fire ants have such broad impacts, cost/benefit analyses often support implementation of programs to eradicate fire ant incursions (Jetter et al., 2002; Klotz et al., 2003; Wylie and Jansen-May, 2016). For example, the potential impact of fire establishment in Australia had benefit-cost ratios ranging from 25:1 to 390:1 with projected potential damage estimates as high as AUD $45 billion (Antony et al., 2009). Currently, Australia is engaged in a 10-year (2017–2027), AUD $411 million eradication program (Wylie et al., 2020).

THE ECOLOGY OF THE PROBLEM

Because fire ants are so numerous, aggressive, and have painful stings, tolerance for them is very low, especially in the presence of children or those allergic to the stings. Thus, the need for effective fire ant control is high around homes, playgrounds, and other high-use areas. Several insecticides formulated as baits can efficiently kill fire ant colonies, allowing them to be cleared from a property. However, fire ants are so widespread in the southern United States that colonies in unmanaged landscapes are a constant source of rapid reinfestation. This cannot be changed because using pesticides to reduce reservoirs of fire ants in unmanaged lands, inaccessible habitats, and sensitive conservation areas is too costly, and depending on the treatment method, often is environmentally unacceptable. Thus, natural enemies from South America that specifically attack fire ants were introduced into the United States as classical, self-sustaining fire ant biocontrol agents. Once established, these natural enemies can reproduce, become more common, and spread. The long-term goal is to provide permanent, widespread suppression of fire ant populations.

Fire ant biocontrol agents can target either the fire ant queens or the workers or both. If the egg-laying queens are killed or incapacitated, the production of workers ceases. If workers are no longer produced in sufficient numbers, the colony cannot maintain essential functions, such as foraging for food and tending brood, and the colony will eventually decline and die off. However, targeting queens is difficult because queens are protected by the workers and typically stay underground within the nest. Thus, a suite of the fire ant decapitating fly species that parasitize workers and fire ant-specific pathogens that debilitate queens and workers have been used for fire ant biocontrol. The goal is to add enough stressors to fire ant colonies to reduce their population-level dominance in landscapes and allow native ants to successfully compete with them (Oi et al., 2015).

Fire Ant Decapitating Flies

Fire ant decapitating flies (Diptera: Phoridae, genus *Pseudacteon*) (**Fig. 4**) inject their eggs into the thorax of the host worker ant via aerial attack. The maggot that emerges from the egg eventually migrates and enters the head of the ant (Porter, 1998; Morrison, 2000). The fly larva develops within the head of its host and, when nearing maturity, releases enzymes that cause the head to detach. The flies complete their development within the detached head capsule, before emerging, mating, and starting another generation of flies (**Fig. 5**). The flies are tiny, no larger than the head of their host. They live only a few days and, in laboratory tests, preferred to parasitize either red or black imported fire ants, rather than several native *Solenopsis* fire ant species that were offered (Porter and Gilbert, 2004; Chen and Fadamiro, 2018; Chen and Porter, 2020). Extensive host specificity and impact studies both before and after release have confirmed that these flies are not a risk to other ants, insects, livestock, or people (Porter and Gilbert, 2004; Chen and Morrison, 2021). Female flies develop 100–200 eggs, (Porter, 1998; Morrison, 2000; Fadamiro and Chen, 2005).

Figure 4. Adult fire ant decapitating fly, *Pseudacteon curvatus.* (S. Porter, USDA-ARS)

Fire Ant Pathogens

Fire ant pathogens found in South America that infect queens and workers have been isolated from fire ants in the United

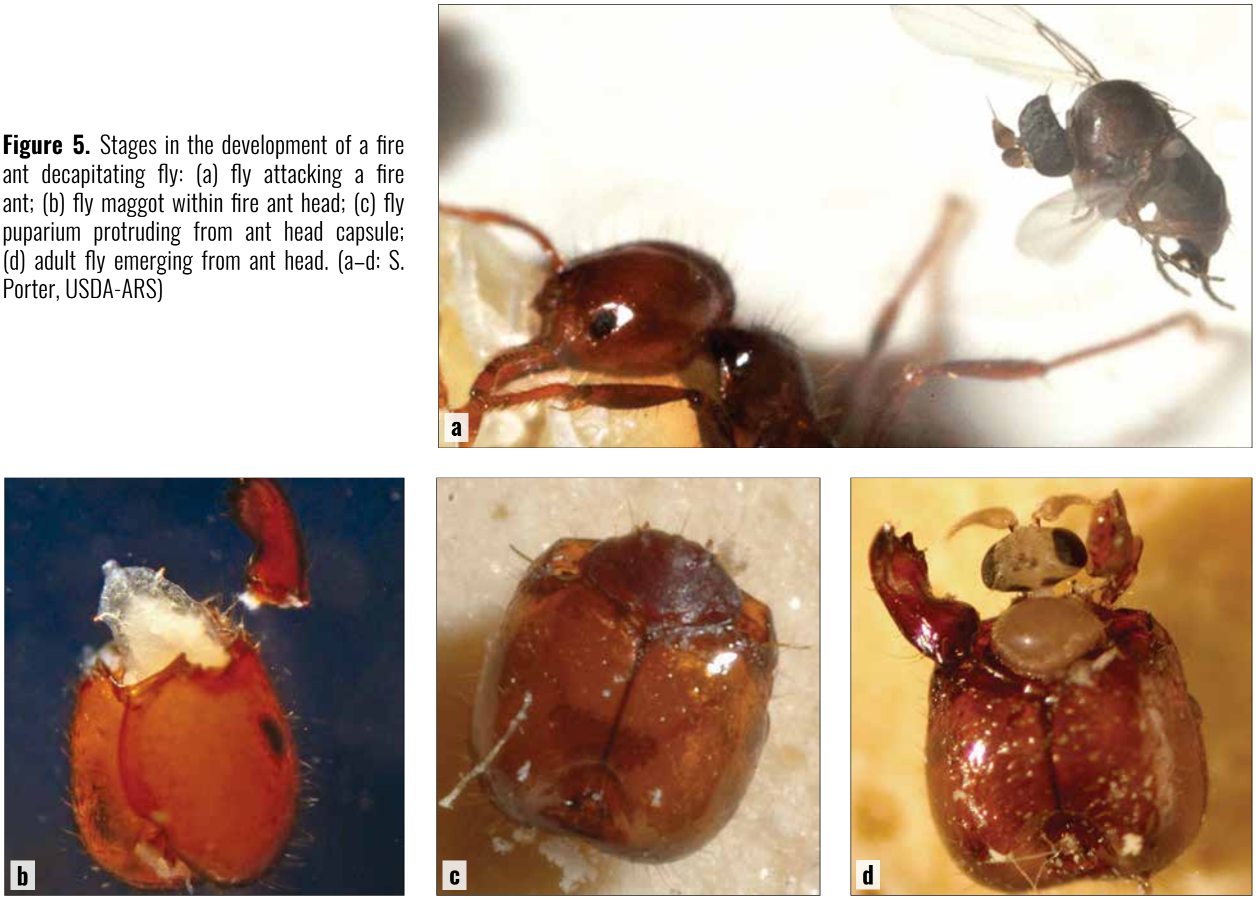

Figure 5. Stages in the development of a fire ant decapitating fly: (a) fly attacking a fire ant; (b) fly maggot within fire ant head; (c) fly puparium protruding from ant head capsule; (d) adult fly emerging from ant head. (a–d: S. Porter, USDA-ARS)

States. The microsporidium (a phylum within or closely related to the Fungi) *Kneallhazia solenopsae* (formerly *Thelohania solenopsae*) infects all fire ant life stages and castes (**Fig. 6**). Microsporidia are intracellular parasites that produce spores that inject their contents into cells of host organisms that ingest them (Han et al., 2020). Fire ant queens infected with *K. solenopsae* lose weight, lay fewer eggs, and die faster than non-infected queens (Williams et al., 1999). The host range of this pathogen is limited to six species in the genus *Solenopsis* found in South America, including both red and black imported fire ants (Oi and Valles, 2009). In the United States, *K. solenopsae* has also been detected in the tropical fire ant (*Solenopsis geminata*) and the *Solenopsis geminata* x *Solenopsis xyloni* hybrid. Further genetic analyses are needed to determine if there are *K. solenopsae* variants among these host ant species (Ascunce et al., 2010).

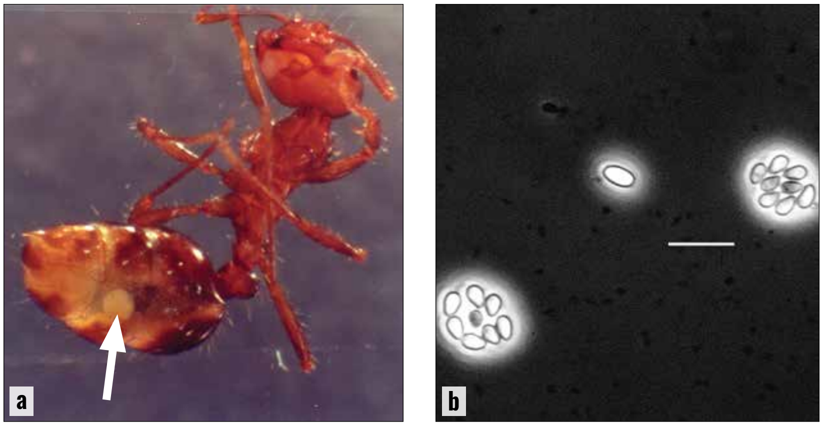

Figure 6. (a) Fat body [white arrow] in fire ant containing (b) spores of the fire ant pathogen *Kneallhazia solenopsae.* (a: USDA-ARS; b: J. Becnel, USDA-ARS)

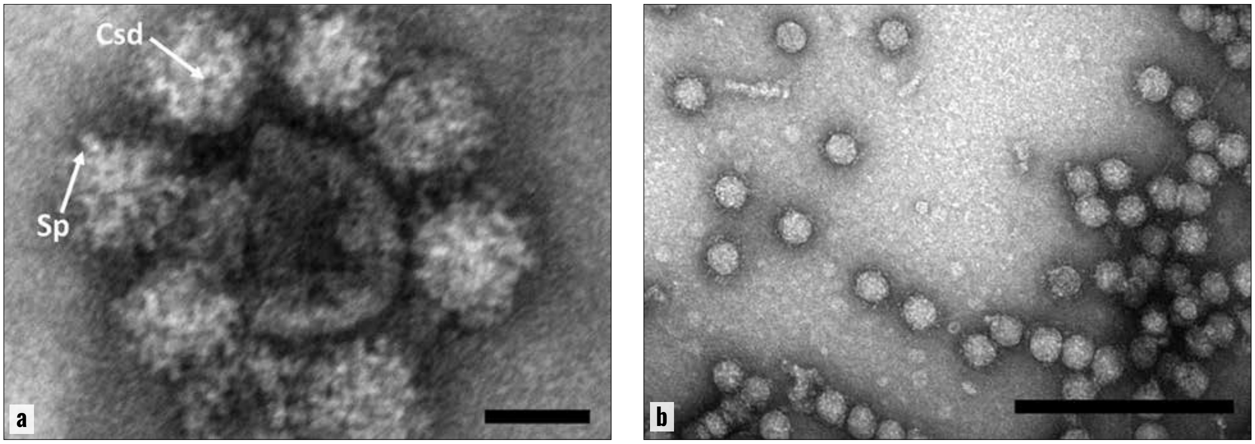

Figure 7. Electron micrographs of Solenopsis invicta virus 3: (a) close-up of virus particle showing surface projections (Sp) and cup-shaped depressions (Csd); (b) numerous virus particles. (a,b: S. M. Valles, USDA-ARS)

Solenopsis invicta virus 3 (SINV-3) is a virulent fire ant pathogen released for biocontrol (**Fig. 7**). Like *K. solenopsae*, this pathogen is found in both South and North American fire ants. It causes colony death by reducing queen fecundity and inhibiting feeding behavior in workers that results in colony starvation (Valles et al., 2013; Valles et al., 2014). Host range studies indicate the virus is specific to red imported fire ants and their hybrid (Porter et al., 2013a; Porter et al., 2015; Valles and Firth, 2021).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Fire Ant Decapitating Flies

Six species of fire ant decapitating flies were collected in South America in collaboration with cooperators in Argentina and Brazil and then exported to quarantine and containment facilities in Florida and Texas (with proper permits), where they were reared and studied. After extensive review by federal and state agencies, the flies were released in Florida, Texas, Tennessee, Alabama, and eventually other southern states from 1997 to 2017 (**Table 1**). Depending on species, the flies are active at different times of the day, locate ants at nests or on foraging trails, and inject their eggs into workers of particular sizes (Pesquero et al., 1996; Morrison et al., 1997; Orr et al., 1997; Morrison and Gilbert, 1998; Morrison and King, 2004; Folgarait et al., 2007; Calcaterra et al., 2008). The release of the various species with different preferences for attacking fire ants permitted increased parasitism pressure on fire ant populations (Porter and Gilbert, 2004; Chen and Morrison, 2021). Releases of different biotypes of the same *Pseudacteon* species also facilitated establishment in both red and black fire ants from populations located in different regions. For example, *Pseudacteon curvatus*, originally collected from black imported fire ants, and *P. curvatus*, collected from red imported fire ants, were more successful when released against their original host species in the United States (Vazquez et al., 2004).

Pseudacteon tricuspis was the first fire ant decapitating fly species released and established in the field [Florida, 1997] (Porter et al., 2004). Three years later, *P. curvatus* was successfully introduced in Tennessee and later in Florida, where it established thriving populations that competitively reduced *P. tricuspis* populations (LeBrun et al., 2009; Porter and Calcaterra, 2013). Subsequently, releases of *Pseudacteon obtusus* (in 2006 and later) resulted in almost complete elimination of *P. tricuspis* in Florida; however, releases of *P. curvatus* and *P. obtusus* in Texas did not always result in reduction or displacement of pre-existing *P. tricuspis* populations (Plowes et al., 2011; Porter and Calcaterra, 2013). The other three *Pseudacteon* species

Table 1. Traits of *Pseudacteon* species established in the USA for fire ant biological control.

Species	Avg. ♀ thorax width [a]	Host size preference	Host location	Year of first successful release	Reference [c]
P. tricuspis	0.51 ± 0.03	medium-large	nest	1997	Porter et al., 2004
P. curvatus	0.33 ± 0.03	small	nest	2000 [b]	Graham et al., 2003
				2003 [b]	Vazquez et al., 2006
P. litoralis	0.57 ± 0.02	large	nest	2005	Porter et al., 2011
P. obtusus	0.53 ± 0.02	medium-large	trail, nest	2006	Plowes et al., 2011
P. nocens	0.49 ± 0.05	medium-large	nest	2006–2010	Plowes et al., 2012
P. cultellatus	0.33 ± 0.02	small	nest	2010	Porter et al., 2013b

[a] mm ± standard deviation, n = 10

[b] 2000 Las Flores biotype; 2003 Formosa biotype.

[c] Additional summarized data from Porter and Calcaterra, 2013; Chen and Porter, 2020

were successfully established in Alabama (*Pseudacteon litoralis*), Texas (*Pseudacteon nocens*), and Florida (*Pseudacteon cultellatus*) (Porter et al., 2011; Plowes et al., 2012; Porter et al., 2013b). Co-occurrence among two or three different fly species at individual sites is common and supports the approach of introducing multiple biocontrol species (Huffaker et al., 1976), each with unique combinations of attack parameters (e.g., host location, time periods of attack, and host size preferences). Early field releases of each species were conducted by the USDA-ARS in Gainesville, the University of Texas at Austin, and various cooperators. By 2002, the USDA-APHIS and the Florida Department of Agriculture and Consumer Services, Division of Plant Industry began large-scale rearing efforts and managed dozens of releases of *P. tricuspis*, *P. curvatus*, *P. obtusus*, and *P. cultellatus* in cooperation with state departments of agriculture, university extension agents, specialists, and researchers from 14 states and territories (Callcott et al., 2011; Callcott, 2017).

Fire Ant Pathogens

Surveys for pathogens of fire ants in South America and the United States have been conducted since the early 1970s, and new pathogens are still being discovered (Oi and Valles, 2009; Valles et al., 2018; Valles and Rivers, 2019). Two pathogens, the microsporidium *K. solenopsae* and SINV-3, have caused significant declines or death of inoculated colonies (Williams et al., 1999; Valles et al., 2014). *Kneallhazia solenopsae* was first isolated from fire ants collected in Brazil in 1973 (Allen and Buren, 1974; Knell et al., 1977). In the 1990s, it was being studied in Argentina as a possible classical biological control agent of fire ants in the United States (Briano et al., 1996; Briano and Williams 1997). However, in 1996 *K. solenopsae* was detected in fire ants in Florida and subsequently in other states (Williams et al., 2003). By introducing live, infected brood into fire ant colonies, *K. solenopsae* infections were successfully initiated in Florida, Louisiana, Mississippi, Oklahoma, South Carolina, and California in 1998–2004 and 2014 (Oi and Valles, 2009; Oi et al., 2019).

Using metagenomics and next generation sequencing, over 15 viruses have been discovered in fire ants from both the native and introduced ranges of fire ants since 2004 (Valles et al., 2004; Valles and Rivers, 2019). Solenopsis invicta virus 3 has thus far been the most virulent virus, and it is capable of causing colony death (Valles et al., 2014). Solenopsis invicta virus 3 was successfully transmitted to field colonies in Florida and California in 2008 and 2014, respectively (Valles and Oi, 2014; Oi et al., 2019; S. M. Valles pers. comm.).

HOW WELL DID IT WORK?

The fire ant decapitating flies are well established, and they are distributed throughout the fire ant quarantine area in the southern United States. Among the six established species, three have spread extensively. *Pseudacteon tricuspis* had covered about 70% of the fire ant regulated area in 2011. However, given the displacement of this species by other *Pseudacteon* species, its effective distribution has declined in Florida, parts of Texas, and perhaps other regions as well. The two biotypes of *P. curvatus* spread rapidly to cover an estimated 90% of the quarantined area in 2011, and they probably cover nearly 100% today. *Pseudacteon obtusus* covers perhaps 25% of the quarantine area, predominately occurring in Florida, Texas, and Georgia (Callcott et al., 2011; Callcott, 2017). The other three species are still expanding, but are probably each limited to a single state.

Death of fire ant workers from direct parasitism by decapitating flies is estimated to be ≤2% per colony (Morrison and Porter, 2005a; Valles et al., 2010, Morrison, 2012). However, the indirect effects of the flies limiting foraging and other activities to avoid parasitism are thought to be more damaging to colonies. In the presence of the decapitating flies, fire ants try to avoid attacks by retreating into their nests, or assuming a curled, immobile posture. Additionally, foraging was observed to be significantly reduced (83%) at food sources by the presence of phorid flies, and in some cases other ant species were able to take over the food source (Porter et al., 1995). However, the impact of reduced fire ant foraging can be compensated by foraging at night when the flies are inactive, accessing food through tunnels, or guarding food sources to prevent access by competitors. Thus, Morrison (2012) considered the impact of the introduced decapitating flies on fire ant populations to be weak because evidence is lacking for consistent parasitism pressure by the flies to depress fire ant populations. Instead, fly abundance fluctuates with the availability of fire ant hosts. Nevertheless, the impacts of *Pseudacteon* decapitating flies on fire ant populations has been sufficient to cause the evolution and maintenance of a suite of defensive behaviors against the flies (Porter, 1998).

Natural infections of *K. solenopsae* and SINV-3 have been discovered in fire ant populations in the United States. Surveys in Texas, Louisiana, and Mississippi, as well as arbitrary sampling at study sites, indicated that *K. solenopsae* is self-sustaining and has been found in at least 12 states (Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, Oklahoma, South Carolina, Tennessee, Texas, Virginia) (Mitchell et al., 2006; Streett et al., 2006; Milks et al., 2008; Oi et al., 2019; D. H. Oi, unpub. data). Solenopsis invicta virus 3 has been documented from five states (Florida, Louisiana, Texas, Tennessee, and California); however a systematic survey of its distribution in the United States has not been conducted (Yang et al., 2010; Valles et al., 2017; Oi et al., 2019).

Kneallhazia solenopsae and SINV-3 have weakened and reduced the fecundity of queens, and the resulting decline of fitness in infected colonies is well documented in the laboratory (Williams et al., 1999; Oi and Williams, 2002; Valles et al., 2013; Valles et al., 2014). In the field, reductions of 63% were reported in *K. solenopsae*-infected fire ant populations, primarily due to smaller colony sizes rather than fewer nests (Oi and Valles, 2009). *Kneallhazia solenopsae* infections are more prevalent in polygyne populations, where the non-territorial behavior and asynchronous infections of queens within a colony provide more opportunities to acquire or produce uninfected ants and sustain the colony, but also allow the pathogen to propagate itself. In contrast, infection of the single queen in monogyne colonies leads to colony decline without recovery (Oi et al., 2004; Fuxa et al., 2005).

Data on the impact of SINV-3 on fire ant colonies in the field is currently limited to a small field test that showed reductions in the size and density of inoculated fire ant nests and local spread to non-inoculated colonies (S. Valles and D. H. Oi, unpub. data). The high virulence and transmissibility of SINV-3 were very evident when this virus contaminated fire ant rearing facilities and killed many colonies. This forced the implementation of extensive mitigation protocols and demonstrated persistence of the virus under laboratory conditions (Valles and Porter, 2013).

OUTCOMES OF THE BIOLOGICAL CONTROL OF FIRE ANTS

Pathogens of fire ants present in their native range of South America, such as *K. solenopsae* and SINV-3, apparently arrived in the United States with their host fire ants. *Kneallhazia solenopsae* and SINV-3 have been released in areas where their infections were not present, and together with the fire ant decapitating flies, comprise a suite of fire ant-specific natural enemies that are exerting biotic stress on these invasive ants. The decapitating flies, *K. solenopsae*, SINV-3, and several other Solenopsis invicta viruses co-occur and appear to be compatible with each other (Valles et al., 2010). However, decapitating flies develop very poorly in fire ant colonies infected with SINV-3 virus (S. D. Porter, unpub. data). Decapitating flies are known to acquire *K. solenopsae*, and although there is no evidence of the flies vectoring the microsporidium, the pathogen apparently does not harm the flies (Oi et al., 2009; Mészáros et al., 2014; D. H. Oi, unpub. data). Nevertheless, the possibility still remains that decapitating flies may be significant vectors of other pathogens.

There is some evidence that fire ant biocontrol agents can delay the reinfestation of land previously cleared of fire ants through insecticide treatments (Oi et al., 2008). Indeed, the ability of queens infected with *K. solenopsae* to survive and found new colonies is diminished, and this impact may contribute to the slowing of reinfestation (Cook et al., 2003; Oi and Williams, 2003; Overton et al., 2006; Preston et al., 2007). Also, several long-term studies of fire ant populations in Florida (S. D. Porter, unpub. data) show moderate declines in fire ant populations (20–30%) over this same period, although the cause of these declines has not been linked to the release and/or accumulation of biocontrol agents in their invasive range (Morrison and Porter, 2005b). This lack of linkage is at least partly due to high natural variability in fire ant populations, the difficulty in accurately measuring fire ant populations, the gradual increase in biocontrol agents over the last two decades, and what is likely to have been only modest to moderate impacts overall.

The efforts described above represent the first concerted effort to utilize biological control to suppress an invasive ant, and there are still promising prospects for additional importations. For example, some species of small-sized decapitating flies (e.g., *Pseudacteon obtusitus* and *Pseudacteon nudicornis*) attack trails of fire ants foraging away from the nest (Feener et al., 2008; Folgarait et al., 2020). That type of agent would add a missing component to the suite of established large and small flies (*P. tricuspis*, *P. litoralis, P. nocens, P. curvatus, P. cultellatus*) that specialize in parasitizing different-sized fire ants at nests and the large species *P. obtusus* that attacks trailing fire ants (**Table 1**). Another microsporidian pathogen of fire ants, *Vairimorpha invictae*, is a promising candidate for importation because it reduces fire ant colony growth (Oi et al., 2005) and, in combination with *K. solenopsae*, is associated with fire ant population declines in the field (Briano, 2005). Finally, the numerous *Solenopsis invicta* viruses are an untapped reservoir of potential biocontrol agents (Valles and Rivers, 2019). Classical and augmentative biological control remain the only economically viable option for long-term regional management of invasive pests like fire ants. Continued importation and release of other biocontrol agents may eventually lead to an acceptable level of fire ant control by natural enemies (Morrison and Porter, 2005b; Vander Meer et al., 2007).

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CHAPTER 24

Biological Control of Leafy Spurge

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NON-TECHNICAL SUMMARY

Leafy spurge, *Euphorbia virgata* (Euphorbiaceae), is a persistent invasive weed causing an expensive management problem that costs U.S. agriculture millions of dollars annually in reduced rangeland productivity and revenue. Chemical control is often only marginally successful, and the repeated applications required carry high economic and ecological costs. In the 1960s, the aggressive spread of leafy spurge led to concerted efforts to develop a biological control program against leafy spurge. Because the weed reproduces both by clonal sprouting and substantial seed production, a long-term, low-input control method was required to impose consistent pressure on the plant and reduce leafy spurge populations to acceptable levels. Today, widespread establishment and biocontrol management with *Aphthona* species of flea beetles (Coleoptera: Chrysomelidae) is a critical component of integrated leafy spurge control across large areas of the western United States.

HISTORY OF INVASION AND NATURE OF PROBLEM

Leafy spurge, *Euphorbia virgata* (Euphorbiaceae), originates from across Europe and western Asia with a center of distribution in the Caucasus (Croizat, 1945; Moore, 1958; Selleck et al., 1962). The first collections of leafy spurge in North America were from Massachusetts in 1827 (Britton, 1921; Bakke, 1936) and Ontario in 1889 (Gusson, 1944), then records show the weed spreading westward to Minnesota in 1890 (Kommedahl and Johnson, 1959), North Dakota in 1909, and California in 1916 (Stevens, 1927; Robbins, 1940). By 1933, it was found coast to coast in the United States and was reported from 19 states (Hanson and Rudd, 1933). Leafy spurge is hypothesized to have arrived in North America via soil used as ship ballast (Britton, 1921) and as a contaminant in oats, wheat, and smooth bromegrass (*Bromus inermis*) seed from Russia and Hungary (Batho, 1931; Dunn, 1985). This weed has likely been introduced multiple times from different regions of Eurasia, as evidenced by the diverse strains and ecotypes that have been noted in North America (Baker and Arnekleu, 1964).

West, N. M. and J. F. Gaskin. 2022. Biological control of leafy spurge, pp. 266–281. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

The first report of the weediness of this species came from an editorial in the New York Herald in 1921 (Feb 9, p. 8). Hanson and Rudd (1933) reported three centers of infestation: one on the east coast; scattered infestations in Montana, Idaho, and Washington; and a large and costly infestation in the northern central prairie regions (**Fig. 1a**). By 1975, leafy spurge was found in 30 states and every province of Canada except Newfoundland (Dunn, 1979; **Fig. 1b**). By the time the first leafy spurge symposium was held in 1979, the weed had invaded an estimated 1 million ha (2.5 million acres), with an associated annual (1978) cost of $10.5 million (Noble, 1979; **Fig. 1c**). Nearly 15 years later, almost 6% of the non-tilled land in North Dakota (approximately 410,000 ha or 1,013,000 acres) was infested with leafy spurge (Leitch et al., 1996). Ten years after that, Duncan et al. (2004) estimated that leafy spurge occupied nearly 1.5 million ha (3.7 million acres) in 17 western states, with an annual spread rate of 12–16%, as well as infesting 400,000 ha (nearly 1 million acres) in the eastern United States. More recent estimates show leafy spurge presence in 37 states (not found in the southeastern United States), and seven Canadian provinces and territories (EDDMapS, 2022; **Fig. 1d**).

Frequent introductions through contaminated seed from Europe and Asia throughout the late 1800s and early 1900s likely laid the foundations for the major invasions in the north-central states and the prairie provinces of Canada (Dunn, 1985). Because leafy spurge invades both cropland and pasture, and because it can establish from seeds, root buds, or root fragments, dispersal through agricultural landscapes was likely common and aided extensively by humans. Seed production peaks around the time of hay harvest in these areas, increasing the potential for movement in hay and forage. Additional dispersal with seed exchange, and through farm equipment and railroads, exacerbated spread, and overgrazing and lack of revegetation left bare soil open for invasion (Selleck et al., 1962; Dunn, 1985; Messersmith et al., 1985). Leafy spurge seeds are physically propelled away from mother plants, ensuring wide distribution in local patches. Seeds are also moved by and germinate in water, which historically led to extensive invasions along waterways (Selleck et al., 1962), and animal dispersal may contribute to over-land movement, though direct evidence is limited (Messersmith et al., 1985). As leaf spurge spread through western North America, land managers attempted to control it by cultivation, manipulating grazing, and applying herbicides, but the invasion continued to double in size approximately every 10 years. Consequently, chemical costs were increasing as well (Anderson et al., 1999). High propensity for both local and long distance spread, as well as the difficulty and longevity of management required to eradicate leafy spurge once established, ensured that this weed remained a priority pest across its introduced range.

WHY CONTROL THIS INVASIVE SPECIES?

Leafy spurge is a perennial, highly self-incompatible forb native to Europe. It grows in dense patches and all parts of the plant contain a milky latex toxic to cattle, which causes cattle to avoid grazing in leafy spurge-infested pastures. The plant also contains phytotoxic compounds that may inhibit competing vegetation (e.g., Qin et al., 2006), although these interactions are not well-studied. Leafy spurge produces extensive root systems that allow it to aggressively outcompete other plants. Impacts on plant communities often persist even after the weed is removed. These invasion legacy effects have been attributed to slow recruitment of desirable species, secondary invasions, or lack of revegetation after control (e.g., Lesica and Hanna, 2009; Larson and Larson, 2010). The intensive management needed to suppress leafy spurge infestations and the residual effects on the soil have long-term effects on the soil microbial community and plant recruitment (e.g., Pritekel et al., 2006; Lesica and Hanna, 2009). Consequently, forage and native species are reduced, and net rangeland productivity declines in invaded areas, reducing cattle production (Hansen et al., 1997; Bangsund et al., 1999; Mangold et al., 2018). Eradications of leafy spurge invasions are unlikely; at best, management primarily reduces weed abundance and impacts to ecologically and economically tolerable levels (Progar et al., 2010). Biocontrol agents, when established at adequate densities, provide necessary enhancements to weed reduction measures. Although leafy spurge remains an important target for control

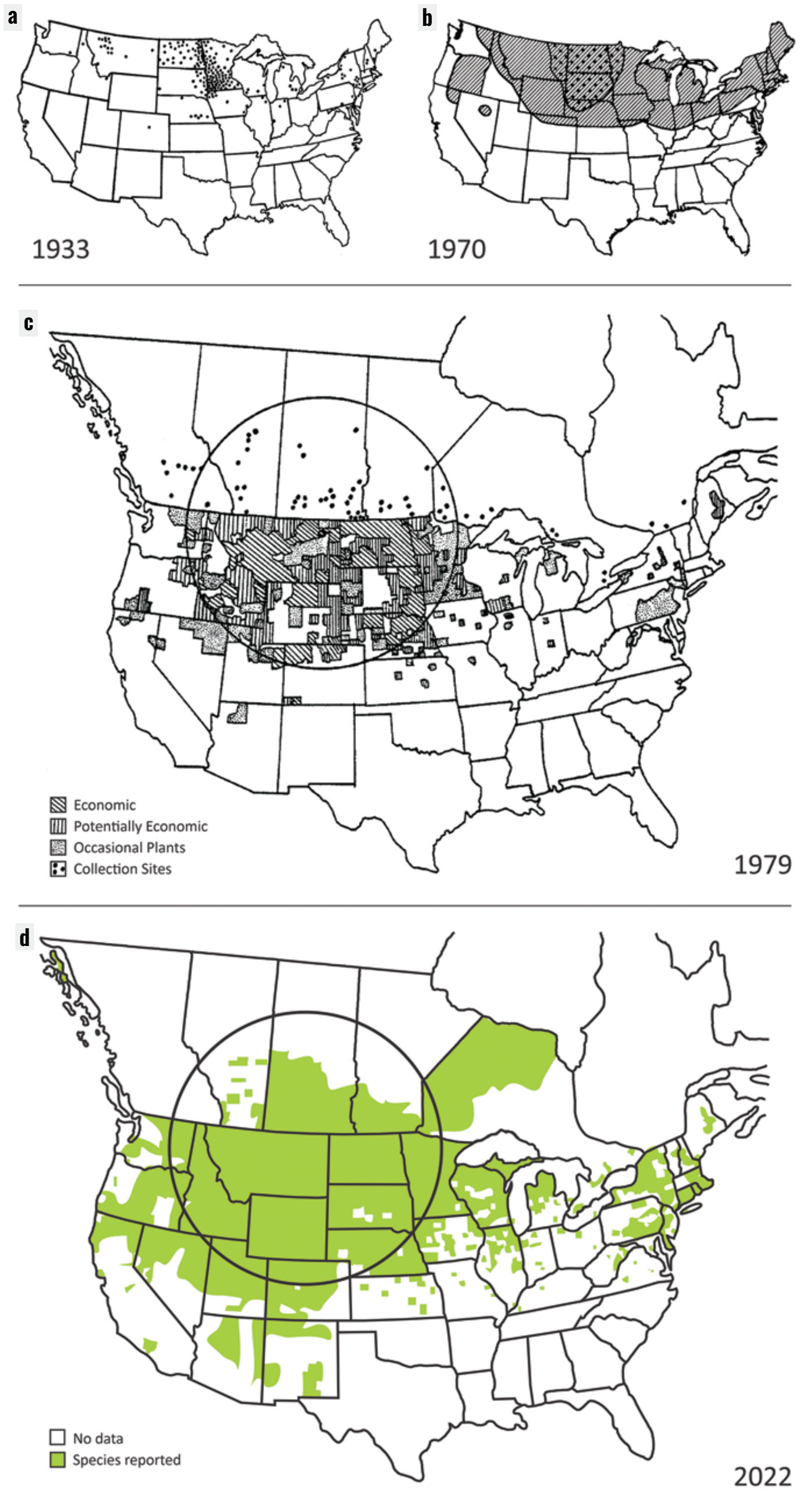

Figure 1. Distribution of leafy spurge (*Euphorbia virgata*) invasions from the 1930s through present day. Hatching reflects different degrees of impact, and the circles in the bottom two figures indicate the region with the highest density of infestations. (modified from a: Hanson and Reed, 1933; b: Dunn, 1979; c: Noble, 1979; d: EDDMapS, 2022)

throughout the western United States (e.g., Van Wychen, 2017; Mangold et al., 2018), it also provides a broadly implemented and well documented example of successful weed biocontrol.

THE ECOLOGY OF THE PROBLEM

Leafy spurge's high reproduction capacity, and the rapidity and intensity with which it becomes entrenched once established, make its control complicated. Frequent root budding and high seed production lead to dense leafy spurge infestations that require several years of intensive effort to manage. More than half of the plant's biomass is often below-ground, and vegetative shoots can emerge from depths of 30 cm (12 in) for several years even if the majority of the root mass is removed. Selleck et al. (1962) estimated that below-ground mass would need to be excavated more than 0.5 m (20 in) deep to have any influence on above-ground biomass. Seeds are dehisced (ejected) from plants to distances of 5 m (16 ft) or more, contributing to site-level spread and reducing the rate at which new plants might be limited by local shoot densities. Seedlings begin producing vegetative buds within seven days after plant emergence. After 10 days, seedlings can survive and resprout from root buds, even if cut to ground level. Patches can reach densities of 200 or more shoots per m^2 (18.6/ft^2), causing declines in forbs and near elimination of annual species in infested areas (Selleck et al., 1962; Messersmith et al., 1985). Moisture availability and seasonal temperatures, and to a lesser degree nutrient availability, influence rates of patch expansion and extent of above-ground cover between years. Individual patch margins are highly variable but often increase at rates of 0.5 to 4 m/year (1.6–13 ft/year), with likely greater expansion below ground. For instance, patches may appear to recede in dry years, but this is not usually associated with below-ground mortality, and vegetative shoots will re-emerge from rootstocks once conditions improve (Selleck et al., 1962). Leafy spurge's high capability for spread and establishment, as well as its tolerance of severe damage and unfavorable conditions, makes eradication generally intractable.

Integrating measures such as herbicide application and grazing can be effective at reducing weed densities, but pressure must be maintained to adequately control infestations and prevent further invasion (Lym, 2005). Leafy spurge's high tolerance of top-kill and above-ground biomass removal, and additional factors such as passive release of herbicides from spurge roots (Hickman et al., 1989), mean that successful chemical control will be temporary unless maintained over a whole season and throughout several subsequent years. For instance, Datta et al. (2013) found that leafy spurge patches began recovering by the third year after treatment. The use of herbicides alone for control is generally not feasible over the long term due to the expense and the cumulative effects on rangeland of the intense herbicide treatments required. Bangsund et al.,(1996) estimated that herbicide treatments for leafy spurge control gave cow–calf operations a positive return only in leafy spurge patches of 0.5 ha (1.2 acre) size or less.

Grazing management, particularly with sheep and goats, reduces leafy spurge top-growth and spread but has limited influence on the root system; new shoots can later be produced, and shoot recruitment can be stimulated by top removal (Lym, 2005). When appropriately timed and trained, targeted grazing might deplete root reserves and reduce infestations, but grazing treatments must be long-term efforts for leafy spurge suppression (Rinella and Bellows, 2016). Bowes and Thomas (1978) reported that more than three years of continuous sheep grazing were required to reduce leafy spurge stem densities, and even after eight years, 5–10 shoots/m^2 (0.5–0.9/ft^2) were being produced by the perennial root systems. Fire may reduce above-ground stems and young plants, but burning is unlikely to substantially harm patches without additional management (Fellows and Newton, 1999). Although most leafy spurge seeds germinate within the first three years after their dispersal, seed dormancy can allow new plants to grow abundantly from a single seed cohort in the seed bank for up to five years, and to some degree for up to 13 years (Selleck et al., 1962). Thus, biological control provides an important and necessary additional tool to maintain suppression pressure of the plant's populations with minimal investment by the landowner in labor or resources (Lym, 1998, 2005).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The low abundance of leafy spurge and high diversity of associated insects found in the plant's native range suggested that classical biological control might be effective in suppressing leafy spurge. Inspired by earlier successful weed biological control projects in western North America, such as the control of St. Johnswort (*Hypericum perforatum*) by species of *Chrysolina* beetles, researchers from CABI (Center for Agriculture and Bioscience International) and the USDA Agricultural Research Service (ARS) European Biological Control Laboratory began work in the 1960s to identify potential candidate biological control agents for leafy spurge. Agent exploration is often done in the center of evolutionary origin of a weed species, which in this case was hypothesized to be the Caucasus Mountains (Croizat, 1945). However, for political and financial reasons, surveys were focused on Hungary, Romania, Austria, and Serbia (Gassman and Schroeder, 1995).

The first attempts at biological control of leafy spurge involved large insects that could do great visible damage to plants in a short time. In 1966, *Hyles euphorbiae* (Lepidoptera: Sphingidae), a hawkmoth from western Europe that can defoliate plants when at high densities, was released in Canada and then in the United States. The hawkmoth was hindered by disease and predation, which lowered its densities. Even when the hawkmoth occurred at high densities, defoliation by this species did not control leafy spurge (Gassman and Schroeder, 1995). Other large insects that were released in the United States in the 1970s and 1980s included three species of *Chamaesphecia* (Lepidoptera: Sesiidae) and the beetle *Oberea erythrocephala* (Coleoptera: Cerambycidae). All three *Chamaesphecia* species are thought to have failed to establish on leafy spurge. *Oberea erythrocephala* did establish, but whether populations reach high enough densities to reduce leafy spurge infestations is still unknown.

Table 1. Biological control agents of leafy spurge (*Euphorbia virgata*) permitted and introduced in the United States. Primary targets are those impacts considered to be the agent's most important effects. The information is summarized from Winston et al., 2014 and Winston et al., 2021. Hosts are either EC (*Euphorbia cyparissiae*) or EV (*Euphorbia virgata*). This list does not include species released only in Canada or only on EC.

Order, Family	Species	Primary damage targets	Primary reproduction target	Hosts
Established				
Coleoptera, Chrysomelidae	*Aphthona cyparissiae* (Koch)[1]	Rootlets/roots	Vegetative Recruitment	EC, EV
Coleoptera, Chrysomelidae	*Aphthona czwalinai* (Weise)[1]	Rootlets/roots	Vegetative Recruitment	EC, EV
Coleoptera, Chrysomelidae	*Aphthona flava* Guillebeau[1]	Rootlets/roots	Vegetative Recruitment	EC, EV
Coleoptera, Chrysomelidae	*Aphthona lacertosa* Rosenhauer[1]	Rootlets/roots	Vegetative Recruitment	EC, EV
Coleoptera, Chrysomelidae	*Aphthona nigriscutis* Foudras[1]	Rootlets/roots	Vegetative Recruitment	EC, EV
Lepidoptera, Sphingidae	*Hyles euphorbiae* (L.)	Foliage	Seed Production	EC, EV
Coleoptera, Cerambycidae	*Oberea erythrocephala* (Schrank)[1]	Stems, Flowering	Seed Production	EV only
Diptera, Cecidomyiidae	*Spurgia capitigena* (Bremi)	Vegetative & flower buds	Seed Production	EC[2], EV
Diptera, Cecidomyiidae	*Spurgia esulae* Gagné[1]	Vegetative & flower buds	Seed Production	EC, EV
Not Established				
Coleoptera, Chrysomelidae	*Aphthona abdominalis* (Duftschmidt)[1]	Rootlets/roots	Vegetative Recruitment	EV only
Lepidoptera, Sesiidae	*Chamaesphecia crassicornis* Bartel	Main roots, stems	Vegetative Recruitment	EV only
Lepidoptera, Sesiidae	*Chamaesphecia hungarica* Tomala[1]	Main roots, stems	Vegetative Recruitment	EV only
Lepidoptera, Sesiidae	*Chamaesphecia tenthrediniformis* (Denis & Schiffermüller)	Main roots, stems	Vegetative Recruitment	EV only

[1] Agents released by APHIS redistribution programs as reported by Hansen et al., 1997.

[2] Establishment documented in Canada only.

The failure of above-ground defoliation by the hawkmoth (*H. euphorbiae*) to control leafy spurge led to a shift in focus towards insects that attack the plant's root system. The spread of leafy spurge at the local (patch) level was thought to come primarily from new shoots arising from lateral roots rather than seed dispersal. The project also shifted towards finding agents with a lower per capita feeding rate but that were present in large numbers. Emphasis was also placed on using many different agents attacking different plant parts, such as the stems or the reproductive structures. Another strategy employed by the program was to look for agents that were effective in particular habitats supporting leafy spurge (Gassman and Schroeder, 1995). A dozen or more species were studied as potential agents, with emphasis on various species of root-feeding *Aphthona* flea beetles (Coleoptera: Chrysomelidae), in part because of the success achieved in other biocontrol projects using beetles in this family. Studies also focused on potential agents from other *Euphorbia* species in Europe. Some of these agents were known to attack, and had been collected from, several *Euphorbia* species. *Euphorbia cyparissias*, for example, was not considered the main target for control in the United States, but this species was invasive in North America, and it ultimately proved to be the source for several agents from Europe. Some insects that were collected from *E. cyparissias* were released against *E. virgata* (mostly in eastern Canada), while other agents were released against both *Euphorbia* species (see Winston et al., 2014, 2021).

In total, 18 species from eight genera were screened for host specificity, approved for release, and subsequently released against leafy spurge in North America. Of these, 11 species are currently considered established in North America. Only 13 of the 18 have been officially introduced to the United States (the remaining five were only introduced to Canada); nine of the 13 species released in the United States have been reported as established (Winston et al., 2014, 2021) (**Table 1**). Five of these nine established agents are species of univoltine *Aphthona* flea beetles that feed on young roots and shoots developing from rootstocks (**Fig. 2**). These five flea beetles were introduced starting in the late 1980s.

The other four established agents include two spurge midges (*Spurgia* spp., Cecidomyiidae) that attack above-ground buds, potentially reducing seed production, the spurge hawkmoth (*H. euphorbiae*),

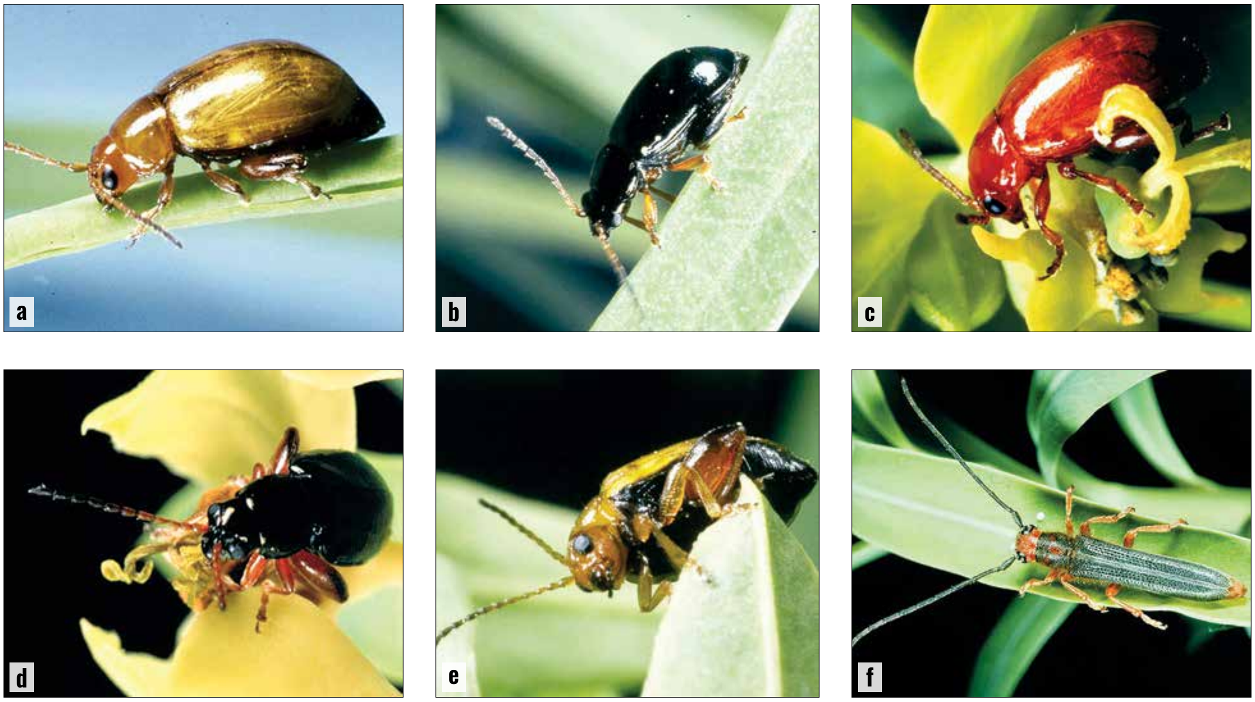

Figure 2. Principal established biocontrol agents for leafy spurge, *Euphorbia virgata*, in the United States. Five of these are flea beetles in the genus *Aphthona* that feed on roots as larvae and above-ground plant parts as adults: (a) *A. cyparissiae*; (b) *A. czwalinai*; (c) *A. flava*; (d) *A. lacertosa*; and (e) *A. nigriscutis*. One additional agent (f) is a stem and root-crown mining beetle, *Oberea erythrocephala*. (a–f: R.D. Richard, USDA-APHIS)

which is a defoliator, and the red-headed stem borer (*O. erythrocephala*) (**Fig. 2f**), which girdles stems and mines the root crown (**Table 1**). The four agents introduced in the United States that failed to establish were a multivoltine flea beetle (*Aphthona abdominalis*) and three species of *Chamaesphecia* moths (**Table 1**).

The various *Aphthona* flea beetle species became widely established and caused dramatic reductions in leafy spurge infestations (**Figs. 3,4**). This success provided the basis for successful leafy spurge biocontrol in North America (Anderson et al., 2003). From the late 1980s to the early 2000s, large-scale release and redistribution programs were funded by the U.S. government, particularly through the USDA-APHIS and ARS, to facilitate the introduction and wide dissemination of these promising biological control agents. These programs were primarily focused on *Aphthona* species, although additional agents, as well as some that subsequently failed, were also released through these efforts. APHIS released seven successful agents and two that failed to establish throughout the Great Plains region between 1988 and 1996 (Hansen et al., 1997) (**Table 1**). More than 48 million biocontrol insects were distributed through an ARS-funded education, outreach, and research program (TEAM Leafy Spurge) (Anderson et al., 2003) that was cooperatively managed by APHIS. In this program, multiple agencies partnered with tribes and private landowners to facilitate releases and the establishment of *Aphthona* insectaries, as well as research on subsequent impacts on leafy spurge (mainly in Montana, Wyoming, North Dakota, and South Dakota). Programs such as these, in combination with state and local distributions, resulted in the widespread establishment of several *Aphthona* species throughout the western United States. For example, of 100 leafy spurge biocontrol release sites surveyed in 2019 in Idaho, Montana, and North Dakota, only five had no *Aphthona* flea beetles present, and these five sites had very low leafy spurge densities (West et al., in review).

In contrast to the successful *Aphthona* species, the relative abundance and fate of the non-*Aphthona* agents in the landscape remains unclear, except for *O. erythrocephala*. Despite variable impacts and uncertain

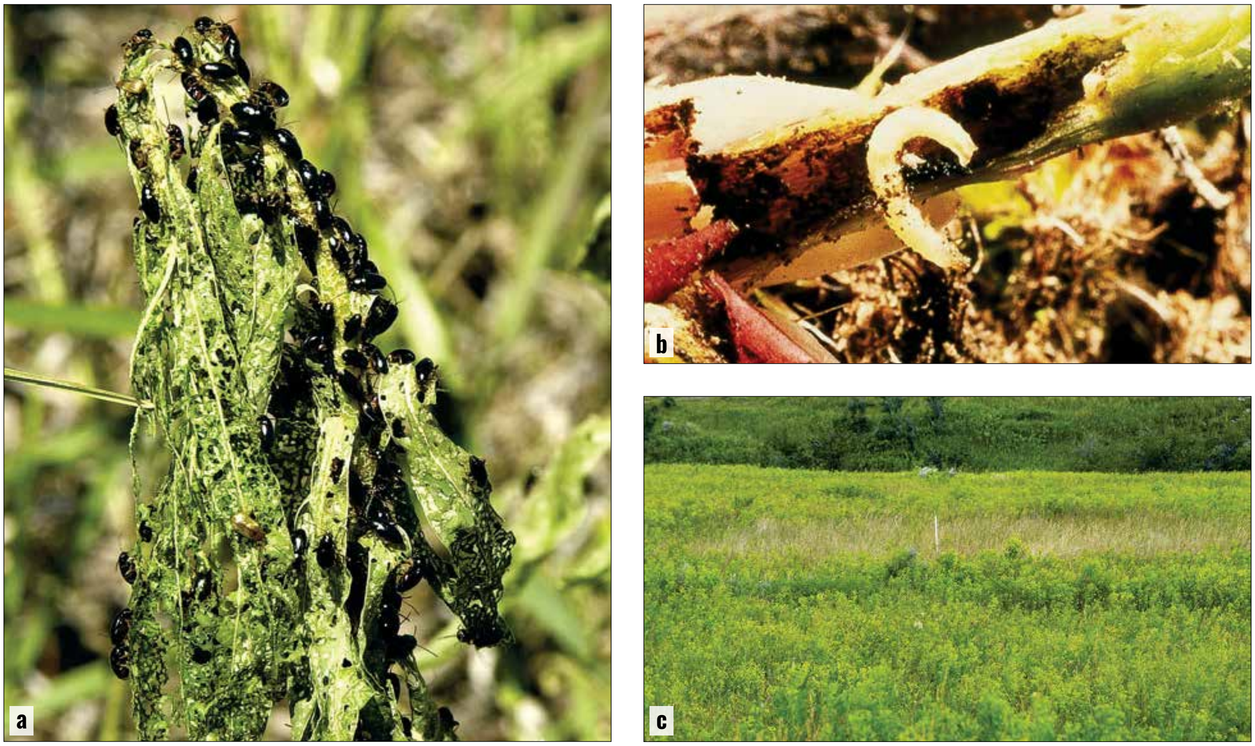

Figure 3. *Aphthona* spp. flea beetle damage and impacts: (a) flea beetle adults attacking a leafy spurge plant; (b) flea beetle larva feeding on roots; (c) Impacts of flea beetles on a leafy spurge infestation. The white pole in the center of the photo is the location of a biocontrol release. Colonization by flea beetles caused high reductions in stem density, resulting in a 'crater' of open habitat within the leafy spurge patch. (a–c: USDA-ARS TEAM Leafy Spurge)

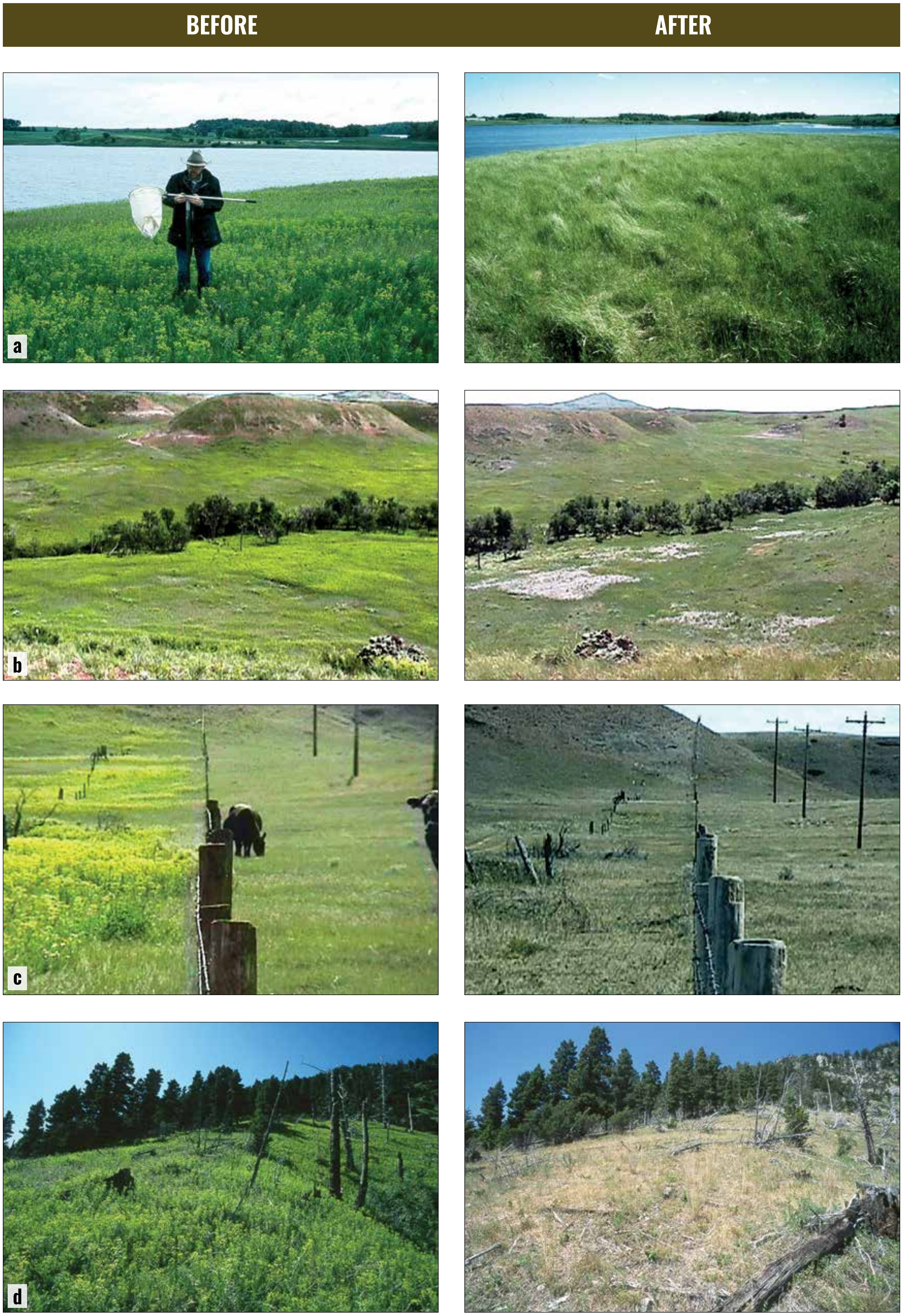

Figure 4. Leafy spurge (*Euphorbia virgata*) infestations before and after the release of *Aphthona* flea beetle agents (a) Lake Ashtabula, ND: herbicides plus *A. czwalinai/lactertosa* release (pictured: Don Mundal, North Dakota State University); (b) Sentinel Butte, ND: *Aphthona* species plus multi-species grazing; (c) integrated management with *Aphthona* species on the left fenceline; (d) Bridger Mountains, MT: *A. nigriscutis* release. (a–d: USDA-ARS TEAM Leafy Spurge)

abundance early in its introduction (Gassman and Schroeder, 1995), *O. erythrocephala* has independently colonized sites (e.g., Anderson et al., 2015), is commonly encountered, and is being redistributed throughout the northern Great Plains and Rocky Mountain West. For instance, more than 23,000 individuals of this species were moved between 2019 and 2021 by the Montana Biocontrol Coordination Project (N. West, unpub. data; M. Maggio, pers. comm.). However, few field studies have quantified this agent's impact or its current distribution or abundance.

Another agent, the hawkmoth *H. euphorbiae*, is also commonly encountered as a generalist pollinator in grassland communities (e.g., Fox et al., 2013); however, its low impact as a biocontrol agent has made it a low priority for any further monitoring. The two spurge midges are difficult to distinguish, and although *Spurgia* species have been detected in the field (e.g., Lym et al., 1996; Hansen et al., 1997), little is known of their distribution or rates of dispersal. Increased monitoring and sampling across the introduced range of these four agents would be needed to determine their extent and abundance in North America.

HOW WELL DID IT WORK?

The leafy spurge control program has been very successful with its emphasis on *Aphthona* flea beetles (**Fig. 4**). *Aphthona* flea beetles established at >85% of the release sites supported by the TEAM Leafy Spurge project, and flea beetle populations increased seven-fold at the project's demonstration sites in Montana, Wyoming, and North and South Dakota between 1998 and 2000 (Anderson et al., 2003). As a result, leafy spurge cover was reduced by 35 to 100% at different sites over the 2–3 years studied (Anderson et al., 2003). Among the 292 release sites supported by TEAM Leafy Spurge, 91% had quantified reductions in leafy spurge stem density (Samuel et al., 2008). Various studies have recorded observing large flea beetle populations that were associated with reductions in leafy spurge cover that then persisted over time (e.g., Kirby et al., 2000 [revisited in Joshi and Olson, 2009]; Butler et al., 2006 [revisited in Butler and Wacker, 2010]; Cline et al., 2008 [revisited in Setter and Lym, 2013; Thilmony and Lym, 2017]). For instance, Lym (2018) reported leafy spurge remained at very low densities (>90% reduction) after 19 years even in the original control plots established by Cline et al. (2008), where insects were not actively introduced. The widespread, rapid success of the *Aphthona* species complex caused most further research and release efforts to be focused on the establishment, impact, and efficacy of these flea beetles.

Integrating biocontrol with other management tools has been demonstrated to work well in rangelands infested with leafy spurge, and integration yields more consistent biocontrol outcomes. Several studies have shown that combining biological control agents with the use of herbicides and grazing reduces leafy spurge populations relative to reliance on a single tool (Lym, 2005). For instance, initial treatments with fall-applied herbicides in combination with biocontrol reduced leafy spurge density 3–5 years earlier than either technique alone, and infestations continued to be controlled by *Aphthona* for the remaining seven years of observation (Lym and Nelson, 2002).

Adding sheep or goat grazing to areas with well-established *Aphthona* populations further reduced above-ground biomass and seed production of leafy spurge (Jacobs et al., 2006). The advantages of using sheep to manage spurge declines with the size and productivity of the pasture (Bangsund et al., 2001), but the gains offered by the faster reduction in spurge cover from grazing can off-set the additional immediate expenses. Also, timing of control activities can affect their efficacy. Spring herbicide applications or grazing too late into the season reduces adult food availability for the biological control beetles and reduces their populations (Lym, 1998). Long-term reduction in above-ground densities eventually reduces leafy spurge seed banks (Thilmony and Lym, 2017); fire may hasten such reductions by triggering large flushes of germination from these seed banks (Larson et al., 2008). Fire can also improve establishment of *Aphthona*, which seems to be improved by reduced groundcover, and fire does not appear to harm larval survival (Fellows and Newton, 1999; Lym, 2005). Resident communities of other plants respond strongly to biocontrol-driven reductions in leafy spurge cover, but this recovery can be slow, and secondary invasions of other weeds often occur (e.g., Lesica and Hanna, 2009; Larson and Larson, 2010). Flea beetle damage may inhibit replacement of spurge by other species to some degree, perhaps due to persistent below-ground competition even with removal of significant above-ground biomass, or perhaps due to chemicals released by the beetles or damaged leafy spurge in the soil (e.g., Kirby et al., 2000; Cline et al., 2008). Therefore, human-assisted revegetation can help increase plant competition and create conditions less susceptible to re-infestation by leafy spurge. However, the intensive tillage and herbicide applications often used to ensure establishment of seeded forage species are not compatible with establishment of the biocontrol agents (Lym, 2005). Thoughtful multi-step integrated management is important to achieving desired plant communities. *Aphthona* beetles are relatively easy to collect and move, and insectaries for their redistribution have been established for decades in most areas with large infestations. *Aphthona* phenology is well suited to integration with several common management tools in rangeland systems. Biocontrol benefits from an integrated context, which increases the likelihood of long-term sustainable weed control in diverse situations.

Despite their overall efficacy, there are limits and nuances to management with *Aphthona* flea beetles and significant knowledge gaps in their practical use. High leafy spurge stem density, soils composed of greater than 80% sand or that are frequently flooded, and shady sites have all been associated with low *Aphthona* efficacy or establishment (Lym, 1998; Jacobs et al., 2001; Samuel et al., 2008). Although it should be noted that in some instances, inundative releases (50+ *Aphthona* per leafy spurge stem) can overcome some disturbance-related limitations, for instance in riparian zones (Progar et al., 2010). Additional ecological interactions can influence agent efficacy and weed prevalence after *Aphthona* releases, such as the structure and composition of the surrounding habitat (Jonsen et al., 2001; Larson and Grace, 2004), soil conditions, and level of precipitation (Jacobs et al., 2001), as well as host plant genotype and habitat selection pressures on agent assemblages (Lym and Carlson, 2002). *Aphthona* species fail to establish long-term populations with the densities needed for weed management at many sites (Lym, 1998). These failures may reflect the limits of *Aphthona* species' tolerance for particular site conditions, but more subtle factors such as plant tolerance to herbivory or mismatches between agent and host plant genotypes may also play a role.

Biological control agents, under current regulations, must be highly host specific. This host specificity can occasionally reach below the species level and manifest itself as preferences for certain varieties or genotypes of the target plant. Also, the degree to which plants can tolerate or resist attack by agents may vary (Gaskin et al., 2011). Lym and Carlson (2002) showed that leafy spurge plant genotype affected feeding and reproduction of some *Aphthona* species. A study with the midge *Spurgia esulae* found that two plant genotypes from Manitoba and Montana were more resistant to galling than others (Lym et al., 1996). Although female midges laid eggs on all genotypes, egg and larval survival varied among genotypes. For both studies, the work was done on seven plant genotypes, each genotype with a different origin (Manitoba, Montana, Nebraska, North Dakota, South Dakota, Wyoming, and Austria), though it is still unclear if certain genotypes are only found in certain areas. Detailed information about the population structure of this weed, and information on where these genotypes are present across the invaded landscape, could be used to better test agent efficacy.

Additionally, the impacts attributable to each distinct *Aphthona* species in different situations and habitats are usually not known. Multiple *Aphthona* species often were and continue to be released together; they co-occur and are not consistently separated beyond their color groups: black (*A. lacertosa*, *A. czwalinai*) or brown (*A. cyparissiae*, *A. flava*, *A. nigriscutis*). For example, brown flea beetle populations at one point appeared to be nearly all *A. nigriscutis*, and both *A. flava* and *A. cyparissiae* appeared to be scarce. However, genetic analyses found both *A. flava* and *A. cyparissiae* at insectary sites in North Dakota, suggesting this assumption was likely based on a lack of morphological distinction rather than a true absence from the landscape (Roehrdanz et al., 2009). The composition of *Aphthona* agent communities can change after the initial introductions in a location due to differences in mobility and density responses (Larson and Grace, 2004). At some North Dakota sites, high populations of *A. nigriscutis* declined while *A. lacertosa* and *A.*

czwalinai populations increased to the point that they became the most common agents (Lym, 1998). In a Wyoming study, *A. nigriscutis* beetles colonized new areas more quickly than other species, but *A. lacertosa* and *A. czwalinai* survived in greater numbers where either species had been established for a year or more, resulting in a turnover in the relative abundance of agents (Kazmer and Marrs, 2005). Pre-release and native range studies suggest *Aphthona* agents should differ in their habitat preferences (Gassmann et al., 1996; Nowierski et al., 2002). We have limited data on how species composition of the agents has changed over time at individual sites or how differences among agent species have interacted to influence agent community composition or dynamics through time. A rigorous assessment of weed and agent populations and associated habitat conditions would be required to allow management recommendations to be based on releases of particular mixtures of *Aphthona* species.

The other four agents established for leafy spurge biocontrol have not been studied as thoroughly. *Hyles euphorbiae* continues to be considered ineffective, particularly as outbreaks of the agent sufficient to cause severe plant damage are too late in the season to affect the population viability of the weed. This agent's impacts have not been well documented in the recent literature. The two *Spurgia* species are established and have been redistributed with the goal of reducing seed production (Hansen et al., 1997)—especially in wooded areas, which are less favorable for *Aphthona* beetles (Lym, 2005). However, biocontrol with *Spurgia* species requires an integrated approach for success (Lym and Carlson, 1994), and the impact of these agents is likely to be limited by complicated genetics influencing the host-agent interactions (Lloyd et al., 2005; Lym et al., 1996). Another agent, *O. erythrocephala*, co-occurs with *Aphthona* beetles and can cause substantial damage in leafy spurge patches. This combination of agents has worked well and has strong potential to reduce stem densities, and recruitment of both seeds and vegetative propagation. Anderson et al. (2015) reported natural colonization and extensive damage by *O. erythrocephala* at *Aphthona* release sites. In contrast, Progar et al. (2011) saw no impact by *O. erythrocephala* for two years after release, during which there was a reduction in agent abundance. APHIS included the *O. erythrocephala* beetle in its redistribution efforts (Hansen et al., 1997), but this agent did not receive the coordinated efforts expended on the *Aphthona* complex. However, a recent study detected *O. erythrocephala* at approximately ⅓ of 100 *Aphthona* complex release sites surveyed (West et al., in review). This finding, combined with the reported movement of the agent by the current redistribution programs, suggests that *O. erythrocephala* warrants more study to determine its impact on leafy spurge populations and its potential role in leafy spurge biocontrol management.

BENEFITS OF BIOLOGICAL CONTROL OF LEAFY SPURGE

Aphthona biocontrol agents have substantially reduced leafy spurge densities across a wide range of sites and provided sustained control for extensive time periods. Thousands of insects continue to be collected annually from local field insectaries and provided to a diverse range of stakeholders and private landowners on free-collection days (**Fig. 5**). Distribution is further supplemented by similar activities led by various state, tribal, and local entities. A potential benefits estimate made at the beginning of the *Aphthona* release program suggested a possible savings of $59 million per year if the biological control agents could suppress the leafy spurge infestation by 65% by 2025 (Bangsund et al., 1999). We are approaching this date, and although no region-wide estimate is currently available, site-level spurge reductions of this magnitude are not uncommon where *Aphthona* agents have established. Furthermore, surveys conducted during and after the TEAM Leafy Spurge program era suggested biological control had met adopters' expectations and led to favorable changes in land managers' perceptions of the prospects for leafy spurge control (Hodur et al., 2006). Considering that the direct economic impacts of leafy spurge before widespread introductions of *Aphthona* beetles were estimated as $40 million annually in four Great Plains states (Leistritz et al., 2004), the addition of biological control as a low-cost sustained option is undeniably valuable.

Figure 5. Insect giveaway at 'Spurgefest II', held in 2001 at Theodore Roosevelt National Park in Medora, North Dakota. (USDA-ARS TEAM Leafy Spurge)

WORK STILL TO BE DONE

Three specific information gaps still need to be filled: (1) a more detailed taxonomic examination of the weed and the agents; (2) an evaluation of how long-term suppression by agents might require shifts in management targets over time; and (3) a better understanding of agent impacts at a regional scale.

Better Taxonomic Information on the Target Weed

Taxonomic characterization of leafy spurge in North America has always been difficult and contentious. The invasive populations appear to be a complex of species native to Europe and Asia (Croizat, 1945), and there may have been multiple introductions of the various species (Dunn, 1985). Radcliffe-Smith (1985) reported some 20 taxa (species and their hybrids) of leafy spurge in North America. The recent treatment of *Euphorbia* in the Flora of North America (Berry et al., 2021) suggests that *Euphorbia esula* L., a name commonly attributed to the invasion, may have occurred sporadically in North America a century ago, but has not persisted, and in its native range in Europe, *E. esula* has little tendency to be weedy. Berry et al. (2021) suggests that *Euphorbia virgata* Waldst. & Kit. is the correct identification of the leafy spurge invasion. This species is more widespread and weedy in Europe and Asia and is morphologically more like the plants in the North American invasion than other spurge species from Eurasia. There has also been speculation that hybridization and polyploidy may have occurred during the invasion, adding to the confusion (Croizat, 1945; Radcliffe-Smith, 1981; Stahevitch et al., 1988). When agents were selected and tested, researchers lacked sufficient genetic information to provide taxonomic clarity, and current agents were likely collected on *Euphorbia* species or biotypes that are not present in the North American invasive population. A robust clarification of the invasion taxonomy would require molecular analysis to complement the existing chromosomal and morphological classifications. Although the biological control program has been successful in many areas, it is possible that by knowing the genetic identification of the invasive populations in more detail, researchers might be able to find more effective, highly host-specific agents.

Long-Term Effects of Biocontrol on Management Targets

Long-term and widespread herbivore pressure by *Aphthona* species may increase the importance of seed production by reducing the plant's ability to reproduce vegetatively and making competition for germination sites more ecologically important. High genetic diversity, which provides a signature of seedling recruitment in clonal species such as *E. virgata*, is commonly encountered in leafy spurge patches (West et al., in review). This diversity suggests that leafy spurge infestation densities are frequently bolstered by seed production, which may indicate the importance of agents beyond the *Aphthona* species complex. For instance, in-depth research on emerging impacts by *O. erythrocephala* may improve biocontrol management with existing established agents. A better understanding of leafy spurge population dynamics in the landscape would inform management decisions about the degree to which active redistribution of agents that target above-ground structures and additional management to reduce seed production are necessary to manage infestations.

Better Understanding of Agents' Impacts at a Regional Scale

Most published studies on leafy spurge biocontrol agent impact in the United States have documented successful reductions in stem density, but the resulting plant communities (i.e., recovery of native or desirable forbs and forage) have rarely been monitored over the long term. Also, existing impact studies are predominately from north central states (i.e., North Dakota, Montana, and Wyoming). Thus, much of what is known about efficacy of the program in the United States is from studies done at local scales along the habitat gradients of the northern Great Plains ecosystem. Without a large-scale assessment of agent impacts and weed populations, it is difficult to evaluate whether local scale reductions in stem density are truly indicative of broader patterns of leafy spurge population control, or if different agents or supporting measures might be needed in other regions.

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Chapter 25

Biological Control of Invasive Mole Crickets in Florida

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NON-TECHNICAL SUMMARY

Neoscapteriscus (Orthoptera: Gryllotalpidae) mole crickets from southern South America arrived in Georgia and Florida from 1899 to the 1920s in dry ship ballast. Over time, they built up huge populations and were targeted for control with many toxic pesticides. In the 1970s, the U.S. Environmental Protection Agency banned the use of one of these pesticides, chlordane, which southeastern ranchers had come to use extensively for mole cricket suppression. Lacking this pesticide, the ranchers asked for help against these invasive mole crickets, which led to the creation of a mole cricket research program at the University of Florida.

Trapping stations were established in 1979 to catch mole crickets so their numbers could be monitored, and while each trap caught thousands of flying mole crickets each year, trapping was unable to reduce mole cricket populations. The lowest-cost control method that researchers could envisage was classical biological control, which, if successful, would provide permanent control without recurrent costs for ranchers. To begin this process, rearing methods were developed for the mole crickets, so that they would be available for experimentation.

In South America, surveys for specialized mole cricket natural enemies found a species of tachinid fly, a crabronid wasp, an entomopathogenic nematode, and a bombardier beetle, each with interesting but incompletely known life histories. Stocks of these four organisms were brought into a quarantine laboratory in Florida, and studies showed that three of the four were proven to be safe to the Florida environment, people, and non-target insects. The three were reared in large numbers and released, starting in 1985.

By fall of 1990, mole cricket numbers began to decline, and by 2000, the average catch in monitoring traps was only 5% of the numbers seen during the 1980s, a permanent 95% reduction of the scope of the problem with no annual recurring costs. Since the conclusion of the research program in 2004, these natural enemies have continued to spread, and they should continue to reduce damage throughout Florida in organic vegetables, pastures, turf, and playing fields, wherever climates are suitable. The program's benefit:cost ratio was 52:1, meaning that the benefits to the public are at least 52 times larger than all the funds expended on the program.

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HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

In the late 1890s, invasive mole crickets were detected in Georgia, and then in Florida. They were not the familiar but harmless native northern mole cricket, *Neocurtilla hexadactyla*, but a species in the genus *Neoscapteriscus*, and it was associated with damage in various vegetable crops (cabbage, green peppers, eggplant, etc.). Initially, these pests were thought to be the West Indian mole cricket, *Neoscapteriscus didactylus*, a pest mole cricket known in Puerto Rico and some other Caribbean Islands.

Eventually, insect taxonomists determined that the mole crickets represented three invasive species, which are now called (1) tawny mole cricket, *Neoscapteriscus vicinus*, (2) short-winged mole cricket, *Neoscapteriscus abbreviatus*, and (3) southern mole cricket, *Neoscapteriscus borellii* (all Orthoptera: Gryllotalpidae). The arrival of these insects was in Brunswick, Georgia, which at the time was a major seaport shipping goods to and from southern South America (Argentina and Uruguay) where all three species are native. It was deduced that these crickets had arrived in ship ballast (Walker and Nickle, 1981). These mole crickets in their native ranges are frequently found along riverbanks, where they could easily be included in sand and gravel dug and used as ballast in ships bound to the southern United States.

Adults of two of the species (tawny and southern) fly readily in spring and fall and spread from Florida to North Carolina and west to Texas. Undoubtedly the southern mole cricket also hitchhiked on vehicles to reach extreme western Arizona, a site in northern Mexico, and California. Short-winged adults cannot fly and are restricted to the eastern and southwestern coasts of Florida with very few inland sites.

Nature of the Problem

For some years, mole cricket damage occurred mainly in vegetable crops, caused by feeding of nymphs and adults on plant roots or stems. In the 1930s, growers tried to control these losses with soil applications of calcium and potassium arsenates. In 1940, Florida growers appealed for federal help, and 1,258 tons of calcium arsenate bran bait were donated to Florida growers in the worst affected counties. In the mid to late 1940s, synthetic chemical pesticides were first developed, starting with the chlorinated hydrocarbons such as DDT. The chlorinated hydrocarbon chlordane provided excellent control of mole crickets, and it was widely adopted for that purpose, not only in vegetable fields, but also on playing fields and pastures where Bermudagrass (*Cynodon dactylon*) and Bahiagrass (*Paspalum notatum*) roots were being heavily damaged. Mole cricket tunneling loosens the roots of grass, and affected pasture grasses die when cattle subsequently uproot them. Use of chlordane on playgrounds and pastures prevented such devastating losses from pest mole crickets. However, chlordane, like DDT, was eventually banned from use in agriculture because its long-lasting residues polluted habitats, leaving toxins that affected wildlife and people's health. In short, this management approach was initially highly effective but was unsustainable because of the pollution it caused.

WHY CONTROL THESE INVASIVE SPECIES?

The control of mole crickets was justified because of the value to the state and nation of the products produced on Florida pastures, which have been important for raising of beef cattle since at least the 1860s. Currently, most cattle operations in Florida are cow-calf businesses with mature calves being shipped to other states for finishing and processing. In 2007, 450,000 cows worth about $400 million were processed. At that time in Florida, beef was produced on 3.2 million acres (1.2 million ha) of pasture land and 1.3 million acres (0.5 million ha) of grazed woodland which was just over 10% of the state.

The need for a biological control project was triggered in pastures in 1978, when the use of chlordane was banned by the U.S. Environmental Protection Agency, because no other chemical insecticides were so effective and inexpensive for killing pest mole crickets. Florida ranchers requested help from the Florida Legislature, which earmarked research funds for this purpose. These funds supported work at the University of Florida Department of Entomology and Nematology, which first carried out the necessary research and then implemented the biocontrol program.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Figure 1. Adult short-winged mole cricket, *Neoscapteriscus abbreviatus.* (Paul Choate, University of Florida)

Figure 2. Sound emitter for attracting long-winged mole crickets (they fall into the bucket below) and *Ormia depleta* flies (they move into the transparent vial at top). (Lyle Buss, University of Florida)

This program ultimately involved the release of three agents—the wasp *Larra bicolor* (Hymenoptera: Crabronidae), the nematode *Steinernema scapterisci* (Rhabditida: Steinernematidae), and the parasitic fly *Ormia depleta* (Diptera: Tachinidae)—against three species of invasive mole crickets: tawny mole cricket, *N. vicinus*, short-winged mole cricket, *N. abbreviatus* (**Fig. 1**), and southern mole cricket, *N. borellii*. The names for these species and other associated species have changed over time and are summarized in **Table 1** for easy reference.

The program first concentrated on understanding the feeding habits of the three invasive mole crickets, which vary. Tawny mole crickets and short-winged mole crickets feed almost entirely on plants, but southern mole crickets are mainly predatory, though they are also destructive to grasses.

Studies were also conducted on the male crickets' songs, which are species-specific and attract the flying females. Electronic sound-emitting devices were built that exactly replicated the songs of tawny mole cricket or southern mole cricket. This was not done for short-winged mole crickets because they neither sing nor fly. When these sound emitters were built into funnel-shaped traps (**Fig. 2**), it made for an effective trap to collect the flying female mole crickets, which allowed researchers to monitor the size of cricket populations (Walker, 1982).

Early on, a new, very low-cost insecticide bait to kill mole crickets was developed that combined chicken feed, some additives, and 2% malathion insecticide (Kepner and Yu, 1987). The product was designed to be formulated by ranchers themselves. However, the Florida Department of Agriculture declared it unsafe because the bait might attract wild birds, and consequently it was banned. The loss of this insecticidal-bait focused attention on classical biocontrol, which was reasoned to be the lowest-cost control method in the long term.

Table 1. Names applied to the organisms in the mole cricket biocontrol program. N = native, I = invasive species, BC = biocontrol.

Common name	Earlier name	Latest name	Feeding habits	Origin (N or I)
tawny mole cricket	*Scapteriscus vicinus* Scudder	*Neoscapteriscus vicinus* (Scudder)	Feeds on plants	I
short-winged mole cricket	*Scapteriscus abbreviatus* Scudder	*Neoscapteriscus abbreviatus* (Scudder)	Feeds on plants	I
southern mole cricket	*Scapteriscus acletus* Rehn and Hebard	*Neoscapteriscus borellii* (Giglio-Tos)	Mainly predatory	I
northern mole cricket		*Neocurtilla hexadactyla* (Perty),	Mainly predatory; not damaging	N
West Indian mole cricket	*Scapteriscus didactylus* (Latreille)	*Neoscapteriscus didactylus* (Latreille)	Feeds on plants	I
None	*Larra americana* Saussure	*Larra bicolor* Fabricius	Parasitoid attacking mole crickets	BC agent
None	*Neoaplectana* sp.	*Steinernema scapterisci* Nguyen and Smart	Mole cricket nematode	BC agent
None	*Euphasiopteryx depleta* (Wiedemann)	*Ormia depleta* (Wiedemann)	Parasitoid attacking mole crickets	BC agent
(*Larra bicolor*'s best nectar plant)	*Borreria verticillata* (L.) G.F.W. Mey	*Spermacoce verticillata* L.	Nectar plant	Debated

Larra bicolor

Larra bicolor (**Fig. 3**) is a parasitic wasp that was already known at the start of the program to attack mole crickets, having been introduced earlier into Puerto Rico from Belém in the Brazilian state of Pará on the Amazon River in the late 1930s to suppress the West Indian mole cricket. However, Puerto Rican entomologists did not develop a method to evaluate this wasp's effects. Efforts to import *L. bicolor* into Florida in the 1940s were unsuccessful. A second attempt, also from Puerto Rico, was made in 1981. A population of the wasp did become established, but only at one site in Fort Lauderdale, from which it spread only slightly. The key factor in this establishment seems to have been the establishment of *Spermacoce verticillata* (Rubiaceae), a plant that produces nectar used by *L. bicolor*. This population of *L. bicolor*, however, had a negligible effect on mole crickets, killing only about 1% of the short-winged mole crickets at the site, and none of the other two species (based on the percentage of crickets in pitfall traps that had wasp eggs or larvae on them) (Castner, 1988).

Figure 3. Adult female *Larra bicolor* wasp attacking an adult *Neoscapteriscus vicinus.* (Lyle Buss, University of Florida)

A third attempt to import a population of *L. bicolor* more adapted to Florida's climate was made in 1988. As the Puerto Rican population imported in 1981 to Florida had been originally from Belém, Brazil on the equator, it may not have been sufficiently cold hardy to survive in northern Florida. So, the same wasp species was imported from Santa Cruz de la Sierra in Bolivia at 18° south latitude and 1,365 ft (416 m) altitude, which suggested that these wasps might have some tolerance to colder temperatures. These wasps became established in Gainesville, Florida (Frank et al., 1995) and then spread in northern Florida. Wasps that had developed in northern Florida were later released at Tifton, Georgia. They established and spread to Alabama and Mississippi, and then along the east coast north to coastal North Carolina. By 2008, *L. bicolor* had been recorded in 48 of Florida's 67 counties (Frank et al., 2009a).

How successful *L. bicolor* was in killing pest mole crickets in Florida was answered by graduate student S. L. Portman in a field study in north Florida (Portman et al., 2009). He found that if a population of the wasp had a convenient nectar source (a patch of a dozen or so *S. verticillata* plants within 650 ft [200 m]) of the pasture, they could out-reproduce mole crickets. Male wasps are typically seen on sunny days feeding on plant nectar, while many females were hunting mole crickets (Portman et al., 2010). Provisioning of pastures with these nectar plants is not hard for ranchers to arrange (Frank and Sourakov, 2002).

The nectar plant *S. verticillata* has been discussed as an invasive plant in Florida. However, under alternate names, this same plant was recorded from Big Pine Key in Florida in 1860 and from the vicinity of Ross Hammock in the Everglades in 1915, perhaps suggesting it is a native species. In 1990, the UF/IFAS Invasive Plant Council was petitioned for an opinion and stated that *S. verticillata* is not invasive, thus allowing the mole cricket researchers to recommend its planting as a nectar source for *L. bicolor*. Planting the species along roadsides would help promote populations of *L. bicolor*. *Spermacoce verticillata* is not toxic to vertebrates and, in fact, it is suitable for grazing by cattle. A decade later, the Florida Exotic Pest Plant Council announced that *Spermacoce verticillata* is invasive; however, weed scientists (Sellers et al., 2019) recommended against trying to eliminate it from pastures because it promotes adult survival and population growth of *L. bicolor*. This decision was based on the same rationale invoked for promoting Bahiagrass, an invasive plant, including giving it exemption from invasive regulations as it is also useful.

Steinernema scapterisci

To explore for natural enemies of mole crickets in southern South America, mole cricket researcher T. J. Walker obtained USDA funds to support a post-doctoral researcher in South America. H. G. Fowler was hired and established a base for his surveys at Rio Claro in the Brazilian state of São Paulo, which had *N. borellii* and *N. vicinus* populations and was only a few hours southwest of the coast of Rio de Janeiro, where *N. abbreviatus*, the third target species, could be obtained. From field-collected mole crickets, Fowler recovered nematodes that he shipped to Gainesville, Florida, but nematode survival was low. Dr. Aquiles Silveira-Guido, a retired professor in Uruguay, was hired to work on the project, and he too found nematodes and shipped some to Gainesville. In 1985, nematologist G. C. Smart and his student K. B. Nguyen visited Fowler and Silveira-Guido. They collected nematodes from the cultures maintained by Silveira-Guido and successfully brought samples of the newly discovered parasitic nematode back to Florida.

Figure 4. *Neoscapteriscus vicinus* with emerging *Steinernema scapterisci* juveniles. (Lyle Buss, University of Florida)

Among the various nematodes collected in Uruguay, there was an undescribed species of *Neoaplectana* that was later described as a new species of *Steinernema*, which proved to be highly specific to *Neoscapteriscus* mole crickets. It was named *Steinernema scapterisci* (**Fig. 4**) (Nguyen and Smart, 1990), and later, its associated bacterial symbiont was described as *Xenorhabdus innexi* (Lengyel, 2005). In this period, entomopathogenic nematodes of foreign origin had a blanket exemption from USDA regulations, being recognized as "harmless to vertebrate animals and to plants." Therefore they could be released without review in the United States. Non-target tests done at the University of Florida in Gainesville showed this nematode had no effect on honeybees, but it did attack a small percentage of the non-native house cricket, *Acheta domesticus*, which does not exist outdoors in Florida.

This new nematode was reared on mole crickets that were available from field monitoring traps or laboratory rearing. The first field releases of the nematode took place in 1985 in small plots in pastures in Alachua County, Florida. Some nematodes were released by burying dead, infested mole crickets, but others were applied in water with a sprinkling can, at approximately 2.7 million per ft^2 (250,000/m^2) based on laboratory experimentation (Hudson and Nguyen, 1989). Both methods placed the nematode beneath the soil surface because such nematodes are known to be quickly killed by exposure to ultraviolet light and desiccation. Both application methods were effective in causing infections in wild mole crickets at the field sites. Mole cricket numbers in those plots were monitored for six years, during which time mole cricket numbers were observed to decline and the nematodes to persist. The plots also contained native northern mole crickets, *N. hexadactyla*, and when these were examined, they were found to be infected with a different, presumably native, nematode that was later described as *Steinernema neocurtillae* (Nguyen and Smart, 1992).

Rearing the new South American nematode at a scale sufficient for widespread release could not be done without the aid of a commercial producer of nematode products. G. C. Smart, through the University of Florida's Office of Technology Licensing, obtained a patent for use of this nematode for control of mole crickets, and Biosys, a California company in the business of mass-producing beneficial nematodes, agreed to develop mass-production methods for *S. scapterisci*. Biosys supplied enough nematodes in a water-soluble gel for trial applications on one large cattle pasture in each of six Florida counties. Nematodes were applied to the subsurface by a tractor-drawn chisel rig in fall 1989 and spring 1990 when mole crickets were adults or almost so (the most susceptible stage). Because chemical control of mole crickets targets small mole crickets in summer (Parkman et al., 1993), the effectiveness of pesticides vs. nematodes could not be directly compared. Dispersal of the nematodes outward from the 2.5-acre (1-ha) treated areas on these six ranches occurred by crawling of infected mole crickets (likely only about 200 ft [60 m] in the 21-month study) or by flight of infested adult crickets, which potentially could reach much greater distances.

In the 1990s and the first decade of the 2000s, commercial production of this nematode was undertaken by several companies and applied in various formulations with various kinds of application machinery. This occurred in two very different settings, including golf courses, with high turf values and relatively small acreage, and cattle pastures, where the opposite conditions prevailed. As with all nematodes attacking soil insects, the formulations and application methods used needed to prevent dehydration of the nematodes being applied by placing them in the soil at a depth where the target pest could be found by foraging nematodes. Other important factors affecting the acceptability of these nematodes were the need to prevent damage to golf course turf by the application machinery and to keep costs very low (for cattle pastures).

In general, long-term impacts of nematode applications on pest density are not expected, and nematode applications are often akin to using an eco-friendly pesticide, for where the only consequence is short-term pest suppression. However, in a few species of nematodes that are more specific to a particular target pest and more persistent, there can be long-term persistence and suppression of the pest. In these cases, the nematode acts like a classical biocontrol agent. *Steinernema scapterisci* turned out to be one such species, which not only persisted from year to year, but spread long distances through flight of infected (but still functional) mole crickets in the early stages of infection. Consequently, as mole crickets were examined in various places after initial releases, new places were found where the local invasive mole crickets were infected by *S. scapterisci*. For example, in Polk County, Florida, nematologist L. W. Duncan (working from the Citrus Research and Education Center at Lake Alfred) developed a genetic probe for *S. scapterisci* and used it to detect the nematode, which was confirmed to be present in many localities where the nematode had apparently never been applied (L. W. Duncan pers. com.)

Evidence was also obtained that showed *S. scapterisci* could persist for many years at sites where it had once been applied, showing it acted as a classical biocontrol agent. At the request of a prominent Florida cattleman, the South American nematode's persistence was assessed at four of a set of six pastures seven years after the nematode's application in 1989–1990. Pitfall traps were installed at the sites to catch mole crickets, and nematode-infected mole crickets were caught at all four sites (Frank et al., 1999). Similarly, in 2001, a Gainesville golf course, where the nematode had been released 12–13 years earlier, was examined, and the nematode was found to have persisted and to be infecting 10–15% of mole crickets. This not only demonstrated the persistence of the nematodes but also showed that they were highly tolerant to chemical pesticide applications, such as are commonly made on golf courses (Barbara and Buss, 2004).

The ability of this nematode to both persist and spread made it potentially useful to ranchers to control mole crickets in cattle pastures. However, even a single application was too expensive for full treatment of large pastures. This limitation led researchers to test the efficacy of applying the nematode in strips across a pasture, with the expectation that the nematodes would eventually spread on their own after a successful application. The degree of pest control provided by this approach was tested in a 25-acre (10-ha) Bahiagrass pasture. Within three years of the application, pitfall trap catches of mole crickets (*Neoscapteriscus* spp. combined) were reduced by 79.2%, and the Bahiagrass cover increased from 33% to 96% in the same period. Such strip applications reduced the cost of the initial nematode application by 88% (eight times cheaper), but also increased the time required for the nematode to reduce the mole cricket density throughout the pasture (Adjei et al., 2006).

Treatment of any given location even once, however, still required some company to produce and market the nematode. Over time, several different companies were involved, starting with Biosys in California, which marketed the nematode as Vector MC™. This production unfortunately violated the University of Florida's patent, and no agreement on licensed use could be reached by Biosys. Later, a British company called Micro-Bio was able to obtain a license for use from the University of Florida. That company was acquired by Becker Underwood, an American Company, that was willing to send technicians/salesmen to attend meetings with ranchers to explain the product's use. In 2009, J. H. Frank obtained a grant to pay for construction of two application machines that would apply the nematodes correctly to the soil subsurface (**Fig. 5**). These machines are currently available for loan to Florida ranchers who wish to apply *S. scapterisci*. In the long term, such applications on pastures are likely to be unnecessary as the distribution of the nematode expands naturally to be more complete.

Figure 5. Tractor-pulled device for applying nematodes beneath the soil surface. (Lyle Buss, University of Florida)

Ormia depleta

Larvae of this South American fly develop as parasitoids inside mole cricket nymphs and adults. The word 'parasitoid' indicates that it kills its mole cricket host. This is the only known tachinid parasitoid of mole crickets. In eastern South America, it exists as far south as Porto Alegre (30°S) in the Brazilian state of Rio Grande do Sul.

The fly (**Fig. 6**) is known to attack only *Neoscapteriscus* mole crickets, and it finds them at night by orienting to their songs. *Ormia depleta* attacks only two species in the United States (tawny and

Figure 6. Adult female *Ormia depleta* fly. (Paul Choate, University of Florida)

southern mole crickets), and perhaps some other *Neoscapteriscus* species in South America. Only male mole crickets sing, so they are the stage the fly attacks most often, but females and large nymphs may be attacked if they are close to singing males. Also, both sexes of short-winged mole crickets can be attacked if they are close to singing males of either of the other two host species, but otherwise the fly cannot find them. Species of *Ormia* that are native to the southern United States do not attack *Neoscapteriscus* mole crickets because they are attracted only to the songs of *Gryllus* or *Neoconocephalus* crickets, not those of *Neoscapteriscus* crickets. Therefore, *O. depleta* is safe to non-target organisms in the United States. The adult flies neither bite nor sting people, and because they are only active at night when mole crickets sing, they are unlikely to be seen by people (Walker et al., 1996).

Adult females of *O. depleta* were first encountered by mole cricket researchers when *Ormia* females were trapped in South America using recordings of mole cricket songs as bait (Fowler and Kochalka, 1985). These flies deposit living larvae (not eggs) on and near singing mole crickets. In quarantine in Florida, work with the native fly *Ormia ochracea* gave clues as to how to get *O. depleta* to reproduce in the laboratory. A rearing method was developed in which larvae were dissected from female flies and placed behind the pronotum of a mole cricket, from where they burrowed into the mole cricket to develop (Wineriter and Walker, 1990).

Using pupae of *O. depleta* reared in the laboratory, we started making field releases of this fly in 1988 at sites near Gainesville and Bradenton. Pupae used in releases were buried approximately ¼ inch (5 mm) in clean, moist sand in a plastic crisper box, which was placed in a cage atop a wooden post embedded in the ground. The placement on a post was done to protect the pupae from disturbance by larger animals. The cage around the plastic crisper box was built of hardware cloth (wire) with a mesh small enough to exclude birds that might eat the fly pupae but that let the adult flies leave. The top of the cage was a piece of plywood with black polyethylene suspended to shade the contents and protect fly pupae from rain. The sand in the release boxes was moistened daily by a spray bottle. Following the release, the sand in the box was sifted to find dead pupae or empty puparia that would indicate successful adult emergence. Based on that information, we estimated more than 90% of the pupae produced adults.

After the initial 1988 trial to develop an effective release method, we started a large program of releases in 1990–1991. Releases in this larger effort were made at 28 golf courses and one sod farm. For these releases more than 10,000 *O. depleta* were reared in mole crickets in the laboratory to supply pupae for releases. At each release site, 200 fly pupae were placed in release cages as described above. The release sites ranged from Ft. Walton Beach in the northwest (near Pensacola) to Naples in southwest Florida (Frank et al., 1996).

In 1992, we began efforts to detect wild fly populations to determine the distribution of *O. depleta* as a measure of the success of the releases. Flies were recovered by attracting them to sound emitters that mimicked mole cricket songs. The emitters were inside heavy plastic bags, and the top of each bag was smeared with “Tack-Trap” (a sticky material used to band fruit trees) to trap the flies arriving to the song source. The 11 sound emitters were positioned along rural roads at approximately 5-mi (8-km) intervals and set to begin operating at sundown. An additional hour was allowed to pass before re-checking the line in reverse. This was done on various dates in June–July 1992, November–December 1992, and November–

December 1994. By the time this survey was finished, the fly had been recovered in 36 Florida counties, all south of approximately 30°N latitude (just south of Jacksonville). Flies did not appear to establish further north, although a few observations of flies were reported there. The maximum number of flies caught in the 'Tack-Trap' was 125, packed 'shoulder to shoulder', at a location in rural Martin County (Frank et al., 1996). That so many flies could have been captured in such a brief time suggests a large population. Efforts to determine what food sources were used by adult flies (like nectar) were unsuccessful. (In the laboratory, we fed them on artificial 'hummingbird' nectar). Walking around at night to try to observe flies feeding on plant nectar achieved one thing: bumping into trees.

Other Potential Biocontrol Agents Tested

Two other agents were investigated in this program, which ultimately were either not successful or unsuitable. One of these agents was *Pheropsophus aequinoctialis*, a predatory ground beetle (Carabidae) in a group known as bombardier beetles. *Pheropsophus aequinoctialis* is widespread in South and Central America, and T. L. Erwin suggested to a member of the mole cricket program that larvae of this beetle might be predators of mole cricket eggs. *Pheropsophus aequinoctialis* was imported from Bolivia and later Brazil into a Florida quarantine facility for study. We learned that larvae of *P. aequinoctialis* can feed on eggs of all four mole cricket species in Florida, but this group included the native mole cricket *N. hexadactyla* (Frank et al., 2009b). Consequently, this beetle was rejected for possible release.

Another agent that was considered was the fungal pathogen *Beauveria bassiana*. In small containers, applications of *B. bassiana* to the surface of mole crickets killed them, and the idea we investigated was to create a bait that could be mixed with fungal spores, which would attract mole crickets. Unfortunately, there appeared to be a trade-off: using a large amount of bait and little fungus attracted mole crickets but did not kill them; using little bait but a large amount of fungus caused the fungus to repel the mole crickets. We needed a cheap but super-attractive bait. As we never found one, this approach was abandoned without being used.

HOW WELL DID IT WORK?

The mole cricket research program ended in 2004 because its objectives had been achieved. In 2004, of the original seven mole cricket trapping stations, five had been terminated due to operators retiring or relocating. The penultimate station was almost abandoned in 2002, when the university farm on which it operated was sold to the county government for development; but mole cricket researchers petitioned the county government to allow its continued operation for two more years until the county government had finished its plans for development. Data from these stations were compiled by Frank and Walker (2006) to document the decline of both mole cricket species caught in traps, which was first noticed in 1990. The data showed that by the end of the program, mole cricket densities had declined by 95% compared to first nine years of trapping (1979–1988), which corresponded to a period before the released natural enemies began showing up at the trapping stations. During the first nine years of this trapping effort, the trend in annual catches was static (**Fig. 7**), showing that trapping of mole crickets itself had no effect on the local population density. Starting in1990, trap catch began to decline, and it continued to drop until about 2000 when it levelled off. Mole cricket decline was clearly due to the natural enemies released (*L. bicolor*, *O. depleta*, and *S. scapterisci*) but the relative contributions of each agent to this control are unknown and may have varied from place to place. (Note that the histogram in **Fig. 7** shows data per 'generational year,' from August in one year through July the next year, to reflect mole cricket reproduction. We considered the average catch during years 1979–1988 to be the 'baseline' because none of the biological control agents showed up at our trapping stations until 1988).

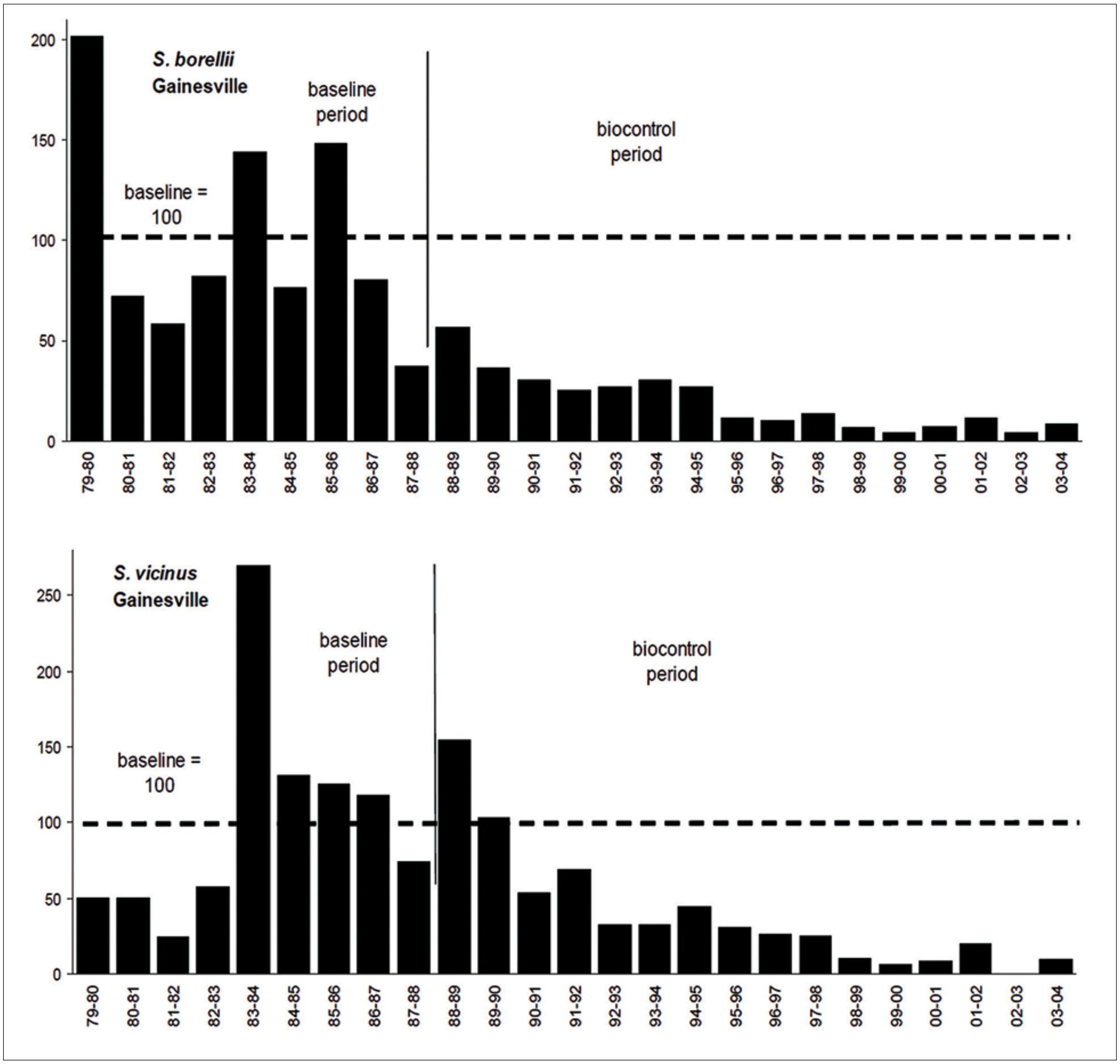

Figure 7. Histograms showing numbers of southern mole cricket, *Neoscapteriscus* (=*Scapteriscus*) *borellii*, and tawny mole cricket, *Neoscapteriscus* (=*Scapteriscus*) *vicinus*, captured (averaged by species and combined over two trapping stations) from 1978–79 to 2004–05. Biocontrol agents were present at the stations by 1990, and they caused the subsequent decline in mole crickets captured. (from Frank and Walker, 2006, reprinted with permission from Oxford University Press)

As a post-datum, we can point out that about 2011, Becker Underwood stopped producing *S. scapterisci* because sales were too low to make an adequate profit to justify continued production. This is likely a case where the very success of the program doomed the future sale of a product that depended for its existence on a continuing pest problem. Regardless, the ability of this nematode to persist and to spread means it is expected to remain a vital part of mole cricket suppression in Florida indefinitely. Furthermore, the nematode is not immobile, as it is spread by newly infected mole crickets, allowing it to spread to new locations over time. Unlike other insect-killing nematodes, this species' populations can survive for many years, if not indefinitely, in a local area if some mole crickets remain. In addition to pastures, owners of organic farms, lawns, and golf courses are expected to benefit from this nematode.

The mole cricket biocontrol program was started at the request of ranchers. A study of the economics of the benefit to Florida ranchers (Mhina et al., 2016) suggested that mole cricket biological control reduced losses from mole crickets in pastures to unimportant levels. The long-term economic benefit of the project had a benefit: cost ratio of 52:1, and its overall annual benefits in terms of avoided pest control and renovation of pastures alone were $13.6 million.

The public benefited from the mole cricket biocontrol program through the reduction of the nuisance that high densities of mole crickets used to cause during outdoor events, such as during nighttime sporting events (in which mole crickets were attracted to outdoor lighting and then landed on people). Mole crickets also no longer swarm around lights in the parking lots of supermarkets and such businesses as convenience stores. Benefits to golf courses were less significant because mole crickets were only one pest among several (although mole crickets for decades have been the most important pests on golf courses in Florida). Only with the decline in numbers of mole crickets did the lesser problem of white grubs surface. Because other pests (such as white grubs) still needed to be treated with broad-spectrum pesticides, suppressing mole crickets with biological control by itself did not reduce pesticide use on golf courses.

The biological control agents released against *Neoscapteriscus* mole crickets in Florida are highly specific to the invasive mole crickets they attack. They do not attack other kinds of mole crickets, such as species of *Gryllotalpa* or *Neocurtilla*, which belong to a different mole cricket subfamily (Gryllotalpinae). Consequently, no effects occurred or are expected in the future on any of these other cricket groups, due to the high specificity of the agents used. Thus, non-target species are protected from harm.

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CHAPTER 26

Biological Control of Invasive *Linaria* spp. in the Western United States

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NON-TECHNICAL SUMMARY

Anecdotal reports indicate that invasive yellow or common toadflax (*Linaria vulgaris*) and Dalmatian toadflax (*Linaria dalmatica*) (Plantaginaceae) were deliberately introduced to North America for ornamental purposes. They were also accidentally introduced as a seed contaminant, as was the case for many early-introduced invasive plants in North America (Mack, 1986, 2003; Mack and Erneberg, 2002; Lehan et al., 2013). In the absence of laws regulating the importation of exotic plants (as opposed to plant pests) into the United States, plant-lovers ranging from homesteaders and miners to horticultural enthusiasts imported and shared non-native flora, including toadflax (Mack, 2004). Widespread co-invasion by both toadflax species in North America resulted in their hybridization, which was first suspected in the early 2000s (Pauchard et al., 2003) and later molecularly confirmed in many western U.S. states (Ward et al., 2009; Boswell et al., 2016).

Most unintentionally introduced toadflax-specialist insects, as well as the approved toadflax biocontrol agents, were initially thought to exploit both *L. vulgaris* and *L. dalmatica*, with preferences for exact toadflax species becoming apparent only after insect establishment in North America (Sing et al., 2016; Smith et al., 2018). In response to this new understanding of agent specificity, concerted efforts were made to find, evaluate, and release host races or biotypes of previously approved toadflax biocontrol agents (Winston et al., 2022). Molecular diagnostics have confirmed the previous introduction of cryptic species, which in turn has explained localized issues with establishment and inconsistent efficacy of agents that, at the time of their introduction, were presumed to attack both *L. dalmatica* and *L. vulgaris* (Toševski et al., 2018). The earliest species introduced for control of toadflax (some flower- or seed-feeding beetles and a defoliating moth) provided minimal control. However, the more recent introductions and establishment of

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stem-mining insects have significantly suppressed toadflax populations throughout North America, which has resulted in widespread and sustained rangeland improvement, reduced weed management costs, and increased protection of non-target organisms.

HISTORY OF INVASION AND NATURE OF PROBLEM

The native range of yellow or common toadflax (*Linaria vulgaris*, Plantaginaceae) (**Fig. 1**) includes temperate areas of Europe and Asia, extending from Scandinavia and the British Isles through northern, central, and southern Europe including the Balkan and Mediterranean regions, to Turkey and southwestern areas in the Russian Federation and China (Saner et al., 1995; CABI, 2022; ISSG, 2022). *Linaria vulgaris* was imported for ornamental, medicinal, and textile dying purposes by early American settlers; it became naturalized in the eastern American colonies of England by 1671, and it was considered a significant agricultural weed both in the mid-western and eastern United States by 1849 (Darlington, 1849; Leighton, 1970; Mack, 2003). First reported in Canada in the early 1800s in southern Quebec, *L. vulgaris* became widespread in the North American prairie regions of Canada by the mid-1900s (Rousseau, 1968; Saner et al., 1995). Historically, *L. vulgaris* spread as a contaminant of crop seed, in baled hay, along railway corridors, and in the ballast of ships (Mitich, 1993; Saner et al., 1995; USDA-NRCS, 2022a). Dissemination of this species as a popular ornamental and medicinal plant was also facilitated by commercial nurseries and seed catalogs (Sing et al., 2016). It is now found throughout North America other than in the Canadian provinces of Nunavut and Labrador (USDA-NRCS, 2022a), and it is considered a nuisance or noxious species as far north and west as Yukon Territory in Canada and Alaska in the United States (Yukon Invasive Species Council, 2020; Alaska Department of Natural Resources, 2021).

Figure 1. Yellow toadflax, *Linaria vulgaris*. (Linda Wilson, University of Idaho, Bugwood.org CC BY-NC 3.0 US)

Figure 2. Dalmatian toadflax, *Linaria dalmatica*. (K. George Beck & James Sebastian, Colorado State University, Bugwood.org CC BY-NC 3.0 US)

Dalmatian toadflax (*Linaria dalmatica*) (**Fig. 2**) was reportedly first introduced in North America in the northeastern United States by 1894, approximately 200 years after *L. vulgaris* was first reported (Alex, 1962). This plant is a Eurasian species from the Mediterranean region, extending from the Balkans to Iran (Robocker, 1974). Like *L. vulgaris*, *L. dalmatica* was introduced as an ornamental plant, both in the United States (to Massachusetts in 1894) and Canada (to Ontario in 1901) (Hatfield, 1894, 1897; Macoun, 1908; Alex, 1962). Escape from horticultural settings has contributed to its current widespread distribution (USDA-NRCS, 2022b), infesting rangelands, open forests, and transportation corridors throughout North America (Lange, 1958; Robocker et al., 1972; De Clerck-Floate and Miller, 2002). The rapid westward spread of *L. dalmatica* is confirmed by voucher specimens from populations that escaped cultivation: collected in California near Sturtevant Camp, Angeles National Forest in 1920, in Washington State near Spokane in 1926, and in Alberta in the towns of Bingen in 1927 and Edmonton in 1933 (Alex, 1962).

Persistent populations of hybrid toadflax (**Fig. 3**), which resulted from cross-pollination of *L. vulgaris* and *L. dalmatica*, have been confirmed since the late 2000s from many sites in Montana, Washington, Idaho, Wyoming, and Colorado, creating a third invasive toadflax target requiring additional management at some locations (Ward et al., 2009; Sing et al., 2016). Hybrid toadflax is not a single defined taxon and is more accurately described as a hybrid complex generated by ongoing cross-pollination between the two parent species (= first generation or F_1 hybrids), crosses of hybrids with other hybrids, and backcrosses of hybrids with one of the parent *Linaria* species (Boswell et al., 2016). Because *L. vulgaris*, *L. dalmatica*, and their hybrid offspring are persistent perennials, there are populations that consist of one or both parent species growing together with multiple-generational hybrids and backcrossed offspring. Controlling these genetically and taxonomically complex populations with biocontrol agents that have specific host preferences can be difficult.

Figure 3. Hybrid toadflax (center) between *Linaria vulgaris* (left) and *L. dalmatica* (right). (Elizabeth Goulet, Cornell University, Bugwood.org CC BY-NC 3.0 US)

WHY CONTROL THESE INVASIVE SPECIES?

Linaria vulgaris can economically harm pastures, rangeland, and cultivated fields, displacing desirable forage and crop plants such as wheat, canola, strawberries, and peppermint (Coupland et al., 1963; Harker et al., 1995; Baig et al., 1999; Volenberg et al., 1999; McClay and De Clerck-Floate, 2002). Yield reductions of 33% in the forage species red fescue (*Festuca rubra*), and 20% in canola and wheat have been recorded in association with yellow toadflax invasions (Darwent et al., 1975; O'Donovan and McClay, 1987; O'Donovan and Newman, 1989).

As a fire-adapted transformer species with the potential to reduce local diversity or transform ecosystems (D'Antonio et al., 2004), *L. dalmatica* has the ability to locally alter vegetation communities following fire (Dodge et al., 2008). Established *L. dalmatica* plants readily regrow from the roots even when their top growth sustains significant burn damage (Smith et al., 2021). Following wildfire, increases in *L. dalmatica* density, cover, reproduction, and distribution have been correlated with reductions in native plant species richness (Dodge et al., 2008) and displacement of native species of concern (Phillips and Crisp, 2001). Jacobs and Sheley (2003) found that prescribed fire increased *L. dalmatica* biomass and seed production without changing toadflax density or percent cover, but it reduced the cover of co-mingled perennial native forbs. *Linaria dalmatica* can also serve as an alternate host for some crop diseases such as Cucumber Mosaic Virus (Pariera Dinkins et al., 2007).

Hybrid toadflax might be a more difficult ecological and management problem than either parent species. Comparisons of *L. vulgaris* x *L. dalmatica* hybrids with the two *Linaria* parent species for traits contributing to invasiveness—including shoot length, flowering stem number, seed capsule production, above-ground biomass, seed germination, and seed viability—all found that hybrids consistently outperformed the parents (Turner, 2012). Results from the same common garden study found that superior adaptation to the local conditions by natural hybrid toadflax genotypes conferred competitive advantages such as earlier emergence from overwintering dormancy and higher rates of seedling germination (Turner, 2012). Species distribution models predict that hybrid toadflax may spread into regions where neither parent species has so far invaded. The potential for increased vigor and rapid adaptation in toadflax hybrids is likely to escalate their threat to native biodiversity in protected areas such as Yellowstone National Park (Pauchard et al., 2003; McCartney et al., 2019).

THE ECOLOGY OF THE PROBLEM

Linaria vulgaris has successfully invaded North American rangelands, grasslands, shrublands, wastelands, agricultural areas (cropland and pastures), and riparian zones subject to chronic, periodic, natural, or anthropogenic disturbance (Coupland et al., 1963; Darwent et al., 1975; Morishita, 1991; Pauchard et al., 2003; Sutton et al., 2007). Although *L. vulgaris* is generally considered a colonizer of disturbed areas, it has also invaded intact native plant communities in remote high-elevation wilderness areas (Sutton et al., 2007). In the Greater Yellowstone Ecosystem, *L. vulgaris* is one of the most invasive plant species, and it remains a significant threat to native biodiversity in open, human- or naturally- disturbed environments in protected areas of the Rocky Mountains (Pauchard et al., 2003).

New invasions of yellow toadflax typically originate from seeds, whereas expansion of established patches occurs primarily through vegetative reproduction (Nadeau et al., 1992; Pauchard et al., 2003). Seeds are produced mid- to late summer, averaging 1,500–30,000 seeds per plant annually, and seeds can remain viable in the soil seedbank for 8–10 years (Carder, 1963; Arnold, 1982). *Linaria vulgaris* is also vegetatively propagated by shoots produced by lateral roots and root fragments (Bakshi and Coupland, 1960; Charlton, 1966; Nadeau et al., 1992). Shoot growth can be initiated from root fragments as small as 10 cm (4 in) and from seedlings as young as three weeks old (Nadeau et al., 1991). Plants can produce 75–694 shoots (typically 90–100) from lateral roots in one growing season, and 200–250 by the second year, with the radius of established patches increasing an average of 1.22 m (4 ft) annually (Nadeau et al., 1991; Saner et al., 1995; Zouhar, 2003; Beck, 2014).

Linaria dalmatica is propagated sexually via seeds produced through obligate outcross pollination and vegetatively by buds formed on the roots of primary and secondary shoots (Vujnovic and Wein, 1997). Mature plants produce up to 500,000 seeds annually, from late June to December, with the seeds remaining dormant but viable in the soil for up to 10 years (Robocker, 1970). Seedling recruitment of *L. dalmatica* is likely limited more by interspecific competition than seed availability (Grieshop and Nowierski, 2002). Vegetative propagation can occur as early as 22 days after seedling emergence, from root and stem fragments as short as 10 mm (0.4 in) (Wilson et al., 2005).

The abundant, small, light-weight seeds of both *Linaria* species shatter out of seed capsules from late summer through winter, and seeds can be further dispersed by wind (Robocker, 1970; Nadeau and King, 1991). Seed viability is largely unaffected by consumption or digestion by livestock or wildlife (Robocker, 1970, 1974), so seed spread in animal droppings may account for isolated invasions or patches in apparently undisturbed areas (Sutton et al., 2007). Dual reproductive modes allow both *Linaria* species to colonize and dominate sites rapidly following disturbance (Pauchard et al., 2003; Dodge et al., 2008). Extensive root systems increase drought tolerance and therefore a competitive advantage over other plants (Sing and Peterson, 2011).

Hybridization between *L. vulgaris* and *L. dalmatica* facilitates introgression of adaptive genes that enhance fitness, expand ecological amplitude, and increase the invasive potential of hybrid and backcrossed genotypes (Ward et al., 2009; Turner, 2012). Gene transfer between invasive populations of both species may be more common than previously realized, as was evident by the frequency with which *L. vulgaris* DNA was detected in field-collected, putative *L. dalmatica* specimens (Boswell et al., 2016). Although *L. vulgaris* and *L. dalmatica* have slightly different habitat preferences (*L. dalmatica* is tolerant of poorer quality soil on dry, open slopes and *L. vulgaris* requires more fertile soil and moister growing conditions), hybrid toadflax readily exploits sites with marginal soil quality and minimal soil moisture, which are common features of Intermountain West rangelands (Boswell et al., 2016; McCartney et al., 2019).

Table 1. List of all biological control species recorded, released, or pending release in the United States, listed according to phase in the history of the toadflax biological control project.

Agent	Order	Site of attack	Introduction				
			Date	Location first record or release	Host found/ released on	Source location	Type of release
Phase 1: First U.S. records of accidentally introduced species							
Rhinusa antirrhini	weevil	seed capsules	1909	MA	yellow toadflax	unknown	adventive introduction
Brachypterolus pulicarius	beetle	flowers	1919	NY	yellow toadflax	unknown	adventive introduction
Rhinusa neta	weevil	seed capsules	1937	CT, NY, NJ, PA, VA, IA	yellow toadflax	unknown	adventive introduction
Rhinusa dieckmanni	weevil	seed capsules	2016–2018	MT	Dalmatian toadflax	unknown	adventive introduction
Phase 2: Introduction of first regulated toadflax biocontrol agent							
Calophasia lunula	moth	foliage	1968	WA	yellow toadflax	Switzerland, via Canada	intentional introduction
Phase 3: Intentional introduction of host-specific biotypes of established agents							
Calophasia lunula	moth	foliage	1990–93	MT, CO, WY, ID	Dalmatian toadflax	Missoula, MT	intentional introduction
Brachypterolus pulicarius	beetle	ovary/ flowers	1992	MT	Dalmatian toadflax	Kamloops, BC	intentional introduction
Rhinusa antirrhini	weevil	seed capsules	1992	MT	Dalmatian toadflax	Canada	intentional introduction
Rhinusa antirrhini	weevil	seed capsules	1996	MT	Dalmatian toadflax	former Yugoslavia	intentional introduction
Phase 4: Intentional introduction of root- or stem-attacking biocontrol agents							
Eteobalea intermediella	moth	roots	1996	MT	Dalmatian toadflax	former Yugoslavia	intentional release
Eteobalea serratella	moth	roots	1996	MT	yellow toadflax	Italy	intentional release
Rhinusa linariae	weevil	roots	1996	MT, WY	yellow toadflax	Rhine Valley, Germany	intentional release
Mecinus janthinus	weevil	stems	1997	MT	yellow toadflax	Germany/France *and* Germany/ France, via Canada	intentional release
Mecinus janthiniformis	weevil	stems	2001	MT	Dalmatian toadflax	former Yugoslavia, via Canada	intentional release
Phase 5: Intentional introduction of cold-adapted agents							
Rhinusa pilosa	weevil	stems	2019	MT	yellow toadflax	Serbia *and* Serbia, via Canada	intentional release
Rhinusa rara	weevil	stems	pending				
Mecinus peterharrisi	weevil	stems	pending				

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The history of the toadflax biocontrol project in the United States evolved through five distinct phases and associated insect species or biotypes (**Table 1**). In the first phase, three exotic toadflax seed- or flower-feeding beetle species invaded North America before efforts began to manage invasive toadflax through regulated classical biological control (Smith, 1959). The first recorded unintentional introduction, in 1909, was of the seed capsule-feeding weevil *Rhinusa* (formerly *Gymnetron*) *antirrhini* (Coleoptera: Curculionidae) (**Fig. 4**). It was first recorded in North America as a Massachusetts field-collected specimen in 1909 (Smith, 1959). Specimens collected in Montreal, Quebec and added to the Canadian National Collection in 1917 represent the first Canadian records for this species (Smith, 1959). *Rhinusa antirrhini* was collected during surveys conducted 1951–57 in British Columbia, Washington, Idaho, and Montana (Smith, 1959). Another seed capsule-feeding weevil, *Rhinusa neta* (**Fig. 5**), first recorded in the United States in 1937 from collections made in Connecticut, New York, New Jersey, Pennsylvania, Virginia, and Iowa, was found to be established in Washington and British Columbia by 1955 (Smith, 1959). Field surveys conducted between 1950 and 1959 found that both of these species were primarily associated with *L. vulgaris* in North America (Smith, 1959; Harris, 1961).

Rhinusa antirrhini collected from *L. dalmatica* ssp. *macedonica* was intentionally introduced to Canada in 1993 and thereafter to the United States in 1996 for biological control of *L. dalmatica* (Winston et al., 2022). *Rhinusa neta* was investigated for its toadflax biocontrol potential by CABI Switzerland in 1996–2001, but the species was never formally petitioned for field release in the United States (Pitcairn et al., 2021). This species was likely not formally pursued as a permitted biocontrol agent due to its perceived limited North American distribution (Smith, 1959; Nowierski, 1995). Recent surveys have found *R. neta* associated with *L. dalmatica* in multiple locations in Montana (I. Toševski, pers. comm.) and California (Pitcairn et al., 2021), well outside of its known historic western North American distribution (Smith, 1959).

Another unintentional introduction, recorded in 1919, was of the ovary-feeding beetle *Brachypterolus pulicarius* (Coleoptera: Kateretidae) (**Fig. 6**). This

Figure 4. Toadflax seed capsule-feeding weevil *Rhinusa antirrhini* (Coleoptera: Curculionidae). (Richard W. Hansen, USDA-APHIS-PPQ, Bugwood.org CC BY-NC 3.0 US)

Figure 5. Toadflax seed-feeding weevil *Rhinusa neta* (Coleoptera: Curculionidae). (Gyorgy Csoka, Hungary Forest Research Institute, Bugwood.org CC BY-NC 3.0 US)

Figure 6. Toadflax ovary-feeding beetle *Brachypterolus pulicarius* (Coleoptera: Kateretidae). (Daniel K. MacKinnon, Colorado State University, Bugwood.org CC BY-NC 3.0 US)

beetle was reported in 1922 as a potential (but eventually unsubstantiated) economic pest due to its presence in damaged strawberry blossoms in the Hudson River Valley of New York. It was subsequently recorded in the flowers of *L. vulgaris* and other plants (Hervey, 1927). *Brachypterolus pulicarius* was collected in 1992 from naturally-established populations on *L. dalmatica* in British Columbia. It was later purposefully released on *L. dalmatica* in Montana, where the beetle successfully overwintered and was recovered from all release sites in 1993 (Nowierski, 1995). This species is now ubiquitous in Canada and the United States on both toadflax species (MacKinnon et al., 2005).

Due to the poor control of toadflax species by seed and flower-feeding species, agents with different sites of action were sought to check the continued, rapid spread of *L. vulgaris* in western Canada (Darwent et al., 1975). Survey efforts by scientists at CAB International led to the collection of the defoliating moth *Calophasia lunula* (Lepidoptera: Erebidae) (**Fig. 7**) from *L. vulgaris* in Switzerland. Following host specificity testing, this moth was released in Canada between 1962 and 1968 (Harris and Carder, 1971), and later in the United States. Initial establishment was restricted to *L. vulgaris* (Harris and Carder, 1971). Individuals collected from *L. dalmatica* in the former Yugoslavia were released in 1988 on *L. dalmatica* in British Columbia and Saskatchewan (Winston et al., 2022). The establishment of *C. lunula* on *L. dalmatica* in North America was first reported in 1989 near Missoula, Montana, when larvae were accidentally discovered feeding on roadside *L. dalmatica* plants (McDermott et al., 1990).

Nearly three decades after the intentional release of *C. lunula*, intensification of the toadflax problem in the western United States and Canada led to a concerted joint effort to identify, test, and gain approval for the release of new toadflax biocontrol agents (Nowierski, 1995). Out of the pool of potential agents identified, permits were approved for the release of four new agent species in North America: the two root-boring moths *Eteobalea intermediella* and *E. serratella* (Lepidoptera: Cosmopterigidae) (**Fig. 8**), the root-galling weevil *Rhinusa* (= *Gymnetron*) *linariae* (Coleoptera: Curculionidae) (**Fig. 9**), and the stem-mining weevil *Mecinus janthinus* (Coleoptera: Curculionidae) (**Fig. 10**) (Nowierski, 1995; De Clerck-

Figure 7. Toadflax defoliating moth *Calophasia lunula* (Lepidoptera: Erebidae) larva. (Gary L. Piper, Washington State University, Bugwood.org CC BY-NC 3.0 US)

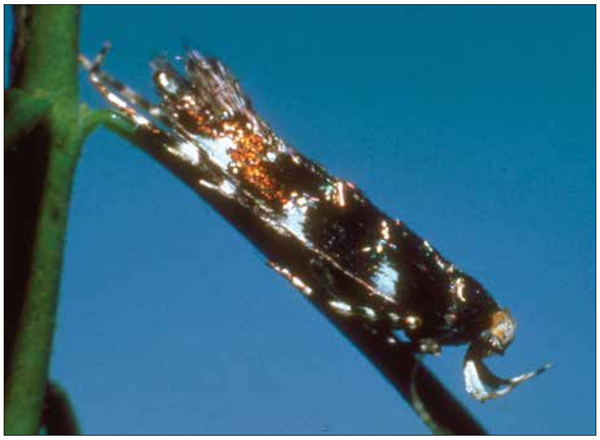

Figure 8. Yellow toadflax root-boring moth *Eteobalea serratella* (Lepidoptera: Cosmopterigidae). (Robert M. Nowierski, Montana State University, Bugwood.org CC BY-NC 3.0 US)

Figure 9. Toadflax root-galling weevil, *Rhinusa linariae* (Coleoptera: Curculionidae). (Bob Richard, USDA-APHIS-PPQ, Bugwood.org CC BY-NC 3.0 US)

Floate and Harris, 2002; McClay and De Clerck-Floate, 2002). These species were selected from the larger pool of potential agents for two reasons. First, no native North American root borers or stem miners were known to attack *L. vulgaris* or *L. dalmatica*. Second, root borers and stem-mining insects were believed to have greater potential for control of toadflax than the defoliators and seed/flower feeders already established in North America (Jeanneret and Schroeder, 1992; Saner and Müller-Schärer, 1994).

Figure 10. Toadflax stem-mining weevil *Mecinus janthinus* s.l. (Coleoptera: Curculionidae). (Bob Richard, USDA-APHIS-PPQ, Bugwood.org CC BY-NC 3.0 US)

Possibly due to their cryptic nature, widespread establishment of the three root-attacking agents has not yet been confirmed in North America (Winston et al., 2022). In 2002, researchers were unable to confirm the continued survival of an *E. intermediella* colony considered established from a 1998 release on *L. dalmatica* in Kamloops, British Columbia (De Clerck-Floate and Turner, 2013). Similarly, although larvae were found one year after a 1995 *E. serratella* release on *L. vulgaris* at Kinsella, Alberta, there was no indication of its continued establishment when the site was resampled in 2007 (De Clerck-Floate and McClay, 2013). Beginning in 2001, *R. linariae* weevils reared from Kamloops, British Columbia propagation plots were abundant enough to make additional field releases on *L. vulgaris* in British Columbia and Alberta (De Clerck-Floate and McClay, 2013) and, later, in Colorado (Winston et al., 2022). Successful overwintering was observed in Colorado in 2016; however, long-term establishment has yet to be confirmed. *Rhinusa linariae* is well-established on *L. vulgaris* at some locations in British Columbia; however, populations have been slow to build and are not widely distributed (Winston et al., 2022).

Twenty-five years after its initial North American release, *M. janthinus* has become widely distributed throughout southwestern and eastern Canada, and the northwestern United States through natural dispersion and active redistribution (Toševski et al., 2018; Winston et al., 2022). Most of the weevils imported under this name from Europe for release in North America are now known to have been *M. janthinus* collected from *L. vulgaris* populations in Western Europe (DeClerck-Floate and McClay, 2013; Toševski et al., 2018). Records indicate that in 1997, 200 weevils of a second, cryptic species (now known to have been *Mecinus janthiniformis*) collected from *L. dalmatica* in Macedonia were shipped for release in Canada (DeClerck-Floate and Turner, 2013; Toševski et al., 2018). This single shipment of *M. janthiniformis* is now believed to have eventually led to outbreak-level populations on *L. dalmatica* throughout western North America (Toševski et al., 2011).

Figure 11. Yellow toadflax stem-galling weevil *Rhinusa pilosa* (Coleoptera: Curculionidae). (R.K.D. Peterson, Montana State University)

Climatic factors in North America, including effects of extreme cold temperatures and lack of insulating snow cover on the overwintering survival of *M. janthiniformis* on *L. dalmatica* (De Clerck-Floate and Miller, 2002) and too short a growing season for *M. janthinus* on yellow toadflax (McClay and Hughes, 2007), spurred a search for additional toadflax biocontrol agents in the 1990s. Several new stem-galling *Rhinusa* weevils, including the closely related *Rhinusa pilosa* on yellow toadflax (**Fig. 11**) and the newly described *Rhinusa rara* on Dalmatian toadflax, were confirmed to be highly host-specific species with good potential as biocontrol agents (Toševski et al., 2015). Newly emerged *R. pilosa* adults aestivate during the hottest part of late summer and then overwinter in leaf litter (Gassmann et al., 2014), which provides them better protection from climatic fluctuations than is the case for the earlier established *Mecinus* species, which are susceptible to freeze-thaw extremes from late fall through late spring and desiccation or physical destruction (when overwintering *Mecinus* adults are in standing toadflax stems). Although *R. pilosa* has been established in Alberta since 2015 (British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2018a), releases made at two sites in southwestern Montana in August 2020 resulted in the first populations of *R. pilosa* to successfully overwinter in the United States, producing galls in 2021 (S. Sing, pers. obs.). *Rhinusa pilosa* adults sourced from a Montana garden-based mass rearing program were released for the first time in 2021 in Oregon, North Dakota, and South Dakota, and at new locations in Montana (S. Sing, unpub. data). Additional *Mecinus* species are also being investigated to discover agents potentially better matched to *L. dalmatica* populations at high-elevation, cooler sites (i.e., *M. peterharrisi*) and for biocontrol of hybrid toadflax and *L. vulgaris* (i.e., *M. heydeni*) (Sing et al., 2016).

HOW WELL DID IT WORK?

Rhinusa antirrhini

A noticeable reduction in *L. vulgaris* infestations in western Canada in the 1950s was attributed to regional build-ups of *B. pulicarius* and *R. antirrhini*, which together exerted population-level impacts on the host weed *L. vulgaris* (Harris, 1961; Darwent et al., 1975). However, attack by *R. antirrhini* alone has not been demonstrated to control *L. vulgaris*.

Brachypterolus pulicarius

Under controlled conditions, exposure of *L. vulgaris* to *B. pulicarius* resulted in significant reductions in total and individual seed weight, percent germination of seeds, and percent viable seeds produced (McClay, 1992). Reductions in stem height, increases of 77% and 95%, respectively in the number of primary and secondary branches, reductions in flower number on a per plant basis of 44–49%, and a 43–93% reduction in seed production were reported for *L. dalmatica* exposed to *B. pulicarius* individuals collected in Kamloops, British Columbia from an adventive population of the beetles established on Dalmatian toadflax (Nowierski, 1995; Grubb et al., 2002). While McClay (1992) found that *B. pulicarius* can reduce flowering and seed production, this beetle generally does not exert enough pressure on plants to effectively control either toadflax species under field conditions.

Calophasia lunula

Although up to 20% defoliation of *L. vulgaris* by larvae of this moth was observed in Ontario (Harris, 1984), only minimal impact was recorded on plant density, possibly because parasitism of the moth's pupae can reach 90% at some sites (McClay and Hughes, 1995). Because defoliation on either toadflax species is seldom lasting, damage by this species alone does not control invasive toadflax.

Eteobalea intermediella, E. serratella, and *Rhinusa linariae*

It has not been possible to assess the impact of either *E. intermediella* or *E. serratella* because long-term North American field establishment has not yet been confirmed for either species (De Clerck-Floate and

Turner, 2013; De Clerck-Floate and McClay, 2013). In an experiment designed to evaluate the potential efficacy of *E. serratella* for control of *L. vulgaris* in commercial mint fields, potted *L. vulgaris* plants receiving the combination of root-mining by the moth *E. serratella* and interspecific competition from inter-planted mint showed reductions in number, weight, and rate of shoot regrowth, following simulated harvest of the mint crop by stem cutting (Volenberg et al., 1999). After multiple field releases of the two *Eteobalea* species resulted in the failure of either species to become truly established, no further attempts were made to establish either of these root-boring moths in the United States or Canada. Consequently, the potential efficacy of *E. serratella* to control *L. vulgaris* infesting mint fields has not yet been field-tested.

In contrast, the third root-feeding species, the beetle *R. linariae*, successfully established in British Columbia where adults feeding on foliage and larvae galling roots both reduce *L. vulgaris* nutrient reserves (British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, 2018b). This has led to heavy impact on *L. vulgaris* in the immediate vicinity of release sites. However, *R. linariae* populations are slow to build and slow to disperse on their own if *L. vulgaris* patches are widely scattered, so redistributions are made manually every 2–4 years (Winston et al., 2022). *Rhinusa linariae* beetles from British Columbia were shipped to Colorado in 2008 and 2015 to initiate insectaries there for redistribution in the United States. This weevil has successfully overwintered at one Colorado release site, but long-term establishment has not been confirmed (A. Norton, pers. comm.).

Mecinus janthinus/M. janthiniformis

Mecinus janthinus populations were slow to build up in both Canada and the United States compared to the rapid, obvious establishment of *M. janthiniformis* (McClay and De Clerck-Floate, 2002; Toševski et al., 2018), and consequently few studies have evaluated the impact of *M. janthinus*. However in Montana, wherever *M. janthinus* became established (either through intentional releases or natural dispersal), a consistent and dramatic decline in *L. vulgaris* abundance has been observed (S. Sing, unpub. data). *Mecinus janthinus* failed either to establish or significantly increase in number at some locations in western Canada and the western United States despite multiple releases (De Clerck-Floate and McClay, 2013; Sing et al., 2016).

In contrast, for *M. janthiniformis*, studies have documented important reductions in *L. dalmatica* biomass and flower and seed production per m^2 (Goulet et al., 2014). Suppression of seasonal stem height growth (Goulet et al., 2013) has also been attributed to attack by *M. janthiniformis*. Similarly, Cariveau and Norton (2014) found reductions in the number of flowers (33%) and seeds (38%) per plant in Dalmatian toadflax subjected to larval feeding by *M. janthiniformis*, compared to control plants. Larval feeding by *M. janthiniformis* is thought to disrupt water and nutrient transport in infested *L. dalmatica* stems. This disruption results in growth inhibition (Schat et al., 2011) and reduction in photosynthesis and transpiration, leading to a reduction in resources available for flower production (Peterson et al., 2005).

In western Canada, the impact of *M. janthiniformis* on *L. dalmatica* has been demonstrated by the regional dispersal of the agent, significant decline in host plant size and density, and widespread fragmentation of host plant patches (Van Hezewijk et al., 2010). The strongest impacts of *M. janthiniformis* on *L. dalmatica* have typically been seen at release sites where environmental factors, such as higher precipitation, are particularly favorable. Such conditions allow greater increases in agent numbers (Weed and Schwarzländer, 2014). In general, the warmer conditions that are commonly found in British Columbia, Utah, and California (Willden and Evans, 2019; Smith et al., 2021) are also more favorable than those typical of Montana and Alberta (De Clerck-Floate and Miller, 2002; Sing et al., 2008; De Clerck-Floate and Turner, 2013).

Parasitism is another factor influencing the success of biological control by species of *Mecinus*. The first New World record for the Palearctic species *Pteromalus microps* (Hymenoptera: Pteromalidae), which was recovered in Wisconsin from populations of *R. antirrhini* on *L. vulgaris*, was assumed to be the result of the adventive introduction of the parasitoid (Volenberg and Krauth, 1996). This parasitoid was later reported from several sites where *M. janthinus* had been released on *L. vulgaris* in Alberta (McClay and De Clerck-Floate, 2002). Parasitism of *Mecinus* spp. by various parasitoid species has been reported with increasing frequency in the United States in recent years (Willden and Evans, 2019), with the generalist weevil ectoparasitoid *Neocatolaccus tylodermae* (Pteromalidae) being the most commonly recovered species to date, having been recorded from both *M. janthinus* and *M. janthiniformis* (Karimzadeh et al., 2021).

Host Races or Biotypes

The possibility that geographically disparate populations of toadflax-feeding insect species occurring either on *L. dalmatica* or *L. vulgaris* may have evolved distinct host races in response to the locally prevalent *Linaria* species has been investigated. This work eventually resulted in permits to import and release or redistribute host races or host specific biotypes of *B. pulicarius* and *R. antirrhini* (Groppe, 1992; De Clerck-Floate and Harris, 2002; Nowierski, 2004; USDA-APHIS, 2022). Although *B. pulicarius* has been field collected from both invasive toadflax species, even the individuals that naturally established on *L. dalmatica* demonstrated a consistent preference for *L. vulgaris* (MacKinnon et al., 2005). However, molecular analyses were unable to detect any significant genetic differentiation based on host plant association in *B. pulicarius* (Hufbauer and MacKinnon, 2008).

Cryptic Species

As stated previously, based on the results of overseas host-specificity testing, the toadflax stem-mining weevil *M. janthinus* was considered a single species that non-preferentially attacked both *L. vulgaris* and *L. dalmatica*, and it was approved for release in Canada and the United States for biological control of both invasive toadflax species (Jeanneret and Schroeder, 1992; De Clerck-Floate and Harris, 2002; McClay and De Clerck-Floate, 2002). Morphological, molecular, and biological evidence has since shown that in its native range, the agent originally known as *M. janthinus* is two closely-related species that are ecologically segregated by host plant (Toševski et al., 2011, 2013). The native ranges of *M. janthinus* and *M. janthiniformis* do not overlap, reflecting the separate distributions of their natural hosts in central and southern Europe, southern Russia, and southwestern Asia (Jeanneret and Schroeder, 1992; Toševski et al., 2011, 2013). Molecular and morphological analyses confirmed that *Mecinus* weevils currently established on North American toadflax commonly belong to haplotype groups present in the native ranges of their populations (Toševski et al., 2018).

Field and garden observations have suggested a strong preference in North American *R. antirrhini* populations for *L. vulgaris* (Smith, 1959). Host-specificity testing of a potential *L. dalmatica* host race or biotype of *R. antirrhini* found that host acceptance and performance for beetles collected from Dalmatian toadflax in the former Yugoslavia were higher on test plants of *L. dalmatica* that originated from Canada compared to *L. vulgaris* plants of European origin (Groppe, 1992; De Clerck-Floate and Harris, 2002). Analyses of *R. antirrhini* collected across its European native range from five *Linaria* species or subspecies concluded that structuring of genetic variation was best explained by the host plant of field-collected specimens rather than their geographic distribution (Hernández-Vera et al., 2010). A contemporaneous phylogeny of the genus *Rhinusa*, based on adult morphological characters and host plants, grouped *R. antirrhini* with *Rhinusa dieckmanni* (Caldara et al., 2010). Molecular information on *R. antirrhini* s.l. collected from *L. dalmatica*, *L. dalmatica* ssp. *macedonica*, and closely related species at multiple locations in the European native range, and from *L. dalmatica* in Montana in the United States suggests that *Rhinusa* spp. individuals found on *L. dalmatica* and its closest non-*L. vulgaris* relatives are more likely to be *R. dieckmanni* than *R. antirrhini* (I. Toševski, unpub. data).

Non-Target Attack

Rhinusa neta, a toadflax seed-feeding weevil that was unintentionally introduced to North America and recorded in the United States since 1937 and in Canada since 1957 (Smith, 1959), has never been permitted for collection and redistribution (Pitcairn et al., 2021). Host specificity of *R. neta* assessed by CABI-Switzerland beginning in 1996 found that the weevil could successfully complete development on several non-native *Linaria* species (*L. vulgaris*, *L. dalmatica*, *L. supina*, *L. reflexa*, and *L. purpurea*) as well as on the California endemic species *Neogaerrhinum strictum* (USDA-NRCS, 2022c; ITIS, 2022) and the annual invasive species *Chaenorrhinum minus* (dwarf snapdragon) (Gassmann and Paetel, 1998; Gassmann, 2001). *Chaenorrhinum minus*, reportedly introduced and disseminated throughout North America in ballast material that was subsequently re-used in transcontinental railway beds (Arnold, 1981, 1991), is now established throughout North America (USDA-NRCS, 2022d). It provides a widely available alternative food source that may facilitate the distribution of this oligophagous herbivore. In recent years, *R. neta* has also been collected from the seed capsules of another endemic species, *Sairocarpus virga* (USDA-NRCS, 2022e), in five California counties (Pitcairn et al., 2021).

Host-specificity testing under controlled conditions showed that the larval stage of the toadflax-defoliating moth *C. lunula* fed on (Karny, 1963), and to a limited extent was able to complete development on (Harris, 1963), three non-target ornamental species: *Antirrhinum majus* (common snapdragon), *Cymbalaria muralis* (Kenilworth ivy), and *Linaria maroccana* (Moroccan toadflax). The former species is ubiquitous throughout North America, while the latter two have escaped cultivation in various U.S. locations, including California (USDA-NRCS, 2022f,g,h). *Calophasia lunula* is also able to complete development on the California endemic species *S. virga* (Wilson et al., 2005). In this context, the risk of non-target attack on *S. virga* is likely amplified by the presence of abundant additional hosts, including *L. dalmatica*.

Hybrid Toadflax

Data and qualitative observations indicate that the stem-mining weevils *M. janthinus* and *M. janthiniformis* are currently the best widely available classical biological control agents for managing *L. vulgaris* and *L. dalmatica* (Sing et al., 2016; Willden and Evans, 2019; Smith et al., 2021). Both species exhibit extreme fidelity to their natural (native range) host, seldom developing or establishing on the other non-host invasive toadflax species (Toševski et al., 2011, 2018). Hubbard (2016) reported a probable olfactory basis for this observed host fidelity, finding a significant correlation between the semiochemical profile of respective natural hosts and host acceptance in female weevils of both *Mecinus* species. Preliminary tests under containment conditions indicate that a yellow toadflax semi stem-galling weevil, *Mecinus heydeni*, was able to recognize, accept, and successfully develop on its natural host and a range of field-collected and hand-crossed hybrid toadflax genotypes, but it fully rejected *L. dalmatica* as a host for feeding or oviposition (Sing et al., 2016).

BENEFITS OF BIOLOGICAL CONTROL OF INVASIVE TOADFLAXES

Published post-release evaluations of biological control of *L. dalmatica* by *M. janthiniformis* in Canada and in the United States report that successful establishment and build-up of agent populations were correlated with significant reductions in stem length, density, and cover, as well as plant biomass and seed production of the target toadflax species (Van Hezewijk et al., 2010; Jamieson et al., 2012; Weed and Schwarzländer, 2014; Weed et al., 2018; Smith et al., 2021). As stated above, similar accounts of *M. janthinus* suppression of *L. vulgaris* have not yet been widely published, although anecdotal reports of both the target weed and agent disappearing from previously productive collection sites are not uncommon in Montana, where it first became established in the United States (M. Maggio, pers. comm.).

Although permitting of any weed biological control agent requires documentation of stringent host specificity, this aspect of toadflax biological control is also one of its main benefits, in that direct non-target effects and collateral damage are not risks associated with the use of this management tool. The high level of treatment selectivity conferred by toadflax biological control is particularly important when plant species of concern (e.g., Flagstaff pennyroyal, *Hedeoma diffusum* [Phillips and Crisp, 2001]) or of economic value (e.g., peppermint [Volenberg et al., 1999]) are intermingled with *L. dalmatica* or *L. vulgaris*.

Finally, once established, toadflax biological control systems can function as sustainable components of integrated weed management. This is particularly important when weed infestations occur at a landscape scale on low-value land, necessitating the use of low- or no-cost and reduced-input weed treatments. *Mecinus janthiniformis*' ability to self-distribute effectively allows for control of unrecorded or undetected toadflax populations and the re-establishment of agent populations on *L. dalmatica* infestations that often dominate sites following wildfire.

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Chapter 27

Success Takes Time: History and Current Status of Biological Control of Purple Loosestrife in the United States

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NON-TECHNICAL SUMMARY

Purple loosestrife (*Lythrum salicaria*, Lythraceae) is a long-lived forb that has negatively affected North American wetlands for decades. Following the introduction of purple loosestrife from Eurasia in the early 1800s, populations gradually spread across North America, eventually leading to the decline of many native birds, plants, and amphibians. Land managers recognized the widespread ecological harm caused by purple loosestrife and called for sustainable control methods, realizing that traditional methods such as chemical treatments had failed to produce desirable outcomes. In response, research to assess biological control options for purple loosestrife began in 1986 in Europe. This biological control program represented one of the first times a plant was targeted for biological control because of its harm to flora and fauna rather than because of its negative impacts to agriculture. This work led to the release of four host-specific insects: two leaf-feeding beetles (*Galerucella calmariensis* and *Galerucella pusilla*; both Coleoptera: Chrysomelidae) and a root-feeding weevil (*Hylobius transversovittatus*; Coleoptera: Curculionidae) in 1992, followed in 1994 by a flower-feeding weevil (*Nanophyes marmoratus*; Coleoptera: Curculionidae). The *Galerucella* leaf-feeding beetles now appear to be widely established and abundant. Data on the abundance and distribution of the root-feeding and flowering-feeding weevils remain sparse. The effect of these insects may vary from site to site, but in many regions across North America, such as the Pacific Northwest, the Great Lakes Region, and the Northeast, biological control of purple loosestrife is now highly effective and economical. For example, long-term data collected from New York document that these insects reduce the density, height, and flower production of purple loosestrife, which in turn allows an increase in native plant diversity. This is the ultimate goal of weed management. Many biological control success stories are anecdotal, and purple loosestrife is one of the first examples for which we have strong evidence that control of invasive plants by insects can result in native plant recovery.

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HISTORY OF INVASION AND NATURE OF PROBLEM

Purple loosestrife (*Lythrum salicaria*, Lythraceae) is a widespread, long-lived wetland perennial forb easily recognized by its dense, showy, purple flowers in summer and early fall. It was introduced from Eurasia to the northeastern United States in the early 1800s, both intentionally through the plant trade and likely unintentionally in contaminated ship ballast and on wool fleece. Purple loosestrife quickly spread westward aided by horticulturalists and beekeepers. Populations built up over time, resulting in dense, nearly pure monocultures. Wetland managers were the first to notice that purple loosestrife was leading to widespread degradation of wetlands across much of the United States (Thompson et al., 1987; Skinner et al., 1994; Blossey et al., 2001). This was confirmed by later experimental studies (see next section for details). Currently, purple loosestrife occurs in all 48 contiguous states except Florida, as well as in nine Canadian provinces. It is listed as a noxious weed in most of the United States and Canadian provinces.

WHY CONTROL THIS INVASIVE SPECIES?

Efforts to control purple loosestrife through flooding, mowing, herbicide application, burning, or discing are unsuccessful. Short-term suppression can sometimes be achieved through herbicide application or flooding, but neither method provides long-term control and both often perpetuate the negative impacts they are trying to mitigate (Malecki and Rawinski, 1985; Haworth-Brockman et al., 1993; Welling and Becker, 1993; Gabor et al., 1995, 1996; Katovich et al., 1996). For example, repeated herbicide applications are necessary to prevent purple loosestrife reinvasion (Malecki and Rawinski, 1985; Balogh and Bookhout, 1989; Gabor et al., 1995, 1996), yet these applications also harm native plants that occur in treated areas, leaving a highly disturbed site prone to future invasion. Furthermore, flooding at depths greater than 30 cm (12 in) kills purple loosestrife seedlings (Haworth-Brockman et al., 1993), yet established plants can thrive under flooded conditions. Moreover, maintaining deep water levels prevents germination of many native wetland plant species (Kettenring and Tarsa, 2020).

Both conventional control and doing nothing therefore lead to continued widespread environmental degradation and ecological harm from purple loosestrife. Thus, using biological control to reduce the negative impacts of purple loosestrife, but not eradicate the target plant, seemed a promising alternative to wetland ecologists and land managers.

THE ECOLOGY OF THE PROBLEM

Ecological Effects of Purple Loosestrife

In its native range in Europe, purple loosestrife typically occurs in mixed wetland communities (Shamsi and Whitehead, 1974). Occasionally, disturbances may create near monocultures of purple loosestrife, but they are short lived, likely because specialist insects quickly consume both above- and below-ground plant tissues, in turn causing declines in reproduction (Lehndal et al., 2016). In contrast, in North America before the biological control program, purple loosestrife outcompeted native vegetation (Gaudet and Keddy, 1988; Weiher et al., 1996; Weihe and Neely, 1997) and permanently reduced wetland plant diversity. The ability of purple loosestrife to directly outcompete native plants was likely compounded by indirect competition mediated by shared pollinators; purple loosestrife has reduced the abundance and distribution of winged loosestrife, *Lythrum alatum*, (the most widespread native *Lythrum* species in the United States) through both these mechanisms, i.e., direct competition and indirect competition via pollinators (Blossey et al., 1994a; Brown, 2002). Furthermore, many native animals already struggling due to habitat loss are unable to thrive in dense and extensive purple loosestrife stands. These species include the American bittern (*Botaurus lentiginosus*), black tern (*Chlidonias niger*), bog turtle (*Glyptemys muhlenbergii*), least bittern (*Ixobrychus exilis*), pied-billed grebe (*Podilymbus podiceps*), sora (*Porzana carolina*), and Virginia rail (*Rallus limicola*) (Thompson et al., 1987; Schneider and Pence, 1992; Lor, 2000).

Once purple loosestrife forms dense monocultures, it also triggers alterations in the biogeochemistry and hydrology of invaded wetlands. For example, when purple loosestrife replaces broadleaf cattail (*Typha latifolia*) as the dominant vegetation type, phosphate in pore water pools in the soil declines in the summer (Templer et al., 1998), with a stronger nutrient flush in the fall (Emery and Perry, 1996; Grout et al., 1997). These changes in timing of nutrient release alter wetland function and may increase rates of downstream eutrophication (Emery and Perry, 1996), in turn causing declines in detritivore populations adapted to plant tissues whose decaying biomass typically drops in the spring (Grout et al., 1997). Changes in timing of nutrient release may also have cascading effects on detrital-based food webs, such as further declines in endangered salmon species in the Pacific Northwest (Grout et al., 1997). Furthermore, shifts in litter quality also appear to negatively affect the development of amphibians such as the American toad (*Anaxyrus americanus*) (Brown et al., 2006; Maerz et al., 2010; Martin et al., 2015).

Purple Loosestrife as a Model of Evidenced-Based Management

Wetland managers and state and federal agencies across North America supported the implementation of biological control of purple loosestrife. Yet a small group of academics initially objected to this program, citing a general lack of evidence of negative impacts of purple loosestrife (e.g., Hager and McCoy, 1998). However, just because impacts are not addressed in the peer-reviewed literature does not mean they do not exist. Land managers have daily connections to specific habitats and their species, and may observe succession and shifting species assemblages that short-term experiments, however sophisticated, are unable to detect. Thus, land managers are an extremely valuable 'early warning system' that may allow us to control invasive species before they inflict irreversible harm. In the case of purple loosestrife, wetland managers were the first to recognize many of the negative ecological impacts that were later experimentally confirmed (as summarized by Blossey et al., 2001; see previous section for more details).

This initial pushback to biological control of purple loosestrife highlights how expected benefits of proposed management actions should always be weighed against their expected costs (both financial and ecological). To be clear, pushback is healthy for the field of biological control and helps maintain and improve rigorous standards that lead to increasingly safe and effective biological control (Hinz et al., 2019, 2020; Szűcs et al., 2019; Paynter et al., 2020; Sun et al., 2020, 2022). Indeed, biological control programs rigorously assess safety and often efficacy of potential biological control agents before their release. However, few weed biocontrol programs quantitatively assess long-term outcomes after insects are released (Crawley, 1989; McClay, 1992; McFadyen, 1998; McEvoy and Coombs, 1999; Morin et al., 2009; Hinz et al., 2020; Schaffner et al., 2020). This information is critical to understanding whether we have achieved sustainable, long-term control of the target weed and successfully mitigated the weed's undesirable effects (Schroeder, 1983; Morin et al., 2009; Schaffner et al., 2020). These types of data also provide evidence of biological control as an effective management tool (Malecki et al., 1993; Morin et al., 2009; Hinz et al., 2020). Invasive species management relies on public trust and goodwill, making data on beneficial outcomes and absence of non-target effects critical to ensuring continued funding for biological control as a management tool.

To help address this knowledge gap, scientists developing biological control of purple loosestrife established a standardized monitoring protocol to evaluate the establishment, abundance, and impact of insects released to control purple loosestrife on both purple loosestrife and its co-occurring plant community. This protocol was first implemented across North America in 1996 and used permanent 1 m^2 (10.8 ft^2) quadrats to assess changes in insect damage, purple loosestrife performance, and plant community composition over time (see Blossey, 2001).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

The biological control program for purple loosestrife was initiated in 1986 (Malecki et al., 1993), beginning with surveys of purple loosestrife in its native European range to look for potential control agents. Insects were identified as potential agents if they had a widespread distribution in their native range, if initial observations suggested they had strong negative impacts on individual purple loosestrife plants, and they appeared to be highly specific (i.e., fed only on purple loosestrife). Of the more than 100 different insect species observed feeding on purple loosestrife in Europe, only six were considered as candidates for follow-up investigations. The life history, native range distribution, impact on purple loosestrife, and host-specificity of these insects were thoroughly investigated and reported in peer-reviewed publications (Blossey, 1993; Blossey et al., 1994a,b; Blossey, 1995a,b,c; Blossey and Schroeder, 1995). Rigorous cost-benefit evaluations and risk assessments are a critical part of the decision process governing which insects, if any, are approved for release in North America to control target weeds. Thus, it was only after a rigorous suite of experimental laboratory and field studies that five insects were identified as posing minimal threat to North American plant species and were approved for field release: two leaf-feeding beetles (*Galerucella calmariensis* and *Galerucella pusilla*, both Coleoptera: Chrysomelidae), a root-feeding weevil (*Hylobius transversovittatus*; Coleoptera: Curculionidae), a flower-feeding weevil (*Nanophyes marmoratus*; Coleoptera: Curculionidae), and a seed-feeding weevil (*Nanophyes brevis*) (Blossey and Schroeder, 1995). The seed-feeding weevil was never introduced because scientists were unable to find nematode-free populations in Europe. The nematode did not appear to harm *N. brevis* yet constituted an unknown, hence unacceptable, potential risk to native North American weevils.

The four introduced insects are complementary, in that they attack different parts of the plant. The two leaf beetles, *G. calmariensis* and *G. pusilla*, share similar life histories and have similar feeding behavior (Blossey et al., 1994a; Blossey, 1995a). They overwinter as adults (**Fig. 1a**) in the leaf litter, then emerge in the spring to lay eggs (**Fig. 1b**) and feed on above-ground plant tissues in ways that result in distinctive damage. Adults often inflict shothole damage (**Fig. 1c**) that is especially obvious in the spring, while larvae inflict window-pane damage (i.e., stripping the photosynthetic tissue from small sections of leaves, while leaving

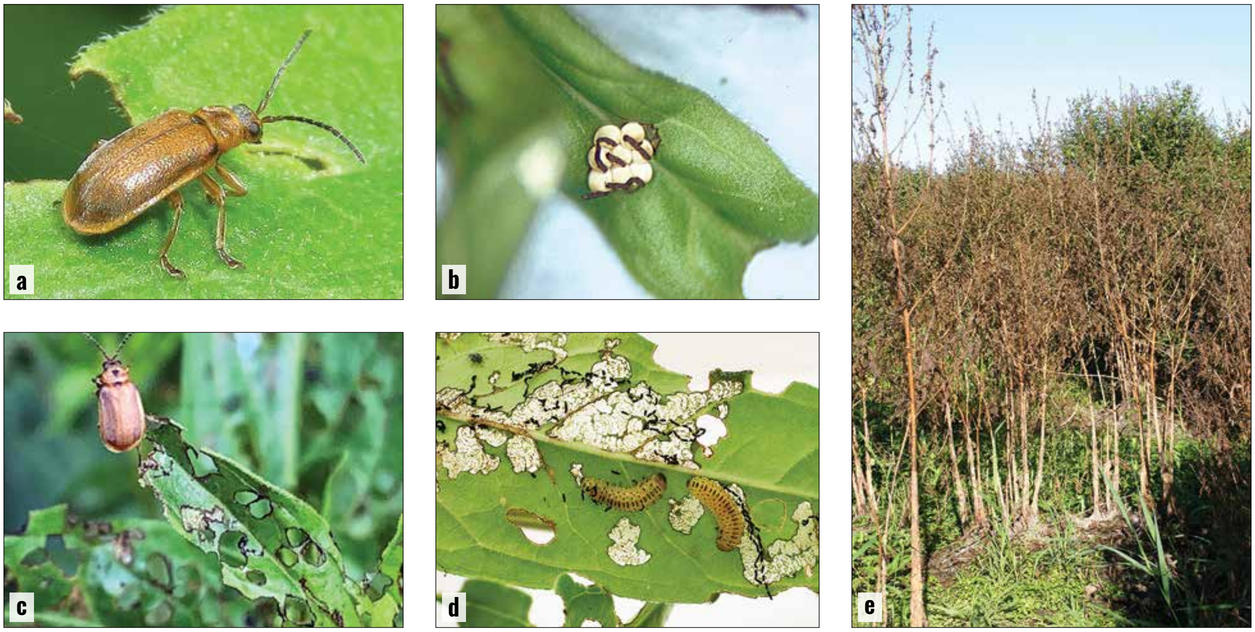

Figure 1. *Galerucella* leaf-feeding beetle: (a) adult; (b) eggs; (c) adult and shothole feeding damage; (d) larvae and window-pane feeding damage; (e) nearly complete defoliation of purple loosestrife stems following release of *Galerucella* beetles. (a: Rob Westerduijn; b: Agriculture and Agri-Food Canada; c: Don Sutherland; d,e: Eric Coombs, Oregon Department of Agriculture; a,c: iNaturalist.org CC BY-NC 4.0; b,d,e: Bugwood.org CC BY-NC 3.0 US)

the upper epidermis intact) (**Fig. 1d**). At high population densities, these insects often completely defoliate entire populations of purple loosestrife (**Fig. 1e**). Both species can also produce a single, a partial second, or even two generations per year depending on day length and climate (Blossey 1995a; Grevstad and Coop, 2015; Wepprich and Grevstad, 2021), potentially resulting in multiple defoliation events per year.

The root-feeding weevil, *H. transversovittatus,* predominately damages the below-ground tissue of purple loosestrife. While adults (**Fig. 2a**) feed on leaves (**Fig. 2b**), the main damage results from larval feeding on root tissues (**Fig. 2c**). Larvae take one to two years to develop and, once mature, create a pupation chamber in the upper portions of the root, emerging as adults from mid-summer to late fall. Adults (10–14 mm in length) are night-active and can live for several years. Females lay eggs in the soil close to a purple loosestrife root or insert eggs in the lower part of the stem. Individuals can be difficult to detect given their nighttime feeding behavior and larval stages feeding below ground. Digging up purple loosestrife roots, however, reveals obvious larval root damage. For detailed descriptions of the life history and ecology of *H. transversovittatus* see Blossey (1993).

Figure 2. *Hylobius transversovittatus*, a root-feeding weevil: (a) adult; (b) adult feeding damage on leaf margin; (c) larva in destroyed rootstock. (a: Jennifer Andreas, Washington State University Extension; b,c: Eric Coombs, Oregon Department of Agriculture, Bugwood.org CC BY-3.0 US)

The univoltine flower-bud weevil, *N. marmoratus* (**Fig. 3a**), predominately damages developing ovaries in flowers before they open, which reduces seed output through flower-bud abortion (Blossey and Schroeder, 1995). In spring and early summer, adults first feed on young leaves—creating a shothole pattern—and then on flower buds once they become available. Adults lay eggs in immature buds (**Fig. 3b**), with attack rates that can exceed 70%. A single larva develops within a single bud, creating a distinctive circular hole at the base of the dead bud when individuals emerge as adults (**Fig. 3c**).

The development of mass rearing methods allowed rapid distribution of insects across the continent (Blossey and Hunt, 1999; Blossey et al., 2000). By 1999, more than three million leaf beetles and more than 100,000 root-feeding weevil eggs and adults had been released in >1,500 wetlands in 30 states and several Canadian provinces (Blossey et al., 2001). Following these releases, statewide and regional mass production efforts continued for many years, allowing for rapid adoption of the program across the United States.

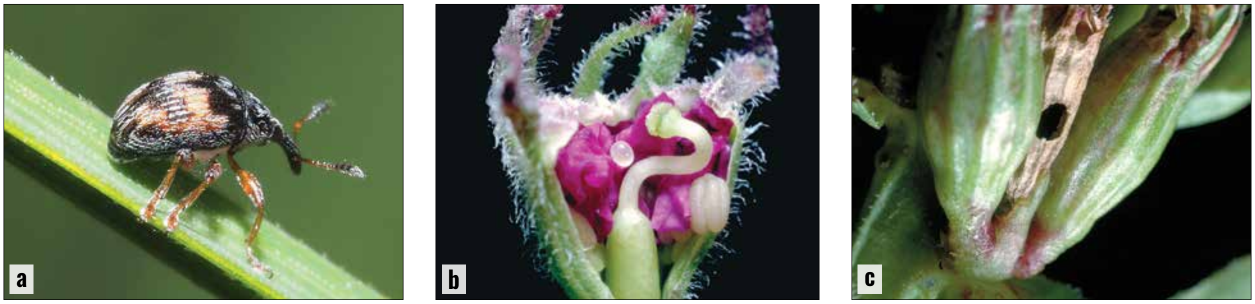

Figure 3. *Nanophyes marmoratus,* a flower-feeding weevil: (a) adult; (b) egg within a dissected flower bud; (c) adult emergence hole from a dead flower bud. (a: Felix Riegel, iNaturalist.org CC BY-NC 4.0; b,c: Gary Piper, Washington State University, Bugwood.org CC BY-3.0 US)

HOW WELL DID IT WORK?

Today (2022), up to three decades have passed since *Galerucella* spp., *H. transversovittatus*, and *N. marmoratus* were initially released to control purple loosestrife in North America. However, formal assessments of the North American distribution and impact of the four species across the continent are not available. While in some regions purple loosestrife remains a priority pest subject to management, we typically lack data (at least in the published literature) to evaluate how the abundance of purple loosestrife or its impact on other biota have changed over time, or whether prioritization of the species for herbicide or mechanical management is justified given purple loosestrife's existing impacts or future threats. We also lack data on whether there is a continued need for insect redistribution and whether local eradication attempts via biological control or other treatments are ecologically feasible, desirable, or actually counterproductive as a management goal. Yet in regions where data have been collected, both anecdotal and empirical evidence overwhelmingly support this program's success. In the following section, we combine published evidence on the impact of these insects with knowledge obtained by our interactions with wetland managers and agencies since inception of the program, focusing on three overlapping categories of success: establishment success, biological success, and ecological success (**Fig. 4**).

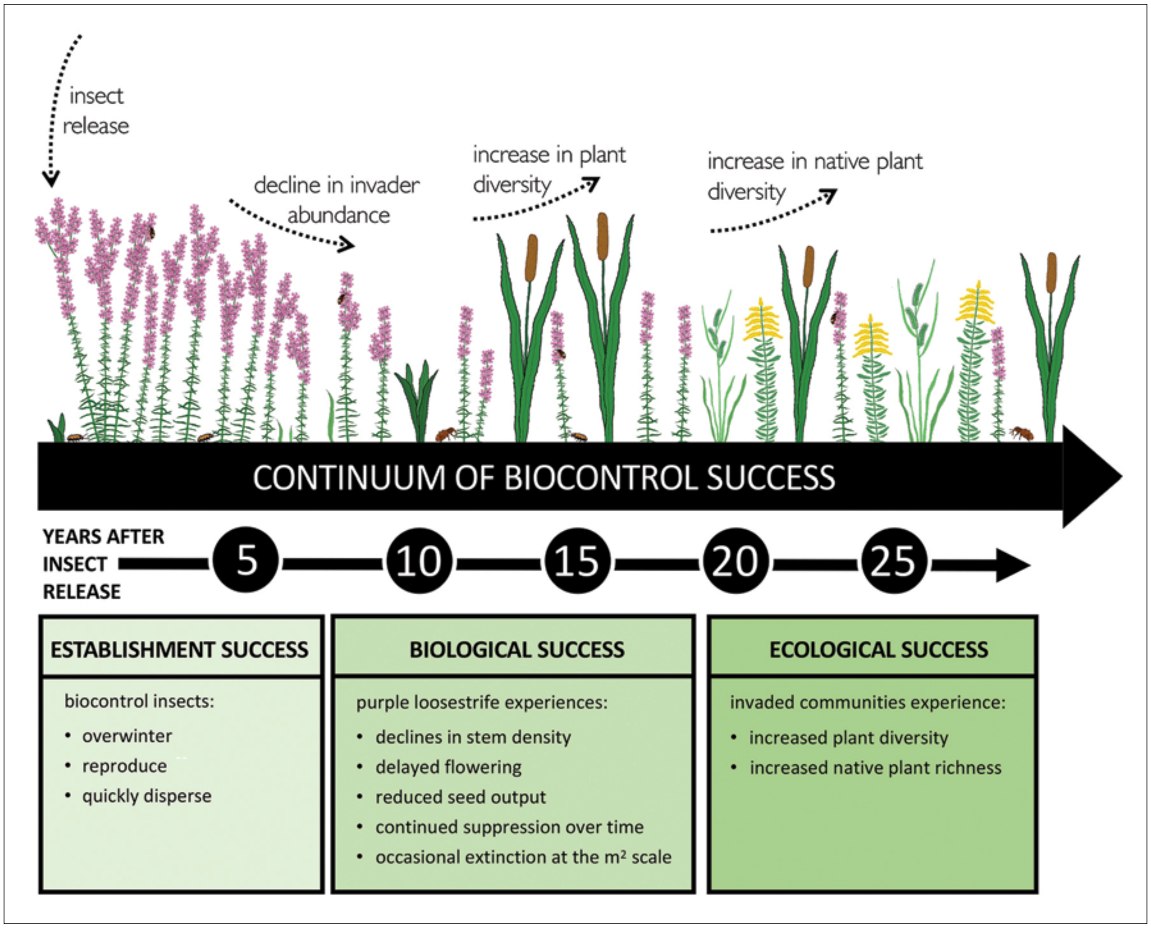

Figure 4. Successful weed biological control represents a slow accumulation of desirable changes across decades and here we summarize evidence that supports the success of biological control of purple loosestrife. Establishment success occurs when species released to control the plant successfully establish, reproduce, and become widespread, resulting in self-sustaining populations. Biological success is achieved through reduced spread or population growth rates of the target plant. Ecological success is achieved when biological control stabilizes or increases the population growth rate of desirable species that are threatened by the target plant. (S. Endriss, Cornell University)

Establishment Success

Establishment success is defined as the ability of released insects to establish self-sustaining populations at release sites. Early evidence suggested that establishment success increases when increased numbers of adults are released (Grevstad, 1999). But early experiments were typically done by releasing a few to a few hundred individuals. The rapid population build-up and mass production capabilities later allowed releases of thousands of individuals per site, and the two *Galerucella* species established self-sustaining populations at many release sites from California to Virginia and north throughout Canada (Hight et al., 1995; Piper, 1996; McAvoy et al., 1997, 2016; Grevstad 1999; Wiebe et al., 2001; Albright et al., 2004; Blossey et al., 2015; Beaulieu et al., 2018).

Anecdotally, *G. calmariensis* appears to be the more common species. When investigated, *Galerucella* establishment was easy to track because adults are readily visible and feeding (larvae and adults) is obvious, often resulting in dramatic defoliation events. However, we know much less about the fate, impact, and persistence of the *Galerucella* species in the Southeast and in areas where purple loosestrife is less abundant.

The root feeder, *H. transversovittatus*, established across temperate North America (Wiebe et al., 2001; Blossey et al., 2015). Populations of this species are difficult to track due to the nocturnal habits of adults and below-ground feeding of larvae. This makes it difficult to determine how well this species has done or how it contributes to the biological control of purple looseleaf. The flower feeder, *N. marmoratus*, received less attention in surveys and in redistribution programs. The limited information that does exist suggests it is now common and established at release sites across temperate North America, including sites in Manitoba and Ontario (Blossey et al., 2015; St. Louis et al., 2020).

All four species appear to be strong dispersers. Adult *Galerucella* disperse and colonize even isolated, single purple loosestrife plants. Their dispersal habits are often driven by food shortages at heavily defoliated stands, male aggregation pheromones, and plant-induced volatiles in response to leaf-feeding damage (Bartelt et al., 2008; Hambäck, 2010). Overall, the two leaf-feeding species can, on average, disperse 50–1,200 m per year (164–3,937 ft/yr) (Grevstad and Herzig, 1997; Ferrarese and Garono, 2010; Swain et al., 2011; McAvoy et al., 2016), with a maximum annual dispersal of 9 km (5.6 mi) recorded in the literature (Ferrarese and Garono, 2010). These documented dispersal distances are achieved by active flight and passive transport on water (Ferrarese and Garono, 2010; McAvoy et al., 2016) and are likely conservative as annual spread of up to 20 km (12.4 mi) was observed in some areas in Minnesota (L. Skinner, pers. comm.). Flower-feeding weevils have been documented to disperse, on average, 3.1 km (1.9 mi) per year, 1.5 km (0.9 mi) of which was across bodies of water (Ferrarese and Garono, 2010). Information on dispersal of the root-feeding weevil is limited, but damage by *H. transversovittatus* has been found throughout New York State, including at sites where it was not released.

Thus, given enough time, all four species regularly colonize small purple loosestrife populations, including those located in less preferred habitats, and can persist in small populations. For example, Carrie Brown-Lima and Bernd Blossey conducted roadside surveys within a 40-km (25-mi) radius of Ithaca, New York in 2001 (ten years after initial insect releases). They found that dispersal of the two leaf-feeding species and of the flower-feeding weevil was limited by topography and was correlated to prevailing wind directions (C. Brown-Lima, pers. comm.). However, when these same roadside surveys were repeated by Stacy Endriss and Bernd Blossey in 2018 (25 years after insect releases), both the leaf-feeding beetles and the flower-feeding weevil were widespread throughout central New York, and these species had colonized purple loosestrife irrespective of differences in roadside habitat or the population size of their host plant.

Biological Success

Biological success is defined as the ability of biological control to reduce the spread as well as the abundance or population growth of the target plant species (Blossey, 2016). Here, we focus on declines in abundance of purple loosestrife, as reductions in spread are difficult to document without landscape-scale studies, and, to our knowledge, no such study exists in the purple loosestrife system. Again, we note that before their release in North America, the impact and host specificity of each of the four insect species were thoroughly investigated using indoor potted plant experiments (in both the native and introduced ranges) and outdoor potted plant and field experiments (in the native range) (Blossey, 1993; Blossey et al., 1994a,b; Blossey, 1995a,c; Blossey and Schroeder, 1995). We also emphasize that short-term studies provide valuable insights into the potential mechanisms and impact of biological control (e.g., Katovich et al., 1999, Katovich et al., 2001; Dech and Nosko, 2002; Hunt-Joshi et al., 2004; Hunt-Joshi and Blossey, 2005), but that single-season field visits (Hovick and Carson, 2015; St Louis et al., 2020), short-term studies, and common garden experiments (Stastny et al., 2020) should not be used to predict the long-term impact and region-wide success of biological control; they provide ecological 'snapshots' that generate insights about a specific time or place but cannot capture long-term dynamics. Thus, we focus here on the results of long-term monitoring efforts.

In the case of purple loosestrife, data collected over periods of at least five years across different parts of the continent (Landis et al., 2003; Grevstad, 2006; Boag and Eckert, 2013; Britton et al., 2014; McAvoy et al., 2016) and information from informal reports, poster presentations, and personal communications demonstrate that leaf-feeding *Galerucella* beetles establish, build large populations, and create widespread and repeated defoliation events within a few years of their initial releases. Further, in response to damage by *Galerucella* beetles and other released insect species, purple loosestrife populations often decline over time, as shown by greatly reduced stem densities (Landis et al., 2003; Britton et al., 2014; McAvoy et al., 2016; but see, Grevstad, 2006) and a much shorter flowering period (B. Blossey, pers. obs.).

For example, eight to ten years following releases of leaf-feeding *Galerucella* beetles in Indiana, the density of purple loosestrife stems and inflorescences had declined at four of four monitored sites (Britton et al., 2014). In addition, in our long-term study (1998–2019) following purple loosestrife-herbivore dynamics for more than two decades across 33 sites in New York (Blossey et al., unpub. data), we observed dramatic insect population fluctuations over time, but purple loosestrife was ultimately no longer present in about half of our 340 permanent meter-square quadrats (1–15 quadrats per site) in 2019. When purple loosestrife did persist, stem densities in permanent quadrats decreased by about 80%, with these declines only becoming statistically significant 7–15 years after insect releases. This finding was similar to that of purple loosestrife populations monitored in Virginia where stem density held steady or increased the first five to seven years following insect releases across three sites, only declining for the two sites that were monitored across longer timescales (McAvoy et al., 2015). Furthermore, in New York, when insects were initially released in 1992, purple loosestrife began flowering in late June with a showy display lasting into September. However, almost 30 years later in 2021, plants begin flowering in late July or even August, severely curtailing seed output. Thus, insect releases appear to have resulted in widespread and sustained biological success in this system.

Importantly, widespread declines in purple loosestrife abundance, and even local eradication (at the square meter level), does not mean that purple loosestrife has disappeared from the landscape. Thriving individuals with heights comparable to those common 30 years ago can still be found. Purple loosestrife populations remain especially robust in areas subjected to chemical control or regular maintenance activities—such as along roadsides, ditches, or mowed strips—as these heavy disturbances can greatly reduce effectiveness of biocontrol agents (Blossey et al., unpub. data). Local site-specific conditions can also prevent insects from thriving and causing effective control, such as those present at tidal or densely shaded sites. In addition, we have limited understanding of the demographic effects of single generations (only F_1 overwintering), partial generations (F_1 adults and their offspring overwintering), or two generations/year (only F_2 adults overwintering) in leaf-feeding *Galerucella* beetle populations, and how this variation interacts with local climates or predation to determine the local impacts of these beetles on purple loosestrife. Regardless of the mechanism, these sites may eventually achieve biological success given time or may function as permanent purple loosestrife refuges. However, sites where purple loosestrife escapes biological control appear to be the exception to the rule and should not distract from the widespread biological success of this program: land managers no longer need to worry about the threat of purple loosestrife in the vast majority of wetlands across temperate North America. Furthermore, when local purple loosestrife outbreaks do occur due to seedling recruitment in response to drawdowns or site disturbances, they are quickly followed by population increases of the insects that then suppress these populations. These dynamics now resemble the situation known from purple loosestrife's native range in central Europe.

How each of the four released insect species contributes to this collective success is less understood. Also unclear is whether biological control has less impact on small populations of purple loosestrife, or sites with low stem densities, as insects may be able to successfully establish but may lack sufficient food to reach outbreak population densities. However, across our 33 monitored sites in New York State, we observed declines in stem density even for purple loosestrife populations with initially low stem densities (Blossey et al., unpub. data). Releases at sites with small purple loosestrife populations may also prevent local increases in stem densities or spread to other areas, even if local declines in purple loosestrife vigor and abundance are not observed. We found that more than a decade after releases, even sites with initially low abundance of purple loosestrife remained diverse, with purple loosestrife as a minor component of the plant community.

Importantly, where data have been collected on the outcomes of biological control, site-to-site variation in the impact of biological control is apparent. Yet insect predators, diseases, site conditions, and regional climate overall do not appear to reduce the potential for biological success across North America, as declines in stem density and shortened flowering times are repeatedly observed in the Pacific Northwest, the Midwest, the Northeast, the Mid-Atlantic states, and in Canada (e.g., Landis et al., 2003; Britton et al., 2014; McAvoy et al., 2016). Furthermore, rapid evolution of purple loosestrife in North America following insect releases has not impeded biological success in this system (Stastny et al., 2020).

We emphasize that long-term studies are critical to understanding the outcomes of biological control. As shown in this system, biological success can take many years to materialize given that control agents only reduce host plant performance once they establish and increase to outbreak population sizes. In addition, boom-and-bust cycles are also typical of biological control (Heimpel and Mills, 2017; Hill et al., 2020), with herbivore populations exceeding their food supply, crashing, and then rebuilding after host plant recovery. **Figure 5a** contains a simplified illustration of the boom-and-bust cycle observed at many sites in the purple loosestrife biocontrol system. Anecdotally, these boom-and-bust cycles are dampened over time in response to local food availability, yet they may also be driven by unpredictable disturbance events.

For example, in the purple loosestrife system, two events almost wiped out thriving *Galerucella* populations in some regions of New York, delaying biological success for many years. The first was extensive mosquito control efforts using aerial insecticide spraying following the arrival of West Nile Virus in 1999. In the Hudson River Valley, this almost eliminated *Galerucella* spp. populations that before these efforts had been continuously

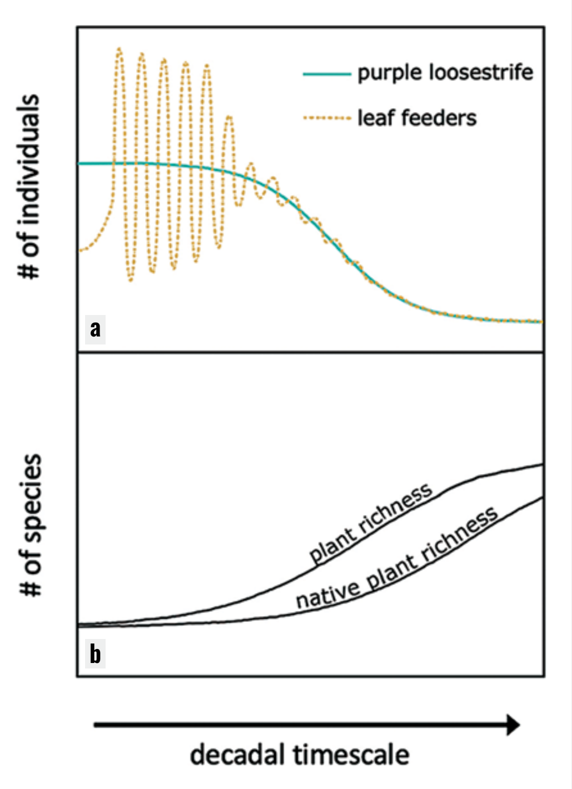

Figure 5. Conceptual diagram depicting the general pattern of purple loosestrife biocontrol through the lens of (a) insect dynamics and changes in purple loosestrife stem densities and (b) the response of total and native plant species richness over time. Note that insect abundances are at least an order of scale higher than purple loosestrife stem densities but are scaled to more easily visualize the relationship between insect dynamics and declines in target plant abundance over time. (S. Endriss, Cornell University)

increasing. It took many years for *Galerucella* species to recover in this region (B. Blossey and V. Nuzzo, pers. obs.). We suspect that mosquito control efforts using aerial sprays have similarly harmed *Galerucella* spp. populations in the Syracuse area (B. Blossey, pers. obs.), even when sprays were conducted in August after most *Galerucella* activity had subsided. Indeed, selected mosquito control larvicides have been shown to negatively impact *Galerucella* survival and development under controlled laboratory settings (Lowe and Hershberger, 2004).

The second event was an unusually early spring in the early 2000s in the Finger Lakes Region of New York, followed by strong frosts in late May or early June that killed most young purple loosestrife shoots. Although plants recovered quickly, *Galerucella* adults had already been active for a few weeks, and this temporary absence of food killed most of the leaf-feeding beetles that year. Eventually, sites in this region were either recolonized or beetle populations gradually rebuilt from the few surviving individuals. Again, single-season field visits and common garden experiments may completely miss such events because they are unable to capture both changes in site histories and in insect and host plant abundance over time.

Ecological Success

Biological success is important, but the ultimate goal of land management is ecological success—a reduction or elimination of the negative impacts the target plant has on native biota. In this case, ecological success requires that native species retain and increase their presence in wetlands that were once dominated by purple loosestrife (i.e., increases in the population growth rate of desirable species that are or were threatened by purple loosestrife invasion [Blossey, 2016]).

Data that can be used to evaluate ecological success of weed biological control programs are rare, especially given the decades-long timescales at which these plant biocontrol impacts are realized. Weed biocontrol programs typically do not have the logistics and funding in place to achieve this desired accountability (Blossey, 1999, 2016). Here, however, we provide strong evidence that biological control of purple loosestrife has resulted in ecological success, making it an important case study in support of biological control as an effective management tool. We acknowledge that this evidence is limited to primary producers, and we lack important information on how birds, amphibians, mammals, or insects have responded to declines in purple loosestrife vigor, stem densities, and/or cover.

In general, the pattern observed in longer-term studies is a slow but persistent increase in total and native species diversity and abundance as insect feeding drives declines in purple loosestrife stem density and cover (Landis et al., 2003; Albright et al., 2004; Britton et al., 2014; conceptually illustrated in **Fig. 5**). This process is gradual, as it requires a significant loss of dominance by purple loosestrife and—once purple loosestrife declines—a sustained recruitment of native species that can take decades. The rate and degree of such events varies across the landscape and over time. For example, species richness of non-target plant species was higher six years after insect releases at four of five sites monitored by Landis et al. (2003) in Michigan. In contrast, at four sites in Indiana, native plant species had not increased after 10 years despite heavy impacts of the leaf beetles as well as increases in overall plant richness across three of the four sites (Britton et al., 2014). Furthermore, at our 33 long-term monitoring sites in New York State, plant diversity and richness increased over time, but became significant only more than a decade after initial insect releases (Blossey et al., unpub. data). Importantly, we were able to find support that increases in the abundance of non-target plant species (including native plant species) were correlated with declines in purple loosestrife stem density. This is especially convincing because without this mechanistic link, it can be hard to distinguish the impact of biological control from other processes that may similarly drive shifts in plant communities over time.

These findings highlight that just as insects need time to establish, build up their populations and achieve biological success, the species that co-occur with purple loosestrife also need time to recover and respond to declines in purple loosestrife abundance (conceptually illustrated in **Fig. 5b**). The gradual decline in purple loosestrife vigor due to biological control allows native species to slowly rebuild their populations and occupy vacated space. This contrasts with the rapid but short-term decline of purple loosestrife following herbicide campaigns, where annuals or other invasive species are favored, and entire sites are reset to the beginning of successional development following treatment. Successful biological control therefore not only takes time, but biological control is likely so successful because it takes time.

However, we caution that increases in plant diversity are not always desirable, especially if incoming species represent new invaders or other species that may not deliver the desired benefits to wetland function or native fauna that were the goal of the biological control program. For example, species that can establish dominance and near monocultures, such as *Phalaris arundinacea* (reed canary grass), introduced *Phragmites australis australis* (Phragmites), or even extensive stands of *Typha* spp. (cattail), may have detrimental effects similar to purple loosestrife. Examples of this undesirable replacement were reported by Hovick and Carson (2015) and were also found for some sites in our long-term study in New York (Blossey et al., unpub. data).

Finally, the safety of non-target species is a crucially important consideration in host specificity screening of potential biological control agents. Early critiques of implementing biological control of purple loosestrife included concerns that the introduced insects would feed upon and harm native populations of *L. alatum* (winged loosestrife) and *Decodon verticillatus* (swamp loosestrife or waterwillow) (e.g., Hager and McCoy, 1998). Pre-release evaluations documented that the root-feeding and leaf-feeding biocontrol insects were able to complete their larval development on these plants under laboratory conditions, as well as inflict small to moderate amounts of feeding damage on these species (Kok et al., 1992; Blossey et al., 1994b). Post-release field studies, however, demonstrated that minor feeding and 'spillover' events are unlikely to result in population-level declines of these species (Corrigan et al., 1998; Blossey et al., 2001). In our long-term monitoring efforts in New York, *D. verticillatus* was present at the start of insect releases at some sites, and two decades later the species was thriving in the presence of biocontrol, accounting for at least 20% of plant cover at these sites. While *L. alatum* was not present in our monitoring quadrats, the species has been rediscovered in the larger Montezuma wetlands in areas where it was thought to have been replaced by purple loosestrife (B. Blossey, pers. obs.; F. Morlock, pers. comm.).

BENEFITS OF BIOLOGICAL CONTROL OF PURPLE LOOSESTRIFE

As detailed above, the benefits of biological control of purple loosestrife are widespread across temperate North America. For example, herbicide use has been greatly reduced or in some areas eliminated entirely because managers no longer need to worry about purple loosestrife outbreaks. Furthermore, fears of sustained non-target effects on native plants that are closely related to purple loosestrife and of severe losses for the beekeeping industry have not materialized. Finally, wetland managers and individuals without specialized knowledge of insects have become aware of the uses and benefits of biological control, a side benefit of the rapid development of techniques for the mass production and distribution of purple loosestrife biocontrol agents across the United States and Canada. Even today, management campaigns and insect distribution networks are maintained by states such as in Wisconsin (Scherer, 2020).

Importantly, biological control programs that are successful tend to be forgotten, in large part because of their success; the invasive species and biocontrol insects now behave like thousands of native plant species and their natural enemies, without the drama of outbreaks or the perceived need to intervene when large monocultures develop. This, in turn, results in reduced attention by land managers, the media, and scientists. We see this in the purple loosestrife program in that the nationwide attention the species had 20–30 years ago now has been diverted to other problems. A few scientific papers continue to be published on this system, but at a greatly diminished rate compared to a few decades ago and, at least within the United States, with increasing focus on non-management issues. Despite this reduced attention, these successful biocontrol programs maintain their success—they continue to 'chug along', characterized by only small-scale annual fluctuations in plant and insect abundance.

This long-term success underscores the need for greater support to collect data on the outcomes of biological control. The United States spends millions of dollars each year on traditional control methods, often with little evidence of long-term success. Effective biological control, including the work done for purple loosestrife, represents a sustainable and overall low-cost management alternative. Yet significant start-up costs, uncertainty whether biocontrol will ultimately be successful, and concerns about safety of non-target species have greatly reduced biocontrol implementation (Moran and Hoffmann, 2015). This is further compounded by a general lack of data on the outcomes of many biological control programs, particularly across the timescales needed to accurately observe biological and ecological success. Biological control of purple loosestrife is, therefore, one of the first examples of successful recovery of native plant species following control by insects and provides strong support that the benefits of biological weed control are worth the investment.

WORK STILL TO BE DONE

Today, 30 years after initial insect releases in 1992, is there still work that needs to be done or can we move on to other management priorities? In both the United States and Canada, local and regional redistribution efforts of the leaf-feeding *Galerucella* species continue. But are they necessary? Purple loosestrife is most apparent when flowering in late summer (August/September), which is when we receive inquiries regarding purple loosestrife biological control and requests for insects to be released the following spring. Yet the need to release biocontrol agents should be assessed in early spring when *Galerucella* adults, eggs, larvae, and feeding damage are most visible, not in the fall when leaf beetles are overwintering and purple loosestrife has recovered from spring and summer insect feeding damage. In some regions of the United States, especially those with isolated populations of purple loosestrife, leaf-feeding *Galerucella* beetles may be absent. However, for the vast majority of cases in the northeastern United States, interest in biological control voiced in the fall is not followed up with actual requests in the spring, as leaf-feeding *Galerucella* beetles are found to already be widely established in the areas of concern.

Requests for additional insects may also be related to unrealistic expectations as to the speed or level of control these insects may achieve. Indeed, despite widespread recognition that biological control of purple loosestrife has been successful and is sustainable, we continue to see recommendations and attempts at eradication, continued herbicide applications (Knezevic et al., 2018), or attempts to integrate chemical with biological control (Henne et al., 2005). However, biological control requires patience, time, and non-interference. Based on our experience in the northeastern United States, and New York specifically, we strongly discourage use of additional management of purple loosestrife (including digging, mowing, and chemical treatments) to allow biological control to be maximally effective. For example, even if herbicide applications do not reduce the efficacy of insects feeding on purple loosestrife plants (Henne et al., 2005), these applications likely have strong, negative non-target effects on the native species we are trying to protect. This is true for purple loosestrife and for other weed biocontrol systems as well (Peterson et al., 2020). Furthermore, as described above, traditional forms of active management facilitate long-term persistence of purple loosestrife and do not achieve sustained suppression. Traditional management also likely has significant negative impacts on non-target species, but these impacts are rarely reported. For example, a review of published evidence found that chemical treatment resulted in more harm than leaving the introduced species non-treated (Kettenring and Adams, 2011). In the purple loosestrife system, herbicide treatments should be discouraged.

Most importantly, at low purple loosestrife stem densities, the negative impacts that drove the development of biological control will have disappeared or have never materialized. We should not expect, or even desire, eradication because remnant plant and insect populations ensure that herbivores are present and can respond should disturbances create ideal conditions for increased germination and seedling recruitment of purple loosestrife. Once insects are well established, booms in purple loosestrife abundance will therefore be short-lived. To this end, if *Galerucella* beetles are already established at a site, no further releases should be made. Adding insects (except for the root-feeding weevil) will not aid in purple loosestrife declines, as levels of suppression are instead determined by local site conditions. As we detailed above, some sites with heavy shade, or tidal sites with repeated daily flooding, may function as purple loosestrife refuges or may only transition from plant communities dominated by purple loosestrife to something different after many decades of slow purple loosestrife declines. However, as purple loosestrife populations expand geographically into areas where they have not previously occurred, insect releases can still be justified. This situation may be particularly relevant in the western parts of the United States and Canada, where purple loosestrife continues to spread to previously unoccupied areas via waterways.

Thus, while a national or continental assessment of ecological and economic outcomes or a cost-benefit analysis of the biocontrol program targeting purple loosestrife would be desirable, the lack of appropriate datasets and methodological problems associated with economic valuations of species makes such an evaluation unlikely to be achieved and, if achieved, unlikely to be reliable. We know of vast datasets in the files of management agencies, but whether they will ever be published in an easily digestible format remains questionable. Many of the land managers and scientists who were instrumental in funding and supporting initial insect releases across North America are retired or no longer accessible. As a result, their expertise on historic baselines in North American wetlands, even before purple loosestrife gained and then lost dominance, has often been permanently lost.

Despite these sources of potentially unrecoverable data, evidence of the long-term outcomes of biological control in this system remains strong. The absence of extensive and detailed evaluations of weed biocontrol implementation outcomes in other systems is a major handicap preventing weed biocontrol from becoming a more acceptable and better financed management alternative, and the collection of similar data across other programs would help ensure that weed biological control continues to be funded and successful moving forward.

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CHAPTER
28

Tamarix Biological Control in North America

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NON-TECHNICAL SUMMARY

The biological control program against *Tamarix* spp. (tamarisk/saltcedar; Tamaricaceae) was initiated in the 1970s to reduce negative impacts of this invasive Old World shrub to riparian biodiversity and ecosystem function in western North America. Field releases of host-specific leaf beetles (Chrysomelidae) in the genus *Diorhabda* were initiated in 2001, with significant establishment and widespread defoliation observed roughly two years after open releases. What followed were a variety of complex interactions among invasive *Tamarix*, its guild of herbivores including *Diorhabda* spp., and the physical and biotic environment, which varied across the western U.S. project area. Defoliation yielded sustained lower evapotranspiration and opened canopies, allowing increases in desired vegetation in some areas, while in other areas beetle establishment failed for reasons that included less-suitable host species, mismatches of environmental cues with diapause development of the beetle, and predation by generalist insectivores. In some regions, such as Texas, agent populations were short-lived, resulting in lack of sustained *Tamarix* suppression. In other areas, beetle populations reached initial epidemic densities but then declined to moderate levels with patchy subsequent defoliation and diminished target mortality. These short-term dramatic impacts to invasive *Tamarix,* but limited sustained control, suggest potential value in releasing additional host-specific agents, some of which have already been studied and readied for petitioning for release.

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Stakeholder enthusiasm for the biocontrol program was high in some locations and allowed reduced expenditures for conventional weed control. At the same time, perceived threats to sensitive wildlife species, particularly the endangered southwestern willow flycatcher (*Empidonax traillii extimus*) in Arizona, New Mexico, and Utah, resulted in legal actions terminating federal involvement in the biocontrol program nationally. A strategic approach to ecological restoration targeting ecosystems with high potential for enhanced wildlife habitat could help resolve these conflicting issues, but progress is currently inhibited by a lack of political support and financial resources, suggesting that renewed collaboration of interested parties across disciplines is needed to more fully achieve long-range benefits.

HISTORY OF INVASION AND NATURE OF PROBLEM

Introduction

Exotic shrubs in the genus *Tamarix* (also known as saltcedar, tamarisk; Tamaricaceae, **Fig. 1**) were introduced to North America in the 1800s and established invasive populations along most waterways in arid western North America in the early 1900s. Ecological damage of sensitive riparian ecosystems due to the dominance of *Tamarix* provided the incentive to develop a biological control program, conducted with multi-agency and stakeholder input (Stenquist, 2000). We now (2022) mark the 20th anniversary of the first open-field releases of the chrysomelid beetle *Diorhabda carinulata* as a *Tamarix* biocontrol agent. Conflicting and contrasting outcomes are apparent at this stage of the program. First, the widespread defoliation and decline of *Tamarix* that followed have made the project one of the most successful and visually stunning in the history of invasive plant biocontrol. At the same time, lack of *Diorhabda* establishment and impact in many regions show that we have far to go to achieve desired results throughout the *Tamarix* range in North America. Second, the highly visible suppression of *Tamarix* and transformation of riparian corridors following defoliation events in certain locations has, among some, resulted in the notion that biocontrol has brought about a decline in

Figure 1. Tamarisk, *Tamarix* spp. (Tamaricaceae). (Eric Coombs, Oregon Department of Agriculture, Bugwood.org CC BY-3.0 US)

riparian ecosystem services, particularly habitat for an endangered bird subspecies. Biocontrol practitioners are presented with the challenge of tracking success while defusing negative perceptions of a beneficial program (Bean and Dudley, 2018).

Despite controversy, it is broadly recognized that *Tamarix* biocontrol is here to stay and should be integrated into riparian management and restoration plans, an approach with nearly universal support among natural resource managers (Dudley and Bean, 2012). In this report, we update program status and success in *Tamarix* suppression. We also provide suggestions for defusing controversy and keeping a long-term project moving in a productive direction. This is not an exhaustive treatment of the topic, as there are reviews that provide an overview of the *Tamarix* invasion and the biological control program (Dudley et al., 2000; Dudley and DeLoach, 2004; Shafroth et al., 2005; Bateman et al., 2010; DeLoach et al., 2011; Dudley and Bean, 2012; Knutson et al., 2012, 2019; Bean et al., 2013a; Hultine and Dudley, 2013; Dudley et al., 2017; Bean and Dudley, 2018; Pratt et al., 2019).

The Species Invasion

Tamarix is a complex of >50 semi-deciduous shrub species (and some arboreal, evergreen species, e.g., *Tamarix aphylla* or athel) originating in Eurasia and Africa (Baum, 1978). In their original ranges, these plants are sources of fuelwood, construction material especially for furniture, and of modest value as livestock forage (especially goats; Abou Auda, 2010). In its native range, *Tamarix* can form extensive and ecologically important stands that provide structural and resource values to wildlife (van Zeist, 1985; Bunbury et al., 2020).

While its path of transport into North America (and other invaded regions with similar climates, e.g., Argentina, South Africa, Australia) is uncertain, by the late-1800s tamarisks were present as horticultural plants and promoted for erosion control across the southwestern United States (Robinson, 1965; Everitt, 1998). With the widespread regulation (via dams and canals) of western rivers in the early 1900s, tamarisk took advantage of the altered hydrologic conditions to proliferate into riparian areas, reservoir margins, seeps, and wetlands. Its expansion was facilitated by reduced competition from native cottonwood-willow vegetation that depended upon ample water and natural flooding regimes for regeneration.

Nature of the Problem

By the middle of the 20th century, *Tamarix* spp. and hybrid forms (Gaskin and Schaal, 2002) occupied roughly 1.5 million acres (607,000 ha) of the western United State (Robinson, 1965; Everitt, 1998; Nagler, 2011) and displaced iconic cottonwood-willow (*Populus* spp., *Salix* spp.) riparian woodlands and other vegetation types, including mesquite bosque, riparian scrub, and saltgrass/halophytic scrublands. Tamarisk plants in northern latitudes tend to be moderately slow-growing shrubs (Sexton et al., 2006), while those in southern regions are fast-growing where adequate moisture is available. In these regions, they are able to reproduce within one to two years and achieve large stature, often as spreading 'trees' greater than 5 m (16 ft) in height. The expansion of this invader was facilitated by the plants being highly plastic in their growth and reproductive phenology and able to tolerate reduced water availability, soil salinization, and other harsh conditions that reduce suitability to sustain native vegetation (Busch and Smith, 1995; Glenn and Nagler, 2005).

WHY CONTROL *TAMARIX*?

Tamarix invasion poses numerous environmental and economic problems (see reviews of Everitt, 1998; Dudley et al., 2000; Shafroth et al., 2005), which increase greatly as the plant comes to dominate riparian ecosystems. Some of the physiological and morphological properties of *Tamarix* lead to alterations of riparian ecosystem function in ways that favor itself over native species, resulting in *Tamarix* acting as a driver of ecosystem change (Johnson, 2013) and leading to near monocultures. For instance, a serious impact of *Tamarix* invasion is increased frequency and extent of wildfire (Busch, 1995; Drus, 2013). Fire is uncommon in native-dominated riparian vegetation in the region (Verkaik et al., 2013). However, *Tamarix* foliage ignites readily and burns with high intensity when senescent and dry, as well as when green and healthy (Drus et al., 2013). This high combustibility turns riparian areas into conduits for, rather than barriers to, wildfire spread (Lambert et al., 2010). Burn severity increases with *Tamarix* density, with a concomitant increase in mortality of associated native woody plants, such that fire drives a positive feedback loop that can lead to *Tamarix* monocultures (Drus, 2013).

In addition to being fire-adapted, other properties of *Tamarix* allow it to outcompete native vegetation. These include high evapotranspiration rates that deplete shallow groundwater (Smith et al., 1998), inhibition of native plant establishment as a consequence of impenetrable litter, and desiccation and salinization of soils by throughfall and deposition of salt-laden litter (Shafroth et al., 1995). River channels are altered by increases in both sedimentation and erosion where dense stands interfere with stream flows (Graf, 1978; Birken and Cooper, 2006). Impacts of *Tamarix* on nutrient cycling and mycorrhizal ecology are just beginning to be understood, but they are substantial and contribute to the decline of native species (Meinhardt and Gehring, 2012, 2013). These factors interact, with the consequence that *Tamarix* establishes a positive feedback system favoring its own dominance, exacerbating the ecological and economic impacts.

Tamarisk can have some beneficial attributes in its invasive range, e.g., stabilizing dry river channels where native vegetation has declined (Norris and Grim, 2022), offering shade for humans, livestock, and wildlife, and providing some food resources and habitat for wildlife (Sogge et al., 2008; Mahoney et al., 2022). Even so, the modest ecosystem services provided by the plant in the invaded range are a poor substitute for those of a native plant assemblage (Dudley and Bean, 2012). *Tamarix* offers lower quality habitat to wildlife than does native vegetation, presenting a simplified vegetative structure and altering understory conditions for birds, reptiles, and other taxa (Ellis, 1995; Shafroth et al., 2005; Bateman et al., 2013). Widespread displacement of native riparian vegetation by *Tamarix* spp. is even cited as a causal factor in declines of sensitive riparian-dependent species, including the endangered southwestern willow flycatcher (SWFL; *Empidonax traillii extimus*) in the desert Southwest (Suckling et al., 1992; DeLoach et al., 1996).

By altering stream flows and channel morphology, *Tamarix* also changes in-stream properties in ways detrimental to native fish, including endangered and threatened species (Keller et al., 2014). Fish, aquatic invertebrates, and salt marsh fauna are also negatively affected by surrounding *Tamarix* dominance via reduced resource value or altered structural habitat (Kennedy and Hobbie, 2004; Going and Dudley, 2008; Moline and Poff, 2008; Whitcraft et al., 2008).

Historically, the primary impact of concern was depletion of water resources, particularly in the arid Southwest, owing to the high evapotranspiration rates (ET) of *Tamarix* and its ability to maintain photosynthesis and water extraction even in non-saturated soils (Sala et al., 1996; Cleverly, 2013). While precision in estimating water salvage from *Tamarix* control is difficult to attain (Shafroth et al., 2010), ET owing to *Tamarix*'s high areal cover is certainly a factor in water conservation in western North America (Shafroth et al., 2005; Hultine and Bush, 2011). Large-scale tamarisk control efforts have been implemented since the 1920s, but with limited effectiveness and sustainability. Major removals of riparian vegetation for putative water 'salvage' in places like the Gila River (Orr et al., 2017a) further facilitated *Tamarix*' expansion in human-altered habitats due to its tolerance of degraded conditions and capacity to outcompete native plants under such conditions (Sher and Marshall, 2003; Shafroth et al., 2005; Hultine and Dudley, 2013). With a drying climate and regional reservoirs at historically low levels, the interest in removing tamarisk for water conservation remains as important as ever, with agencies and political representatives seeking more effective and less costly means for doing so (Hultine et al., 2015).

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Overview of the *Tamarix* Biological Control Program

Foreign exploration to find potential biocontrol agents for tamarisk was initiated in the 1970s and expanded in the late 1980s and 1990s (Pemberton and Hoover, 1980; DeLoach et al., 1996). From over 300 arthropods considered host-specific to the family Tamaricaceae (DeLoach et al., 1996), host range testing was completed for three candidate agents: the weevil *Coniatus tamarisci* (Curculionidae), the mealybug *Trabutina mannipara* (Pseudococcidae), and a leaf beetle considered originally to be *Diorhabda elongata carinulata* (Chrysomelidae) from Central Asia (**Fig. 2**) (DeLoach et al., 1996, 2004). The *Diorhabda* beetle was chosen for further development owing to its host specificity, ease of handling, rapid growth and reproduction, and substantial host impact via feeding on *Tamarix* green tissues and subsequent foliage desiccation (Lewis et al., 2003; DeLoach et al., 2004, 2011; Pattison et al., 2011).

Figure 2. Tamarisk beetle, *Diorhabda* spp.: (a) adult; (b) larva. (a: Robert D. Richard, USDA-APHIS-PPQ; b: William M. Ciesla, Forest Health Management International; a,b: Bugwood.org CC BY-3.0 US)

At the outset, broad support was garnered through involvement of private stakeholders, federal, state, and local agencies, Native American tribes, university scientists and nonprofit groups organized into the Saltcedar Biological Control Consortium (SBCC) (Stenquist, 2000; DeLoach et al., 2004). The SBCC provided an instrument to address the needs of biocontrol end-users and evaluate and subsequently promote biocontrol as an option for *Tamarix* control. The SBCC goals included long-term monitoring and follow-up restoration, which were strongly advocated for as essential components of *Tamarix* biocontrol (Stenquist, 2000). As the program moved from host range testing to implementation, the SBCC proved invaluable in regional development and coordination of release and monitoring efforts.

The biocontrol program increased in complexity with the development and release of additional geographical ecotypes of what was then believed to be a single species, *Diorhabda elongata*, but which was subsequently reclassified as a species complex specializing on *Tamarix* (Tracy and Robbins, 2009). To suppress *Tamarix* in regions where the Central Asian *Diorhabda* species, (now classified as *Diorhabda carinulata*, i.e., the northern tamarisk beetle) had not been successful, three other species were introduced. The more southern-adapted species were (1) *Diorhabda carinata* (the larger tamarisk beetle) from eastern Europe and western Asia, (2) *Diorhabda sublineata* (the subtropical tamarisk beetle) from North Africa and the western Mediterranean basin, and (3) *D. elongata* (the Mediterranean tamarisk beetle) from the eastern Mediterranean basin (Tracy and Robbins 2009; Dalin et al., 2010; DeLoach et al., 2011; Bean et al., 2013a).

This aspect of the program also proved successful, with widespread *Tamarix* defoliation occurring across Texas and neighboring states (Pattison et al., 2010; DeLoach et al., 2011; Michels et al., 2013), although populations of the three additional species have declined dramatically following initial successes (Knutson et al., 2019), as discussed later.

Other *Tamarix*-specific herbivores unintentionally transported to North America can have significant impact on their hosts as well as interacting with intentionally released agents; these herbivores include a widespread leafhopper, *Opsius stactogalus*, and the scale insects *Chionaspis etrusca* and *Chionaspis gilli* (Wiesenborn, 2005; Louden, 2010; Uhey et al., 2020). More interestingly, the splendid tamarisk weevil, *Coniatus splendidulus* (a foliage-feeding weevil related to the host-specific species *C. tamarisci* that was originally considered for release [Fornasari, 1997]) was first detected in central Arizona in 2006 and subsequently spread through the southwestern states (Eckberg and Foster, 2011; Dudley et al., 2017). Pathways of introduction for these insects are unknown, but these taxa have the potential for synergistic and in some cases antagonistic interactions with *Diorhabda* species (e.g., Swope and Parker, 2012).

The *Diorhabda* Field Release Program

Initial field trials with *D. carinulata* were conducted in 1999 (one Colorado site was initiated in 1998) as a multi-site experimental caging study involving 10 sites in six states (Nevada, Utah, Colorado, Wyoming, Texas, and California) to evaluate capability of this species to develop and reproduce across the exceptionally broad geographic and climatic range of invasive *Tamarix* (Dudley et al., 2001; DeLoach et al., 2004). It is notable that cage studies were not conducted in areas where the endangered southwestern subspecies of willow flycatcher was known to nest in *Tamarix*. This was part of the plan to delay *Tamarix* biocontrol in areas where the shrub may provide limited ecosystem services, allowing restoration to proceed in advance of *Tamarix* decline (Stenquist, 2000).

Establishment process and dispersal

Figure 3. New release of northern tamarisk beetles, *Diorhabda carinulata.* (A. Gaffke, Montana State University)

At the outset of the program, details were unknown regarding release protocols, such as numbers of beetles required for establishment, timing of releases, and optimal storage conditions before release, and releases were further limited by the number of beetles available at the time (**Fig. 3**). The initial release made at the (later, successful) Humboldt Sink site in Nevada was of 1,400 beetles (Carruthers et al., 2008). Later the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) recommendation was for releases of 2,000 adult beetles (Kauffman, 2005). The Colorado Department of Agriculture now recommends 5,000 adult beetles as the minimum number for establishment (Kennard et al., 2016). More important than number released is the physiological state of the released beetles. Adult *D. carinulata* are more active and likely to be flying in the late morning and through the afternoon (Bean et al., 2007a), when releases should be avoided to minimize flight losses (Cossé et al., 2005; Bean et al., 2013a). Beetles collected late in the season are destined for reproductive diapause and are unsuitable for initiating establishment (Bean et al., 2007b). Establishment success can be enhanced by ensuring that beetles are not greatly stressed, as they might be under pre-release conditions typically used for biocontrol agents (cold, dark, and crowded). Since the reproductive and dispersal behavior of *Diorhabda* species is mediated by a male-produced pheromone blend (Cossé et al., 2005), conditions that inhibit production of the pheromone severely diminish establishment efficacy (Gaffke et al., 2020). Distribution of reproductive beetles, handled with minimum disturbance, and released in quantities exceeding 5,000 adults is recommended for establishment. In addition, use of the aggregation pheromone at releases sites enhances establishment (Gaffke et al., 2020).

Where *Diorhabda* species have successfully established and expanded their ranges, the highly visible defoliation has attracted substantial attention (**Fig. 4**); however, most releases across the western United States did not result in agent establishment (Bean et al., 2013b). Establishment was most dependable where tamarisk stands were large, usually greater than 100 plants, in a near-monoculture and contiguous state, and located north of approximately 38°N latitude (Bean et al., 2013a). Initially, field colonies of beetles were localized with only limited adult dispersal to adjacent trees and generally following the stand's edges, including river margins, followed by gradual expansion outward into closed stands (Pattison et al., 2011). *Diorhabda* adults disperse over both short and long distances. The success of this dispersal pattern is facilitated by the male-produced aggregation pheromone, which stimulates swarms of beetles to move small distances (1–30 m [3–98 ft]) when food is abundant, and long dispersal distances (ranging from several to over 100 km [62 mi] per year) when food sources become limiting (Cossé et al., 2005; Nagler et al., 2014; Wenjie et al., 2017; Knutson et al., 2019). The aggregation pheromone facilitates population regrouping after longer-distance dispersal (Cossé et al., 2005; Gaffke et al., 2018, 2019).

Figure 4. Defoliation of tamarisk from tamarisk beetle feeding (brown foliage) adjacent to healthy, undamaged native plants (green foliage). (William M. Ciesla, Forest Health Management International, Bugwood.org CC BY-3.0 US)

Initially, the dispersal rate of *Diorhabda* spp. was predicted to be about 1–2 km (0.6–1.2 mi) per year. However, observed dispersal distances outpaced this prediction, with Utah and Colorado populations of *D. carinulata* now having dispersed as far south as the Mexico-U.S. border (D. Bean and T. Dudley, unpub. data). Remote sensing of defoliation patterns along western river corridors has enabled tracking of dispersal and impact (Nagler et al., 2012, 2014; Wenjie et al., 2017; Bedford et al., 2018).

Constraints on establishment

Many *Diorhabda* releases failed to establish or established weakly, disappearing after one or two years. Some such failures appear to be caused by biological constraints. Other factors restricting regional implementation were policy-related, particularly the cessation of permitting, and other regulatory roadblocks. Below we describe both types of constraints.

(1) Phenological mismatch. This problem was an important reason for failures of the northern-adapted *D. carinulata* at southern locations in the western United States, due to mismatched cues for diapause induction (Lewis et al., 2003; Bean et al., 2007b). Intuitively, the warmer temperatures and longer growing season in the south should promote a long *D. carinulata* reproductive season with multiple generations, but this was not the case because declining photoperiods induce reproductive diapause. Day lengths that are normally encountered in midsummer at higher latitudes (approximately 44°N or higher, the latitude of origin in Central Asia) indicate that winter conditions will soon follow. However, they induced diapause at the wrong time of the year at lower, more southern latitudes, causing beetles to enter diapause prematurely. The shorter summer day lengths combined with longer growing seasons resulted in phenological mismatches between *Tamarix* and *D. carinulata* at southern latitudes (Hultine et al., 2015), limiting the useful range of *D. carinulata* to areas north of about the 38th parallel (Bean et al., 2007b). This phenological asynchrony resulted in a commonly observed pattern in which high initial beetle population densities were followed by major declines (DeLoach et al., 2011). The phenological mismatch constraint was circumvented in parts of the southwestern United States by using the southern-adapted *Diorhabda* species, for which diapause induction was triggered at shorter day lengths, allowing beetles to continue feeding and reproduction later into the season (Milbrath et al., 2007; Dalin et al., 2010). These southern species also had a greater chance of success because of their multivoltine life histories, which they exhibited at more southern latitudes (Dalin et al., 2010). In addition, as *D. carinulata* populations moved incrementally southward in the Colorado River Basin, their day length cues for diapause evolved to become shorter and better-matched to southern ecological settings, and this rapid evolution may have facilitated population recovery within the new range (Bean et al., 2012).

(2) Host utilization. Mismatches between the target plant and the agent have been an impediment to establishment in some areas. Five species of *Tamarix* were targeted by the biological control program. Of these, three species, *T. parviflora*, *T. ramosissima*, *T. chinensis*, as well as *T. ramosissima* x *T. chinensis* hybrids, were the most commonly encountered forms in the western United States. Beetles failed to establish at field sites in coastal California drainages due to a host plant mismatch between *D. carinulata* and *T. parviflora*, which is the target species at many Californian sites (Dudley et al., 2012). This form of *Tamarix* is from the Mediterranean Basin, while *D. carinulata* is from Central Asia and did not coevolve with this host. Consequently, this beetle species may not perceive a chemical cue that *T. parviflora* is a suitable host, as further suggested by cage experiments in which oviposition by *D. carinulata* was rare on *T. parviflora* (Dalin et al., 2009). In contrast, one river drainage dominated by *T. parviflora* has supported a population of *D. elongata* (also from the Mediterranean region) for fifteen years, although damage to the plants has not resulted in suppression (Pratt et al., 2019). Hybridization between *T. ramosissima* and *T. chinensis*, resulting in a latitudinal gradient with a higher representation of *T. ramosissima* in northern populations, can also affect the performance of *D. carinulata* as well as its impact in the field (Williams et al., 2014; Long et al., 2017). Athel (*T. aphylla*) was similarly avoided by beetles in field experiments, although its hybrid form with *T. ramosissima* proved viable as a host (Moran et al., 2009).

(3) Predation. Invertebrate predators also negatively affected some *Diorhabda* populations on the southern plains (Berro et al., 2017; Knutson and Campos, 2019). In particular, the red imported fire ant, *Solenopsis invicta*, was an efficient predator on *Diorhabda* pupae in the leaf litter (Knutson and Campos 2019), which may limit the range of its use to areas outside of the range of fire ants. Cage trials indicated that, where common, the exotic Argentine ant, *Linepithema humile*, also restricted the ability of *D. carinulata* to establish (Dudley et al., 2012). Many species of generalist arthropods (even land crabs) routinely feed on both adults and larvae of *Diorhabda* species and likely limit their capacity to build up sufficiently large populations to persist at release sites (Moran, 2010; Strudley and Dalin, 2013).

Larvae of *D. carinulata* were subject to predation by arboreal ants in the genus *Formica* at field sites in eastern California and western Nevada (Herrera, 2003). Similarly, thatch ants of the *Formica* 'rufa' group appeared to be the primary reason for the failure of establishment of *D. carinulata* following open release at the Owhyee River site in southeastern Oregon (T. Dudley, unpub. data). In many cases, insecticide treatments to reduce ant populations were helpful for promoting establishment of *Diorhabda* species. Studies have also indicated that the presence of predators, particularly *Formica* ants, results in the cessation of the emission of the aggregation pheromone by male *D. carinulata* beetles (A. Gaffke, unpub. data). This means populations of *D. carinulata* will not be retained in patches of *Tamarix* with high densities of ants, limiting the biocontrol potential of the agent in these instances.

(4) Regulatory constraints to the *Tamarix* biocontrol program. The regulatory roadblocks to beetle establishment have stemmed largely from a moratorium on permitting of interstate movement of *Diorhabda* species or holding *Diorhabda* species in culture, which included revoking all existing permits (APHIS, 2010). This drastic measure came about as the result of a lawsuit filed by two environmental groups, the Center for Biological Diversity and the Maricopa Audubon Society, with the USDA-APHIS and the U.S. Fish and Wildlife Service as defendants. The issue was movement of beetles into areas where they could affect SWFL nesting success through defoliation of *Tamarix*. As a result, funding was cut for monitoring *Tamarix* and *Diorhabda*, and implementation efforts terminated with the threat of a $250,000 fine should someone be found in violation of the moratorium (APHIS, 2010).

The moratorium has not only limited interstate movement of beetles for new releases but has also eliminated the possibility of research to determine if other *Diorhabda* species would be better for distribution in areas where *D. carinulata* has failed to thrive. As an example, no further work could be done to test southern-adapted *Diorhabda* species in the Arkansas River Basin in Colorado, far from areas of concern where SWFL are known to nest in *Tamarix*. Overall, the moratorium has affected almost all aspects of tamarisk biocontrol and has made it difficult or impossible for biocontrol practitioners to carry out field trials needed to test factors affecting establishment and impact. The initial and follow-up lawsuits have been described elsewhere (Bateman et al., 2010; Dudley and Bean, 2012; Bean and Dudley, 2018), but in summary it is clear that the moratorium has resulted in a breakdown of the biocontrol program at a national level with little or no impact on the natural movement of beetles southward (Dudley et al., 2017).

Regional Patterns in Establishment, Impacts, and Controversies

One of the difficult aspects of summarizing the *Tamarix* biocontrol program is that there were major regional differences in implementation, monitoring, outcomes, and the level of controversy, making general conclusions impractical. We here highlight some of the regional patterns that we feel are instructive from both biological and regulatory perspectives.

The Intermountain West

The implementation of *Tamarix* biocontrol using *D. carinulata* has been most heavily concentrated in the Intermountain West, which includes the Great Basin Desert, the northern Rockies, and the upper

Colorado River Basin. Initial experimental releases were made, and establishment monitored, at three sites in the Great Basin Desert, and these provided collection sites for further widespread distribution, including implementation programs conducted by local and state officials in Utah (Dudley and Bean, 2012) and Colorado (Kennard et al., 2016).

Establishment of *D. carinulata* in Wyoming and Montana required release of more agents than was required at the more southern sites, with 27,000 adults released before establishment was achieved at the Lovell, Wyoming site (DeLoach et al., 2011). Releases of several thousand beetles in 2007 failed to establish along Fort Peck Reservoir, Montana (Deloach et al., 2011). Releases began again in the state of Montana in 2016, using beetles that had naturally dispersed into the state from established populations in Wyoming. New field releases in Montana, especially when made in conjunction with application of the aggregation pheromone, have now resulted in establishment, although population density has remained low (Gaffke et al., 2020). The number of generations of *D. carinulata* is 1–2 per growing season in Montana and Wyoming, limiting their impact. In areas where growing season limits the biocontrol potential, lures utilizing *D. carinulata* aggregation pheromone are being used to purposefully aggregate the beetles and increase their impact (Gaffke et al., 2018, 2019, 2021).

In western Colorado, *Tamarix* plant mortality from *D. carinulata* herbivory exceeded 30% on average, with some monitoring sites reaching 50% and with widespread biomass reduction exceeding 70% (Kennard et al., 2016). While the overall impact of *D. carinulata* is high throughout western Colorado, beetle populations fluctuate with widespread defoliation common during some years but absent in others. Noting the success of *D. carinulata* in suppression of *Tamarix* in western Colorado, riparian ecosystem managers have incorporated biological control into larger-scale plans for invasive species management on the Dolores River as well as the Yampa and Green Rivers within Dinosaur National Monument (Williams, 2016; Bean et al., 2021). Large defoliating populations of *D. carinulata* established in western Colorado but have failed to become well established in the eastern part of the state, despite a massive release program conducted within the Arkansas River Basin (Bean, 2017).

Successful establishment followed open releases in 2001 at three sites, namely the Sevier site in Utah and two sites in western Nevada (Dudley et al., 2001). The Humboldt Sink site in Nevada was the first of the *Tamarix* Biocontrol Program to achieve biocontrol agent establishment, with approximately 1 ha (2.5 acres) of monotypic *T. ramosissima* defoliated in 2002, which expanded to 100 ha (247 acres) in 2003. Beetles continued to disperse across northwestern Nevada within the Humboldt River Basin and into the adjacent Carson Basin, while from a separate release, beetles defoliated several thousand hectares of tamarisk in the adjacent Walker River Basin (Carruthers et al., 2008; Pattison et al., 2011).

In Utah, there was a delay of over two years between *D. carinulata* releases and substantial defoliation within the Sevier River terminal basin (DeLoach et al., 2011). The Sevier site served as a source for beetles distributed throughout Utah (Bateman et al., 2010; Henry et al., 2018). The initial releases at the Sevier site were of *D. carinulata* collected from Chilik, Kazakhstan, whereas the other original release sites all received beetles from Fukang, China (Tracy and Robbins, 2009).

Lower Colorado River Basin

The Washington County Public Works Department in St George, Utah, transferred *D. carinulata* from the Sevier River site to the upper Virgin River in 2006, which is the primary source of beetles moving into the lower Colorado River (Bateman et al., 2010). Outbreak populations developed by 2008, with widespread defoliation and dispersal into other watersheds (Dudley and Brooks, 2011). Subsequent dispersal, facilitated by the evolution of better adapted phenology cues (Bean et al., 2012), led to the incremental expansion of the beetle population over the next four years through the length of the Virgin River watershed to Lake Mead (Bateman et al., 2010; Dudley and Bean, 2012).

While the release at the upper Virgin River was by far the most publicly visible route of biocontrol introduction into the lower Colorado River Basin, it wasn't the only one. Beetles moved down the Colorado River from established sites upstream of Grand Canyon National Park and most likely reached the shores of Lake Mead from that direction. In addition, the genetic signature of beetles in the Virgin River Valley revealed an avenue of introduction through the Muddy River drainage that originates in central Nevada. Since the Chilik ecotype was introduced into the St. George area, the occurrence of the Fukang ecotype near the confluence of the Virgin and Muddy Rivers pointed to a Nevada origin for a portion of the population in the lower Virgin River Valley (Stahlke et al., 2022).

The upper Virgin River was the first location where tamarisk biocontrol overlapped known nesting of the endangered SWFL. The interactions of tamarisk biocontrol and endangered species are covered in more detail elsewhere (Bateman et al., 2010, 2013; Paxton et al., 2011), but the perceived loss of nesting habitat in the St. George area resulted in the 2009 lawsuit filed by the Center for Biological Diversity in an attempt to stop the program and, possibly more productively, to pressure federal agencies to support riparian wildlife habitat restoration (Bean and Dudley, 2018). In contrast, the Virgin River Conservation Partnership, a working group of agencies and stakeholders formed to address resource issues in this watershed (including flood risk, water conservation, sensitive species protection, and reducing the impacts of invasive species [CCDCP, 2000; USACE, 2008]), were enthusiastic about the arrival of *Diorhabda* beetles in the watershed, as were participants in the Clark County Nevada Desert Conservation Program (DCP, 2011). There was a widely publicized instance where *D. carinulata* defoliated a *Tamarix* shrub in which a SWFL had nested, causing overheating of the brood and failure to fledge. On the other hand, SWFL returning to the Virgin River have been shown to switch from *Tamarix* to native plants for use as nesting substrate when presented with the option. This observation, along with the continued loss of flycatcher nesting territories due to fires promoted by *Tamarix* (Finch et al., 2002; Dudley and Brooks, 2011), provides compelling evidence for *Tamarix* suppression and riparian restoration (Dudley et al., 2012, 2017). This ongoing controversy appears to have no resolution in the absence of a concerted effort by both sides to support riparian restoration efforts and the recovery of habitat dominated by native vegetation, which is far superior to the current tamarisk monocultures that characterize some reaches of the Virgin River as well as the Colorado River downstream of Lake Mead (Dudley and Bean, 2012).

Despite the political situation, *Diorhabda* beetles continued to disperse southward, from Lake Mead where beetles traversing the Virgin River watershed appear to have met with those from the mainstem Colorado River (derived from the Virgin River population via tributaries north of the Grand Canyon or from separate releases near Lake Powell). From that location, dispersal was sporadic owing to the disjunct nature of *Tamarix* patches in the reaches below Hoover dam, followed by steady, longer-distance expansion as beetles encountered more extensive stands that facilitated larger populations and greater impacts. By 2018, beetles had colonized National Wildlife Refuges between Needles, California and Yuma, Arizona, and crossed the Colorado River Delta region of northern Mexico in 2019.

Figure 5. Dead tamarisk plants after multiple defoliations from tamarisk beetles. (D. Bean, Colorado Department of Agriculture)

The impact of defoliation on *Tamarix* survival and green biomass has been characterized at more northern sites (**Fig. 5**) (Hultine et al., 2014; Kennard et al., 2016), but it has not yet been quantified in the lower Colorado River Basin, below Lake Mead. Increased impact of defoliation has been documented in faster-growing plants (Hultine et al., 2013), presumably because such plants devote more resources to immediate growth and less to storage of metabolites that would otherwise support recovery from herbivory. In the southern range of *Tamarix*, where the genetic background is predominately *T. chinensis* (which devotes more resources to growth [Williams et al., 2014]), we expect that herbivory will deplete carbohydrate reserves more quickly (Hudgeons et al., 2007), and this loss should result in higher levels of mortality and branch dieback. This appears already to be the case, although quantification is needed.

Texas and New Mexico

The dynamics of *Tamarix* biological control in Texas and New Mexico appeared to differ from other regions in several aspects of implementation and outcome. While biocontrol has only recently been implemented in the region, reduction of tamarisk for water salvage has a long history in arid New Mexico and west Texas. From 1999 to 2005, areawide programs in Texas expended approximately $8.5 million to apply herbicides to about 51,000 acres of *Tamarix* along the Canadian, Colorado, and Pecos Rivers. Water authorities viewed biocontrol as an inexpensive method to reduce *Tamarix* re-invasion into herbicide-treated areas leading to program funding from state soil and water conservation boards, USDA Natural Resources Conservation service (NRCS), and water districts, although water savings can be difficult to quantify (Gregory and Hatler, 2008; Shafroth et al., 2010).

The USDA Agricultural Research Service (ARS) Grassland, Soil and Water Research Lab at Temple, Texas was the lead agency for the biological control program for this region. Texas A&M AgriLife Extension provided educational programing, while Sul Ross University, Oklahoma State University, New Mexico State University, and Texas A&M University assisted in project research and implementation. The annual meeting of the Texas/New Mexico Saltcedar Biological Control Consortium (re-constituted from the original, nationwide SBCC) brought together these partners along with a wide range of stakeholders (regional water districts, U.S. Fish and Wildlife Service, The Nature Conservancy, National Park Service, USDA-NRCS and others) to review program progress, discuss critical issues, and plan activities related to research, implementation and funding (Carruthers et al., 2008).

As discussed earlier, *D. carinulata* failed to establish in Texas and other sites south of the 38th parallel due to mismatched developmental phenology (Lewis et al., 2003; Bean et al., 2007b). Subsequent studies using the three southern-adapted species (Mediterranean tamarisk beetle, *D. elongata*; subtropical tamarisk beetle, *D. sublineata*; and larger tamarisk beetle, *D. carinata*) resulted in better phenological matching with *Tamarix* and seasonality in Texas, with as many as five generations per season recorded in the field (Milbrath et al., 2007; Dalin et al., 2010). In New Mexico, *D. elongata* was released at three sites, but releases were discontinued in 2009 in response to the emerging concerns about potential impact of *Tamarix* defoliation on the nesting success of the endangered SWFL.

In Texas, approximately 1.1 million adults of the three *Diorhabda* species were field-collected and released at 99 sites in west Texas during 2003–2013. All three species established and reached densities sufficient to defoliate large expanses of tamarisk. In the upper Colorado River of north central Texas, *D. elongata* caused areawide *Tamarix* defoliation 3–5 years after establishment (2008–2010). In southwest Texas, *D. sublineata* established on the Rio Grande and Pecos River and within a year of initial release (2009) defoliated extensive stands of *Tamarix* along 32 km (20 mi) of the Rio Grande. Areawide defoliation continued during 2010–2013 with Landsat satellite imagery showing 75–96% reduction in tamarisk canopy cover along 600 km (373 mi) of the Rio Grande (Wenjie et al., 2017).

Beetles from these populations rapidly dispersed westward into southern New Mexico and northern Mexico. In northwestern Texas, *D. carinata* did not achieve areawide defoliation until 4–6 years after initial releases, but by 2012–2014 *Tamarix* stands were defoliated throughout the Colorado and Red River drainages, and beetles dispersed into Oklahoma, Kansas, and New Mexico (Michels et al., 2012; Sanchez-Peña et al., 2016; Knutson et al., 2019).

By 2014, these three species were present in New Mexico, along with *D. carinulata* from the northwest (Knutson et al., 2019). With the dispersal of these species across the Texas/New Mexico region there was the potential for these species to interact, and we found the first field evidence of hybrid phenotypes for *D. carinata*/*D. elongata* and *D. sublineata*/*D. elongata* soon after contact of the parental species (Knutson et al., 2019; Stahlke et al., 2022). These results confirmed the laboratory mating studies suggesting that hybridization would occur among these three species (Bean et al., 2013b), but the implications of hybridization for the biocontrol program remained uncertain (Bitume et al., 2017).

Another concern was the observation of *Diorhabda* spillover onto a non-target host, the congeneric evergreen species known as athel, *T. aphylla*, commonly grown as a shade tree in towns along the Rio Grande in Texas and in northern Mexico. Athel is a suitable but less preferred host relative to the deciduous *Tamarix* spp. for *Diorhabda* species (Milbrath and DeLoach, 2006, 2007; Moran et al., 2009). The original Rio Grande releases were done in consultation with Mexican officials, as beetles were expected to disperse into Mexico, and in 2010, *D. sublineata* defoliated tamarisk along about 135 km (84 mi) of the river. However, the beetles oviposited on athel following defoliation of the preferred tamarisk host (Sanchez-Pena et al., 2016). As has also been noted further west with *D. carinulata* (Dudley et al., 2017), athel defoliation created public concerns that the horticultural trees were dying. These concerns were largely allayed by a combination of public outreach to explain that impacts were minor, and short-lived as beetle populations quickly subsided following their initial irruption, and athel trees re-foliated, often within the same season (Knutson et al., 2019).

Although initial results of *Tamarix* biocontrol in Texas and New Mexico held promise, high densities of beetles sufficient for areawide defoliation persisted for only 3–4 years for each species. Surveys of Texas and Oklahoma in 2016 found only a few, small populations of *D. elongata* and *D. carinata* and no extensive defoliation. Populations of *D. sublineata* persisted on the Rio Grande in Texas westward into New Mexico but had not recovered to densities sufficient for areawide *Tamarix* defoliation (Knutson et al., 2019). Consequently, defoliated *Tamarix* regrew canopy lost to earlier defoliation (Wenjie et al., 2017).

The cause(s) for drastic declines and in some cases extirpation of these three beetle species is unknown and is similar to, but far more dramatic than, the situation in the Intermountain West and Colorado River Basin where *D. carinulata* maintain persistent, albeit reduced, populations with attendant defoliation. The widespread appearance of hybrids followed by the areawide collapse of *Diorhabda* populations led to speculation that loss of fitness in these hybrids could be responsible (Knutson et al., 2019). Studies of the population genetics and hybrid fitness are needed to determine if hybridization played any role in the population collapse of these species. If hybridization can be shown to explain the loss of effective *Diorhabda* populations in this region, the risks of releasing closely related species in future biological control programs would merit careful consideration.

Host suitability has also been related to poor population performance elsewhere, such as avoidance of *T. parviflora* as a food or oviposition host (Dalin et al., 2009; Dudley et al., 2012), but this Mediterranean species of *Tamarix* is uncommon in the Texas/ New Mexico region. Also, generalist predators may have had significant impacts on local *Diorhabda* populations (Knutson et al., 2019), but this factor on its own seems unlikely to be responsible for the widespread, sustained decline observed in regional *Diorhabda* populations. As a result of the loss of defoliating populations across the region, support for the biological control program faded, and without funding, researchers moved on to other issues.

Pacific coastal areas

The California Bioregion does not have the massive tamarisk stands of the desert regions, but both the *T. ramosissima/chinensis* type and the Mediterranean *T. parviflora* are common in many low gradient, lower elevation riparian systems throughout the region (Dudley and Collins, 1995). Tamarisk is largely absent in the Pacific Northwest except in the arid interior (Columbia River system including the Snake and Owhyee Rivers). Hence, concerns about its invasive impacts are less than in other parts of the West, but it is still considered a high priority invasive plant for removal across the region, particularly because of the increased risk of wildfire and its low value as wildlife habitat (Lovich, 2000).

Caged release of *D. carinulata* at two of the region's original research areas (San Antonio Creek on the Ft. Hunter-Liggett Army Base in central California and in the Cache Creek watershed in the North Coast Range) did poorly in part because the target was the less preferred *T. parviflora* (Dudley et al., 2012). The Mediterranean tamarisk beetle, *D. elongata*, was used instead at Cache Creek and established following repeated inundative releases (Herr et al., 2014). Since then, populations have remained low with little long-term impact. Exhaustive attempts by the California Department of Food & Agriculture (CDFA) to re-distribute this beetle species more broadly in central and northern California where most tamarisk populations are also *T. parviflora* failed (Pratt et al., 2019).

After a several-year hiatus brought on by the legal constraints associated with perceived risks to listed bird species, many stakeholders across the state are again requesting broader implementation of *Tamarix* biocontrol. The CDFA attempted to secure from APHIS permits for importing another species of *Diorhabda*, *D. sublineata*, from Texas because of its broader host range among *Tamarix* species, including *T. parviflora*, but thus far approval has been denied. The California Alliance for Tamarisk Biocontrol (CATB) was formed with support from the state Department of Pesticide Regulation on the basis that biocontrol of pest plants can reduce the use of herbicides. The CATB introduced *D. carinulata* collected within the state (Colorado River) into 24 tamarisk-infested sites on private and public lands, excluding federal lands; many failed for a variety of reasons, including *T. parviflora* being the more common invasive form in most areas (Norelli, 2017). The CATB was, however, successful in promoting *D. carinulata* establishment in the southern California desert at the Mojave River where the Mojave Resource Conservation District and Quail United have stopped using conventional herbicide treatments against the weedy tree. Introductions were also successful at the Salton Sea where a release requested by the Imperial State Wildlife Area has expanded to much of the surrounding area, including the Imperial Valley Water District where biocontrol implementation had been requested for many years, and where tamarisk is implicated in frequent wildfire.

Ecosystem Responses to *Tamarix* Biocontrol and Management Implications

Target impacts

Herbivory by *D. carinulata* is shown in several areas to reduce *Tamarix* green biomass by more than 50% (Hultine et al., 2014; Kennard et al., 2016). Plant mortality is also site-dependent, but it may also exceed 50% at some locations (Kennard et al., 2016) and reached over 70% at one of the original experimental sites (Dudley and Bean, 2012). This level of impact is sufficient for resource managers to incorporate *Tamarix* biocontrol into long-term management plans.

In western Colorado, the Dolores River riparian corridor (infested with *Tamarix* and other invasive plants) was targeted for control by a coalition of stakeholders through the Dolores River Restoration Partnership (DRRPartnership.org), an organization dedicated to restoring native vegetation and improving habit for native fish and other wildlife. A major management component within the restoration program has been the suppression of *Tamarix* with *D. carinulata*, present there since 2006. In this area, *Tamarix* has steadily declined, both where biocontrol was used alone and where biocontrol was combined with other methods (Sher et al., 2018). The dam-regulated Green River, with headwaters in Wyoming, and the largely unregulated Yampa River, with headwaters in Colorado, join within Dinosaur National Monument. Long-term invasive species management and restoration of the river systems includes intensive mechanical removal of *Tamarix* at strategic locations, including high-use riverside camping areas, combined with biological control of the invasive shrub along most of the remote invaded reaches of the two rivers (Williams, 2016; Bean et al., 2021).

It appears that *Tamarix* does not respond as well to disturbance once *Diorhabda* are established within the system, which is important since the river systems of western North America are dynamic, with intermittent flooding, which brings about scouring or sediment deposition, reshaping channels and altering riparian vegetation (Hultine and Bush, 2011). A recent study showed that, following flooding on the Virgin River, native plants reestablished more readily than *Tamarix* with *D. carinulata* present in the system (González et al., 2020a), which was likely the result of decreased competitive pressure from *Tamarix*. In Colorado, it has been noted that *Tamarix* flowering is diminished in the years following defoliation by *D. carinulata*. This could have an impact on post-flood vegetation profiles if there are native plants that can serve as seed sources in the system (Dudley and Bean, 2012). Long-term studies indicate that evaluation of *Tamarix* control, and biocontrol in particular, takes longer than a few seasons (González et al., 2020b). In one study, tamarisk decline was associated with an increase in Coyote willow, *Salix exigua*, a desired species, without increases in undesired noxious weeds (González et al., 2020b). In addition to flooding, fire is a major component of riparian disturbance, and *Tamarix* that has experienced beetle herbivory is less likely to recover from fire (Drus et al., 2014).

The first widespread *Tamarix* biocontrol success was in the Humboldt Sink, in Pershing County, Nevada, as noted earlier. Success of the program led the Pershing County Water Conservation District to suspend their use of herbicides for tamarisk reduction in favor of the more benign biological method, and the beneficial role of biocontrol was noted in a federal EIS concerning transfer of the water management program to local control (USBOR, 2005). In the same system, water savings from reduced evapotranspiration were estimated to be roughly 3.1 million m^3 (2,500 acre-ft) during the first year (Pattison et al., 2010).

South of the Humboldt site, on the western edge of the Great Basin, another cage-trial site was established in the lower Owens Valley where the Inyo County Water District, Los Angeles Department of Water & Power, and local conservationists had been conducting tamarisk removal for decades (ICWD, 2022). Although that initial trial had proven to be unsuccessful, additional releases were made in 2017 at other Owens Valley locations, supported by a grant from the California Department of Pesticide Regulation, and these releases resulted in beetle establishment in several areas associated with the Owens River and Lake, such that resource managers plan to reduce expenses directed to conventional weed control measures in favor of biological control (LADWP, 2019).

Herbivore-plant system

Safety, measured as host specificity, was a primary concern from the outset of the program. Twenty years after agent's field release in North America, there have been no recorded instances of feeding by *Diorhabda* beetles on plants outside of the genus *Tamarix*. As mentioned previously, there has been some feeding by *D. sublineata* and *D. carinulata* on *T. aphylla* (athel), an exotic evergreen *Tamarix* species used for shade at more southern locations. However, this feeding occurred in locations where the local beetle density on deciduous *Tamarix* species nearby was high, from where beetles moved onto athel. Beetles on athel have tended to decline in subsequent years (Moran et al., 2009; Estrada-Muñoz and Sánchez-Peña, 2014; Knutson et al., 2019).

In laboratory settings, some feeding by beetles was recorded on the distantly related alkali heath, *Frankenia salina* (Herr et al., 2009). In the field, however, beetles avoid *F. salina* (Dudley and Kazmer, 2005) in keeping with an emerging pattern in which biocontrol agents appear to be less host-specific under laboratory conditions than in the field (Hinz et al., 2014). Another instance of high beetle host specificity is the poor performance by *D. carinulata* on *T. parviflora* under field conditions (Dudley et al., 2012). While beetles will feed on this species of tamarisk, they rarely reach defoliating densities and generally avoid oviposition on *T. parviflora* if other targets are available. This was unexpected since *D. carinulata* readily feed and oviposit on *T. parviflora* in laboratory settings, and *T. parviflora* was routinely used as a host plant for rearing *D. carinulata* (Bean et al., 2007a).

Wildlife responses and habitat restoration

Amphibians, reptiles, and birds are consumers in riparian food webs and can provide a tool to evaluate how biocontrol may lead to ecosystem-level changes. Extensive defoliation and dieback of *Tamarix* from *Diorhabda* herbivory has clearly resulted in altered conditions, which is unsurprising given the extent of *Tamarix* dominance in many systems. Ecosystem responses were not only anticipated, they were the desired result of *Tamarix* biocontrol via facilitation of riparian recovery. In some cases, undesired responses may follow the rapid defoliation of large stands of *Tamarix*. For instance, there was a general decline of the herpetofauna in defoliated areas along the Virgin River due to habitat (Bateman et al., 2015). However, defoliation did result in increases by some taxa that respond positively to a more open canopy. Paxton et al. (2011) suggested that loss of tamarisk foliar cover may increase nest predation or abandonment by birds such as the SWFL, and increased temperatures in defoliated trees may exceed critical limits of developing eggs. Although undesirable, such effects could be expected in early stages of the program during the period required to restore ecosystems dominated by native plant assemblages (Mahoney et al., 2022).

In addition to altering habitat, tamarisk biocontrol can affect food resources available to wildlife. When abundant, *Diorhabda* larvae and adults can provide food for rodents, lizards, and birds (Bateman et al., 2013; Longland and Dudley, 2008), and Mexican free-tailed bats (*Tadarida brasiliensis*) have been observed capturing adult beetles during mating aggregations (T. Dudley, unpub. data). Some studies suggest that other herbivores on *Tamarix* (e.g., tamarisk weevils and *Opsius* leafhoppers) may be preferred over *Diorhabda* by migratory warblers, raising a concern that loss of *Tamarix* could mean the loss of these other insects as food resources (Paxton et al., 2009). When flowering, *Tamarix* is attractive to generalist nectar-feeding insects, and while flowering is ephemeral, these insects also provide useful food resources to wildlife (Cohan et al., 1978) that could be diminished by biocontrol. Changes in the invertebrate assemblage owing to biocontrol needs to be better documented. However, native riparian trees, in systems where they are still intact, are more valuable than *Tamarix* for supporting insectivorous species such that wildlife stand to benefit from their restoration (Shafroth et al., 2005; Strudley and Dalin, 2013). Ultimately, *Tamarix* may even have a beneficial role in systems where it is suppressed but remains a significant component of vegetation structure (Sogge et al., 2008).

In this context, the SWFL was listed as endangered by the US Fish and Wildlife Service about the same time (1996) as *Diorhabda* was approved by APHIS for field release, and in some regions, SWFL will use *Tamarix* as a nesting substrate owing to its suitable branching structure (Sogge et al., 2008). The potential for biocontrol to come into conflict with flycatcher management was known and extensively discussed well before any field releases (DeLoach and Tracy, 1996; Stenquist, 2000). It was anticipated that given the physiological limitations imposed by mis-timed entrance into diapause by the northern tamarisk beetle, there would be time for active restoration in key areas where flycatchers were known to nest in *Tamarix*. The speed with which *D. carinulata* evolved and became more capable of southward movement was unanticipated (Bean et al., 2012), but it was assumed that natural selection would eventually enable southward colonization (Bean et al., 2007b), necessitating active restoration in some key areas. The important concern is whether financial resources, resolve, and programmatic leadership can facilitate restoration of SWFL habitat in a timely fashion. Nonetheless it appears that negative impacts of biocontrol on the flycatcher may be modest overall (see York et al., 2011) and overshadowed by the impacts of climate change, diminished stream flows, increased fire frequency, and invasive species more broadly (including *Tamarix*), all of which have diminished habitat quality for the bird and yet can potentially be mitigated by ecological restoration.

Given these challenges to riparian ecosystems and associated wildlife, and the limited success of past efforts to restore riparian habitat following tamarisk reduction (González et al., 2020b), there is need for a strategic approach to riparian habitat restoration in response to tamarisk biocontrol that incorporates the complex physical and biological interactions influencing riparian recovery (Shafroth et al., 2008; Orr et al., 2017a). A restoration strategy should comprise evaluation of major stressors in river systems, including factors associated with *Tamarix* invasion and unintended effects of its suppression, to enable conservation or restoration of natural processes where feasible. Restoration should leverage existing biotic elements such that active intervention can be strategically targeted where needed to jump-start natural recovery processes or to steer the ecosystem towards a more desirable trajectory (Beechie et al., 2010, Downs et al., 2011; Palmer et al., 2014; Johnson et al, 2020).

Implementing riparian restoration to maintain or enhance habitat for wildlife, particularly SWFL, in conjunction with tamarisk biocontrol, should thus incorporate how biocontrol itself affects tamarisk and associated riparian habitat, including variation in these effects across regions and the multiple objectives of riparian managers in different ecological contexts. 'Ecohydrological Assessment' provides an effective framework for addressing such issues in strategic riparian restoration planning and implementation (**Fig. 6**). It incorporates evaluation of the biophysical drivers, at multiple scales, that affect the restoration potential of the targeted area, enabling the identification and development of appropriate restoration strategies and targeted actions most likely to be successful under current and anticipated future conditions (Orr et al., 2017a,b).

For example, in some situations such as the lower Virgin River, biocontrol has led to substantial mortality of *Tamarix*. In other cases, mortality has been much more limited (Bean et al., 2013a; Dudley et al., 2017) resulting in varying amounts of living and dead standing tamarisk biomass. Such variable conditions can affect the ability of desirable native and undesirable nonnative plants to establish and thrive on sites subject to biocontrol. Some rivers (e.g., portions of the upper Gila River in Arizona and New Mexico) retain a substantial component of native riparian vegetation, while others are dominated by tamarisk monocultures where the scarcity of native trees limits the potential for natural recruitment and recovery (e.g., the lower Virgin River in Nevada). In the latter case, an ecohydrological assessment would incorporate existing hydrologic processes coupled with strategically located patches of active restoration to guide creation of 'propagule islands' of native plants, such as cottonwoods and willows, that can provide propagules to recolonize areas opened up by biocontrol and substrate conditions (Dudley and Bean, 2012; Orr et al., 2017a). Where patches of native vegetation remain, limited active restoration could enhance the supply of native propagules and increase the value of the native stands as refuges of habitat for species such as SWFL (Orr et al., 2017a,b), a strategy that allows managers to focus resources on those sites with the highest potential for success and the highest conservation or biodiversity value. Thus, the ecohydrological framework facilitates application of triage in developing restoration priorities, with lesser attention to systems with diminished flows and depleted shallow groundwaters where benefits are less likely to accrue.

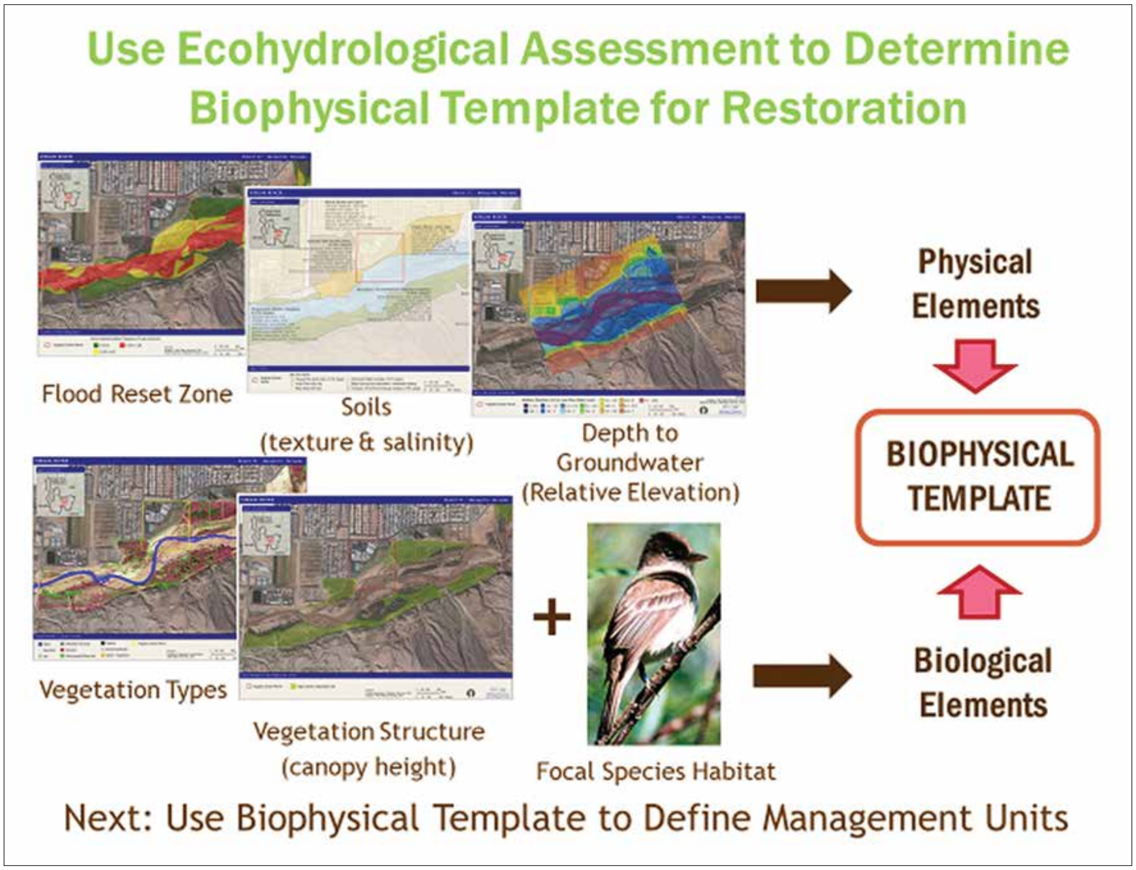

Figure 6. Diagram of an ecohydrological framework to assess the key physical and biological drivers used to develop a management plan for restoration. Physical elements such as hydrology, water availability, geomorphology, and flood pathways, and biological elements such as wildlife presence, habitat condition, and proximity to habitat, can be used to maximize likelihood of successful restoration.

WHAT COULD HAVE BEEN DONE TO IMPROVE THE PROJECT?

The potential for success of biological control as a means of suppressing *Tamarix* is evident on a large scale, and biocontrol has been incorporated into riparian management and restoration planning by resource managers. However, there were numerous missed opportunities to improve project outcomes and perceptions, as well as major caveats regarding the many landscapes (e.g., in Texas) where *Tamarix* biocontrol was ephemeral and ineffective over the long term. Likewise, basic and applied questions remain regarding the biology and ecology of the system that a fuller understanding of the mechanisms underpinning these complex interactions could have resolved. For instance: Why do *Diorhabda* populations fluctuate so dramatically and what impact does this have on programmatic success? What factors led to areawide population collapse of *Diorhabda* species and subsequent failure to recover that has left large *Tamarix* stands to partially recover? What is the management significance of hybrid phenotypes as observed in the Texas/New Mexico program? Other questions concern the variability in the decline of *Tamarix* (and sometimes recovery) and how resource managers can best incorporate biocontrol into large-scale riparian recovery programs. Research questions could evaluate the impact of *Tamarix* decline on wildfire dynamics or the impact of climate change on biocontrol efficacy and ecosystem recovery.

Following the APHIS moratorium, it has become increasingly difficult to secure resources necessary for riparian restoration and the basic research needed to support restoration efforts. The project has also been deeply harmed by negative perceptions, despite lack of substantiating data to validate those perceptions. There was a flood of negative press surrounding *Tamarix* biocontrol that reached a peak following the APHIS moratorium of 2010 (Dudley and Bean, 2012). While it is easy to dismiss it as colorful and inconsequential, the stakeholders who benefit from biological control, and whose support we need, were confused by the negative information in the press and questioned the value and direction of the project. Evaluation of the program has been haphazard and often done without input from biocontrol practitioners. For instance, the program is frequently evaluated from a perspective in which the problem is *Diorhabda* and not *Tamarix* (United States District Court, 2017). To address these problems and advocate for *Tamarix* biocontrol, it would be useful to bring back an updated coalition composed of stakeholders, scientists, and agencies, similar to the disbanded SBCC.

The magnitude and impact of the *Tamarix* invasion and the importance of riparian ecosystems to biodiversity in western North America make this a high-value, critical project. Results, either success or failure, will have long-lasting ecological consequences, particularly on the backdrop of climate change and other anthropogenic impacts that threaten the region. The new version of the SBCC would assist in obtaining long-term resources to support research and restoration, something required for settling the above lawsuits but thus far has been unmet. They would also help organize and coordinate monitoring efforts and provide accurate information to the press. Annual meetings could provide a place to present and discuss new findings and new technologies, as well as to articulate goals and outcomes to the public through press releases, videos, and informational brochures. The new SBCC could also help make available new technologies, such as semiochemicals for manipulation of *Diorhabda* populations to temporarily protect nesting birds (Gaffke et al., 2018, 2021). Although we believe these measures should have been in place even after the 2010 moratorium, we still have the chance to improve program outcome. In particular, the Ecohydrological Assessment approach to strategic habitat restoration has been recommended to USDA-APHIS for addressing the court-ordered mandate to facilitate restoration of habitat for listed species (Bean and Dudley, 2018), but as yet it has not been implemented.

A further important way to improve the North American program will be more active participation with the international effort to control invasive *Tamarix*. The search for and development of additional agents to fill regional needs should continue, and ideally would be part of an ongoing effort, linked with similar efforts on other continents where *Tamarix* is invasive (McKay et al., 2018; Marlin et al., 2019). A network of overseas cooperators should be supported in their efforts to locate and collect agents, conduct testing as feasible in the source countries to enhance ecological realism (Schaffner et al., 2018), and enable the development and implementation of agents complementary to *Diorhabda*, as has been shown to improve efficacy of weed biocontrol in other projects (Denoth et al., 2002). These agents could include *T. mannipara*, a mealybug previously cleared through the Technical Advisory Group for Biological Control Agents of Weeds in North America, and other agents already known to be *Tamarix*-specific. For instance, overseas cooperators have identified and tested organisms that could be used to affect other plant parts, such as the stem-galling midge *Psectrosema* spp. (Cecidomyiidae), a defoliating moth *Agdistis tamaricis* (Pterophoridae), and several other *Tamarix* specialists (Sohbian et al., 1998).

The future success of the *Tamarix* biocontrol program will be marked not simply by reducing *Tamarix* abundances to tolerable levels. It will be considered a success when *Tamarix*, even if still present, is a subordinate element in riparian ecosystems with a functional role in supporting a diverse trophic assemblage of consumers and associated wildlife, as it does in its native range.

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Chapter 29

Successful Melaleuca Biological Control in the Florida Everglades

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NON-TECHNICAL SUMMARY

Non-native plant invasions are often the result of intentional introductions through the horticulture trade. Beginning in the early 1800s, extensive effort was made to explore the world in search of plants for use in ornamental horticulture. *Melaleuca quinquenervia* (Myrtaceae) is one such plant that was brought into south Florida beginning in 1886 for use as a landscape tree.

During the next fifty years (1905–1955), *M. quinquenervia* (or melaleuca) was used to reforest edges of swamps where cypress and pine had been removed by settlers, planted extensively in urban settings, used to stabilize dikes for large U.S. Army Corps of Engineers projects, and seeded from planes in an attempt to make Florida wetlands more hospitable for development. Gradually, however, the landscape melaleuca was invading, especially in the Everglades which became an icon for imperiled North American ecosystems. Soon thereafter in the 1960s, Florida and the federal government began large-scale efforts to preserve this unique subtropical wetland. A major cause of the degradation of the Everglades was the invasion of plant species, especially melaleuca, but also others such as Brazilian pepper tree (*Schinus terebinthifolia*), Old World climbing fern (*Lygodium microphyllum*), and air potato (*Dioscorea bulbifera*).

Over the past forty years or so (1980–2022), extensive efforts to introduce biological control agents against melaleuca (to reduce further expansion and prevent regrowth by limiting reproduction) and to integrate those agents with mechanical and chemical removal reduced the size of the melaleuca-dominated area from 400,000 ha (988,000 acres) to less than 100,000 ha (247,000 acres). Melaleuca control is currently in a maintenance mode in south Florida. Fire appears to spur new seedling recruitment events, but most large, mature stands are dwindling or have been treated and have not returned due to the ability of biological control agents to drastically reduce recruitment of new seedlings.

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HISTORY OF INVASION

Melaleuca quinquenervia (Myrtaceae) is an evergreen subtropical tree from Queensland and New South Wales, Australia (Blake, 1968). In Australia, *M. quinquenervia* (hereafter melaleuca) grows in wet swampy environments adjacent to various species of gum (*Eucalyptus*) and acacia (*Acacia*) trees. Melaleuca is emblematic of the horticultural expansion in Florida during the late 1800s through the early 1900s, when plant collectors such as Dr. David Fairchild in Miami and the Reasoner Brothers of Royal Palm Nursery in Manatee County began importing new taxa that could survive in Florida's subtropical climate (Dray et al., 2006). Several initial introductions and subsequent dispersal efforts using melaleuca seeds and seedlings took place throughout the early 1900s, including by the U.S. Department of Agriculture (USDA), the U.S. Army Corps of Engineers, the Koreshan Unity (a utopian society in southwest Florida), and several nurseries (Dray et al., 2006).

While the initial introductions were made in central Florida, the introductions along the east and west coasts of southern Florida appear to be the source of the invasion and where the bulk of the impacts were later felt. By the 1920s, twenty years after the USDA imported melaleuca into the Miami test station, it had escaped from cultivation (Dray et al., 2006). Melaleuca is now widespread throughout the Greater Everglades ecosystem and affects important conservation areas such as Everglades National Park, Big Cypress National Preserve, and Picayune Strand State Forest, which collectively support a unique subtropical/temperate flora and fauna (**Fig. 1**).

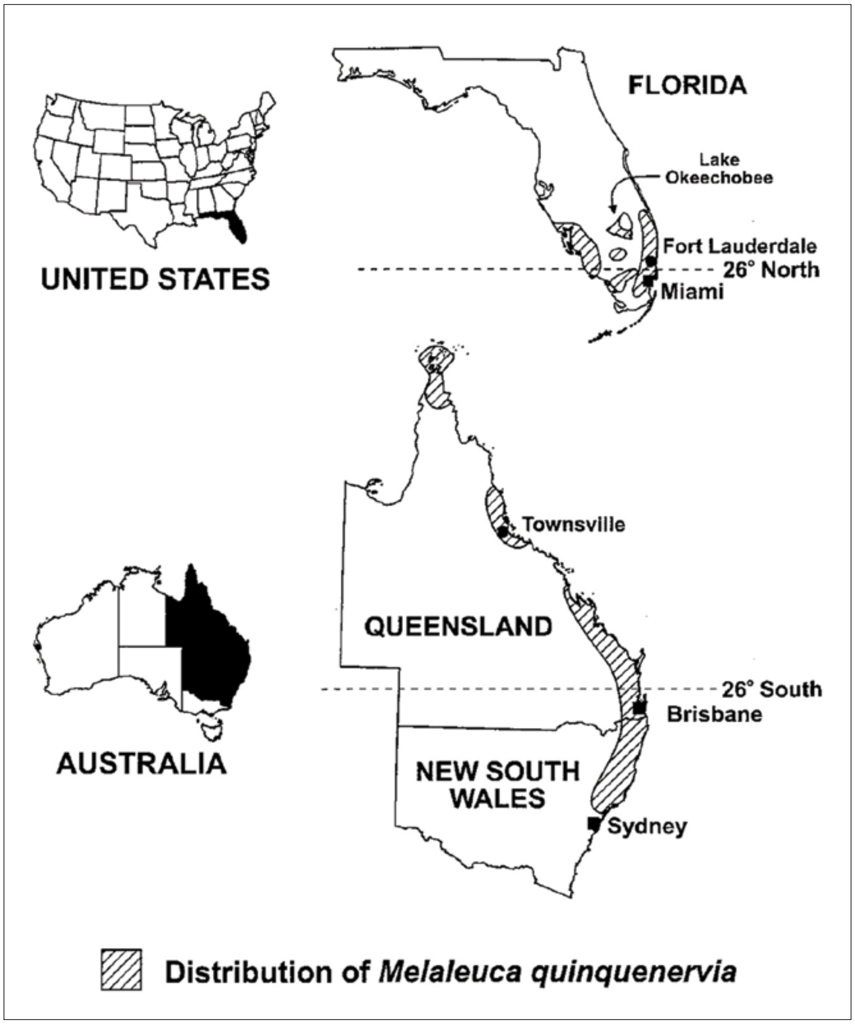

Figure 1. Distribution of melaleuca, *Melaleuca quinquenervia*, in Florida and Australia. (reproduced with permission from Turner et al., 1997)

THE NATURE AND ECOLOGY OF THE PROBLEM

Melaleuca's impact may be limited geographically to southern Florida and several nearby islands (e.g., Bahamas, Cuba, Puerto Rico), but its invasion has important ecological consequences for south Florida's natural ecosystems and human inhabitants. Melaleuca outcompetes and displaces native vegetation, alters fire frequency and severity, and changes the hydrology within the Greater Everglades ecosystem (Martin et al., 2011). By reducing water flow through increased evapotranspiration and loss of any intercepted rainfall, melaleuca effectively reduces the laminar flow over the coral cap rock that is fundamental to the physical basis of the Everglades (Lodge, 2016). Melaleuca reduces both the available surface water and the inputs into the region aquifer that supplies water to the growing human population of south Florida (Chin, 1998).

Fire, water, climate, and topography are the chief natural environmental factors that determine the ecology and structure of the Everglades ecosystem (Lockwood et al., 2003). Based on char in the soil record, fire historically occurred in the region every 2–3 years in some habitats and every 10–15 in others (Lockwood et al., 2003). But the advent of major hydrological diversion projects changed the spatial structure, timing, and intensity of fires in the southern tip of the Floridian peninsula. The expansion of melaleuca and its conversion of grass and sedge marshes to swamp forests further changed the fire dynamics and brought more severe fires closer to the wildland-urban interface. Restoration of the Everglades was initially focused on "Getting the Water Right," but that focus quickly widened to embrace restoration of both the fire and water regimes, as well as efforts to undo the large-scale plant invasions that affect both factors (Sklar et al., 2005).

WHY CONTROL THIS INVASIVE SPECIES?

The Greater Everglades ecosystem extends from the Kissimmee River south to Florida Bay, and it encompasses most of the east-west expanse from the Atlantic Ocean to the Gulf of Mexico (Ogden et al., 2005). By the 1970s, melaleuca had become a severely ecologically damaging species in the Everglades. The same ecological features that produced the largest graminoid-dominated wetland in the world (fire, water, and temperature) made it particularly vulnerable to the melaleuca invasion. Melaleuca is fire-adapted and thus does well in the fire-prone Everglades; mature trees resprout after fires, and millions of seeds are released that quickly germinate in the nutrient-infused post-fire conditions (Conde et al., 1981; Turner et al., 1997). Increased fire frequency and severity create a positive feedback loop in which melaleuca germinates and grows faster and quickly suppresses shade-intolerant native species (Laroche and Ferriter, 1992).

While it is a common misconception that melaleuca was introduced to 'dry up the Everglades,' the plant has indeed had large impacts on the hydrology of the invaded wetlands, resulting in a net loss of 0.2–0.6 million ha (0.5–1.5 million acres) of wetland habitat (Bodle et al., 1994). These newly formed forested areas exclude native understory vegetation and wildlife, including migratory and wading birds such as the endangered wood stork (*Mycteria americana*) (Catling, 2005).

In addition to the well documented ecological impacts of melaleuca, its invasion incurred millions of dollars in control costs and lost economic revenue. The South Florida Water Management District alone spent $13 million ($22 million in 2022 dollars) between 1991 and 1998 attempting to control melaleuca (Serbesoff-King, 2003). Diamond et al. (1991) estimated that economic losses due to melaleuca were nearly $168 million (fire control costs and lost tourism revenue). Balciunas and Center (1991) extrapolated the costs associated with melaleuca invasions to 2010 and estimated that melaleuca would cost up to $2 billion annually if left uncontrolled.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

In January 1990, as a response to the growing call for Everglades restoration, the Melaleuca Task Force was formed to guide efforts to control melaleuca (Laroche, 1994). In a shift from previous control efforts that relied solely on chemical and mechanical methods, the Melaleuca Task Force added biological control as a key part of the control strategy. Foreign surveys for natural enemies of melaleuca in Australia were initiated in 1986 (Laroche, 1994). Over three decades of research (1986–2022) culminated in the establishment of three insects: *Oxyops vitiosa* (Coleoptera: Curculionidae), *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae), and *Lophodiplosis trifida* (Diptera: Cecidomyiidae). A fourth insect, *Fergusonina turneri* (Diptera: Fergusoninadae) and its obligate nematode *Fergusobia quinquenerviae* (Tylenchida: Neotylenchidae) were also approved for release in 2002, but they failed to establish after several release attempts (Pratt et al., 2013). A fifth species, *Lophodiplosis indentata*, was approved for release in early 2022 and is planned for release in the fall of 2022 (Center et al., 2012; Smith et al., 2020). In addition to these releases, the sawfly *Lophyrotoma zonalis* (Hymenoptera: Pergidae) was also evaluated for host-specificity and impact, but it was ultimately dropped from further consideration due to concerns over the mammalian toxicity of sequestered plant poisons found in the larvae (Buckingham, 2001; Oelrichs et al., 2001). The melaleuca project illustrates several innovative approaches to biological control of weeds, including ranking of agents based on mode of attack and how well these insects could be integrated with other management tools (e.g., chemical, mechanical, and biological) (Tipping et al., 2018). While some biological control projects successfully suppress their target weeds solely through the agents' direct and indirect effects (e.g., projects against air potato, *Dioscorea bulbifera*, and golden wattle, *Acacia longifolia*), many others, including melaleuca, require the use of additional methods of suppression to achieve satisfactory control.

By the 1980s, surveys for herbivores and pathogens that attack *M. quinquenervia* were well underway in Australia, and these surveys culminated in the release of the melaleuca snout weevil, *O. vitiosa*, in 1997 (**Fig. 2**). This weevil quickly established at several release sites. Later, several factors were found to influence establishment and impact of this weevil (Center et al., 2000; Balentine et al., 2009). Seasonal changes in foliage growth and hydroperiod appear to have the largest influence on whether *O. vitiosa* would establish at a site (Center et al., 2000). New flushing foliage provides high quality oviposition locations and food for developing larvae and adults. However, Purcell and Balciunas (1994) observed that *O. vitiosa* requires dry soil for its pupation. Immediately after its release, *O. vitiosa* began to have a measurable impact (Pratt et al., 2005). While *O. vitiosa* is arguably the most effective of the biological control agents released against melaleuca, its inability to establish in persistently wet areas created a need for additional agents that could persist under such conditions. Foreign exploration for agents resulted in the discovery of biological control agents that do not require soil

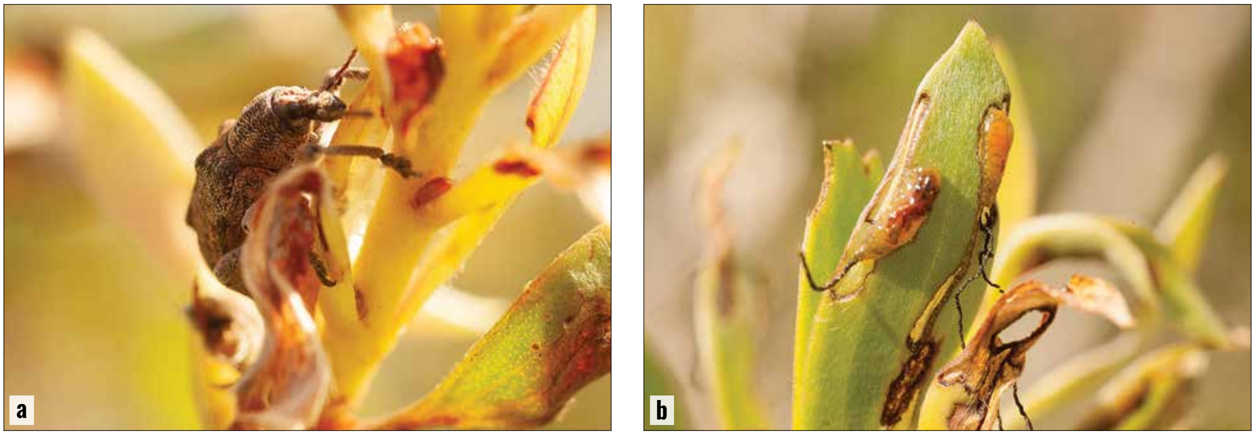

Figure 2. (a) An adult and (b) larvae of *Oxyops vitiosa* feeding on new melaleuca foliage. (M. C. Smith, USDA-ARS)

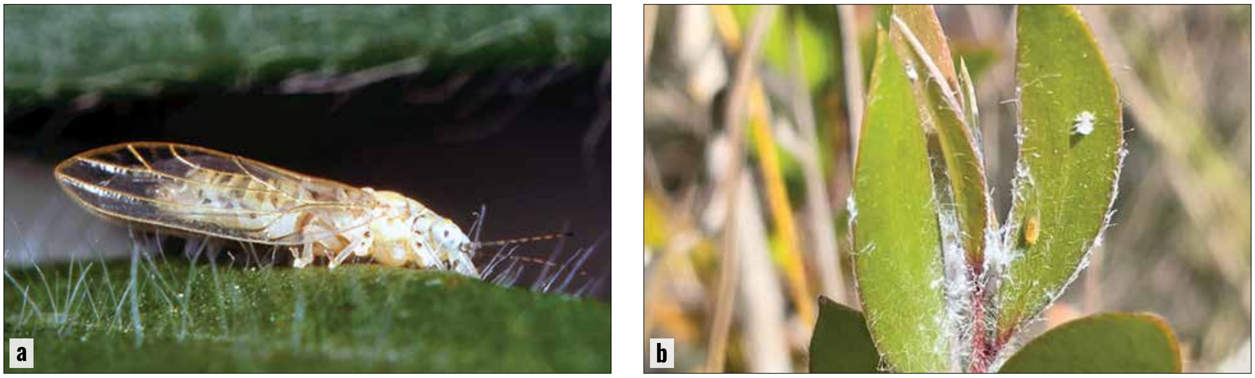

Figure 3. (a) Adult melaleuca psyllid, *Boreioglycapsis melaleucae*, and (b) adult psyllid with nymphal flocculence on a melaleuca leaf. (a: S. Wineriter-Wright, USDA-ARS; b: M. C. Smith, USDA-ARS)

for pupation, primarily the melaleuca psyllid, *B. melaleucae* (**Fig. 3**), and the melaleuca tip-galling midge, *L. trifida* (**Fig. 4a**). These agents were released in 2008 and 2012, respectively. *Lophodiplosis indentata* was issued a release permit in April 2022, and releases were anticipated to begin shortly thereafter. Both *L. trifida* and *L. indentata* are gall midges, with *L. trifida* galling buds (**Fig. 4b,c**) and *L. indentata* attacking leaves (**Fig. 4d**). Several studies in Australia showed that these species have similar impacts on saplings and seedlings, but they do not seem to compete for resources (Kumaran et al., unpub. data). The impact of *L. indentata* on mature trees may be greater than *L. trifida* due to the greater area of leaves available for attack (M. Purcell, pers comm.).

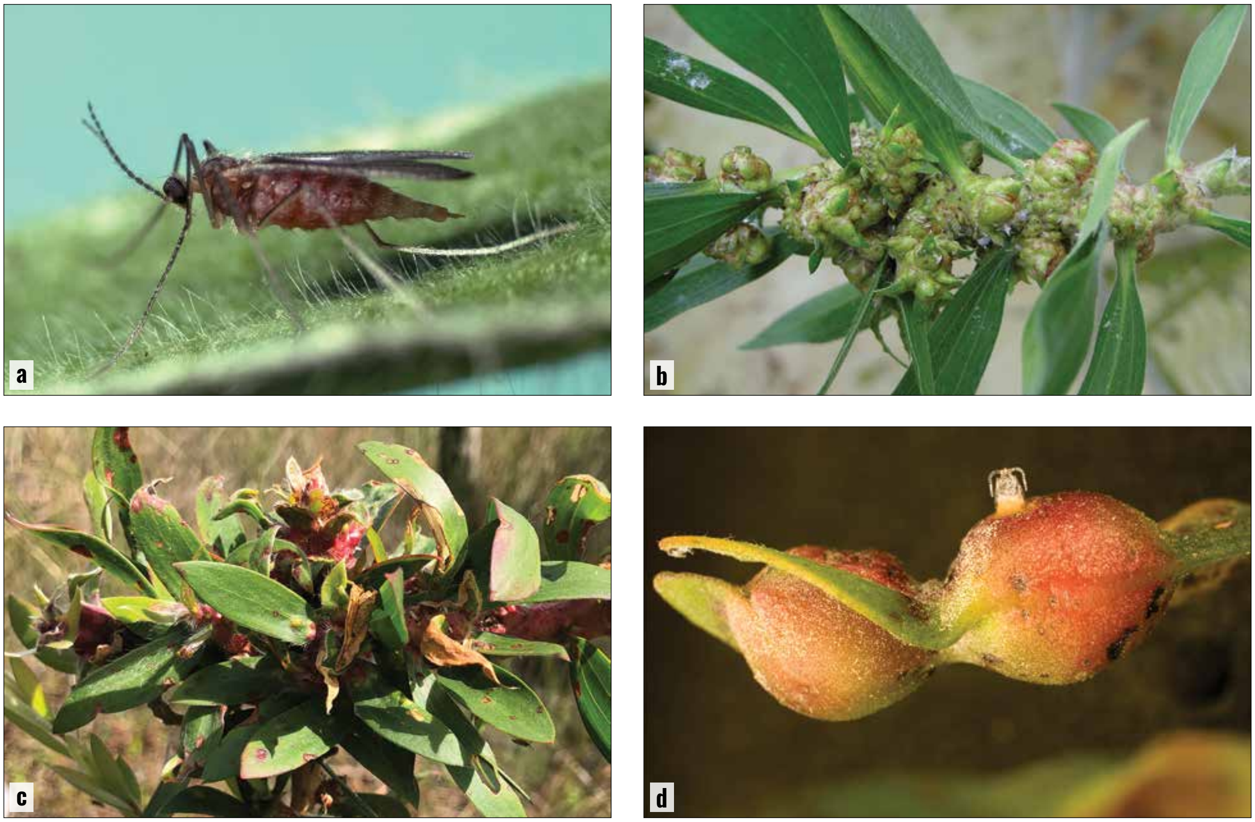

Figure 4. (a) Adult *Lophodiplosis trifida*. Females lay eggs and larvae grow in and emerge from (b) galls that become woody and cease to grow; (c) heavy *L. trifida* galling on melaleuca seedling in Big Cypress National Preserve; (d) an adult *Lophodiplosis indentata* emerges from a leaf pea-gall formed as a response to larval feeding. (a: S. Wineriter-Wright; b: M. Purcell, USDA-ARS Australian Biological Control Laboratory; c: M. C. Smith; d: P. Clark; a,c,d: USDA-ARS)

HOW WELL DID THE INTEGRATED CONTROL PROGRAM WORK?

Measurable impacts of the biological control agents individually and in combination are well documented experimentally (Pratt et al., 2005; Franks et al., 2006; Center et al., 2012; Tipping et al., 2018). *Oxyops vitiosa* attacks the seed-producing plant parts and the new growth, while *B. melaleucae* causes considerable senescence of both young and mature leaves (Franks et al., 2006). *Lophodiplosis trifida* debilitates mature trees, saplings, and seedlings by re-directing resources away from tree growth and reproduction into galls, resulting in plants with 10% less biomass, 10% shorter stature, and 40% lower leaf biomass (Tipping et al., 2016).

Turner et al. (1997) recognized early that large areas of mature trees were unlikely to be suppressed by any one method (chemical, mechanical or biological) given that the biological control agents affected tree reproduction, seedling survival, and growth while chemical and mechanical controls removed large stems effectively but had no effect on plant reproduction. Therefore, a coordinated effort to implement a program of integrated measures began in 1990 with the writing of the Melaleuca Management Plan (Thayer, 1990). The Florida Exotic Pest Plant Council (now the Florida Invasive Species Council) convened various stakeholders to create the Melaleuca Task Force (federal, state, local land managers, scientists, and others) with the goal of developing a comprehensive long-term plan for reducing and managing melaleuca in South Florida (Thayer, 1990). In 2001, The Areawide Management and Evaluation of Melaleuca (TAME Melaleuca) Project was formed and produced a handbook for integrative control of melaleuca utilizing chemical, mechanical, and biological control (Scoles et al., 2006).

The Melaleuca Task Force's objectives, as outlined in 1990, stated that controlling melaleuca would require the full complement of control tactics, including biological control, herbicides, flooding, fire, and mechanical removal (Laroche, 1994). Following this recommendation, the South Florida Water Management District (SFWMD) initiated an aggressive removal campaign focused on the Everglades Water Conservation Areas (WCA) and the Lake Okeechobee marsh. Within seven years, many of the large trees had been removed from several of the WCAs, and the sites were under maintenance control (Laroche, 1994). Manual removal alone, however, would be inadequate to control melaleuca and would need to be followed by herbicide treatment or biological control (Stocker, 1999). The melaleuca biological control insects readily attacked coppicing stumps, seedlings, and saplings, as well as new growth on mature trees (Center et al., 2012). Although areas that do not become reinvaded with melaleuca cannot be specifically attributed to the biological control agents, many of the areas that were mechanically cleared of larger melaleuca trees early on continue to remain essentially free of melaleuca (L. Rodgers, pers. comm.). Also, in areas with formerly dense, mature, melaleuca stands (e.g., Pennsucco, Florida) that were not mechanically cleared, the older trees are now beginning to die due to damage from hurricanes and attack of the biological control agents, and the native understory plants are returning, with few if any new melaleuca seedlings (Rayamajhi et al., 2009; Smith et al., unpub. data). Additionally, the melaleuca biological control agents proved particularly effective at finding and attacking difficult-to-reach melaleuca stands, attacking newly emerged seedlings (which erupt in massive numbers after fire) and reducing subsequent seed production and dispersal (Pratt et al., 2005; Balentine et al., 2009; Center et al., 2012; Tipping et al., 2018) (**Fig. 5a,b**).

Through the combination of tree cutting, herbicide applications, and the impacts of the biocontrol agents, melaleuca coverage in South Florida was reduced from nearly 400,000 ha (988,000 acres) in 2000 to just under 100,000 ha (247,000 acres) in 2015, and likely less in 2021 although this has not been directly measured because it is no longer a management priority (Rodgers et al., 2014; L. Rodgers, pers. comm.). Several invaded areas still have persistent melaleuca populations, but these have not been actively managed for decades. Additionally, areas with melaleuca trees producing seeds can still experience large seedling recruitment events such as those seen in 2018 after the Raccoon Point complex fire in Big Cypress National Preserve (**Fig. 6**). Seedling recruitment events are often very discouraging for land managers, but two separate

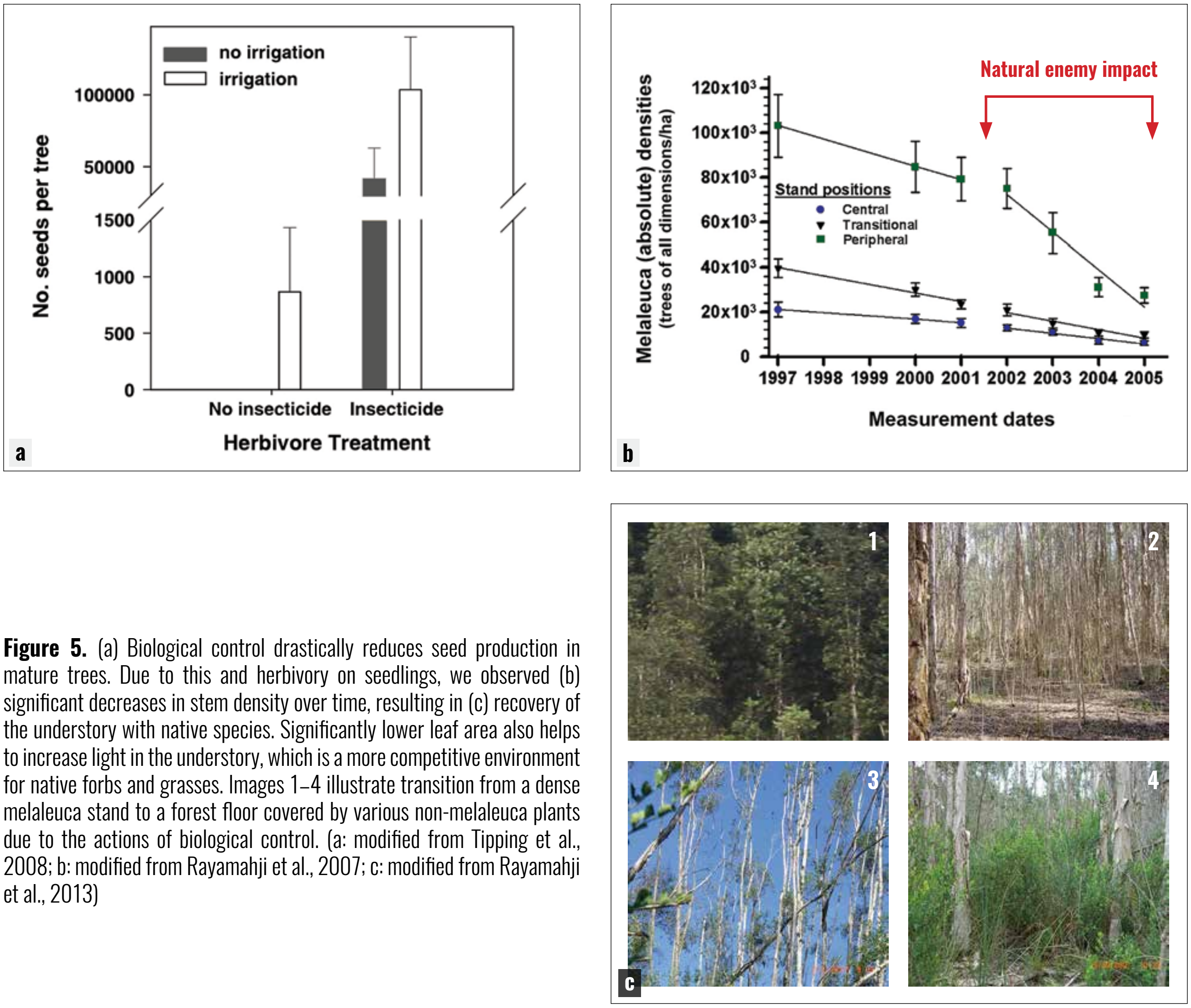

Figure 5. (a) Biological control drastically reduces seed production in mature trees. Due to this and herbivory on seedlings, we observed (b) significant decreases in stem density over time, resulting in (c) recovery of the understory with native species. Significantly lower leaf area also helps to increase light in the understory, which is a more competitive environment for native forbs and grasses. Images 1–4 illustrate transition from a dense melaleuca stand to a forest floor covered by various non-melaleuca plants due to the actions of biological control. (a: modified from Tipping et al., 2008; b: modified from Rayamahji et al., 2007; c: modified from Rayamahji et al., 2013)

experiments, one done before the *L. trifida* release and one done afterwards, illustrate two overarching findings: (1) melaleuca seedlings decline more than 90% over five years even without insect attack and (2) the biological control agents make the surviving seedlings smaller and still non-reproductive at five years after germination (Tipping et al., 2018; Smith et al., unpub. data). In areas where *O. vitiosa* cannot pupate successfully (e.g., persistently wet areas), seedlings are taller and may reach seed-production size (Center et al., 2012). This is due to a lack of persistent herbivory from the weevil. Evidence from post-fire events in these areas suggests that remnant trees are still somewhat reproductive and contribute to the melaleuca seed bank in the soil (Smith et al., unpubl. data). Combining fire with removal of individual remnant trees will likely stop the replenishment of the seed bank at these sites. Research is currently underway to determine if successive burning of these sites and removal of the remnant mature trees will exhaust the seed bank and reduce seedling recruitment events.

Figure 6. A persistent seed bank produces post-fire melaleuca seedlings in excess of 500/m^2. Biological control agents readily attack seedlings (see *Oxyops vitiosa* feeding and *Lophodiplosis trifida* galling damage visible on leaves), which increases mortality and decreases height and biomass. It also impedes the development of seedlings into reproductive trees. (M. C. Smith, USDA-ARS)

BENEFITS OF CONTROL OF MELALEUCA

Melaleuca harmed one of the most imperiled wetland ecosystems in the world, which happens to be next to one of the highest density population centers in the United States. Controlling melaleuca and increasing or restoring ecosystem function in the Everglades have both intrinsic and extrinsic values. For example, laminar water flow over the bed rock is critical for maintaining aquifer recharge and ensuring the safety of municipal water sources (Lockwood et al., 2003). Additionally, millions of people come to south Florida not just for the beaches, but also to visit Everglades National Park, Big Cypress National Preserve, and Arthur R. Marshall Loxahatchee National Wildlife Refuge. The loss in income from lower tourism was estimated in the millions at the start of the TAME melaleuca project. The direct economic costs associated with melaleuca in 2003 were more than $25 million ($39 million in 2022 dollars) (Carter-Finn et al., 2006). Based on the costs of the TAME melaleuca project and the yearly costs of funding the melaleuca biological control project, the total combined costs for biological control development and implementation for melaleuca control were estimated at $7 million. While the benefit-cost analyses for melaleuca biological control vary over time, they range from 2:1 at the start of the project to 40:1 after 15 years (Carter-Finn et al., 2006; McFadyen, 2008). Furthermore, controlling melaleuca with biological control has helped restore an ecosystem badly damaged by human disturbances.

CONCLUSIONS

Melaleuca quinquenervia is often called the 'poster child' for successful integration of biological control into a comprehensive management strategy. Biological control agents were specifically chosen not just for their specificity, but also their mode of attack, point of attack, and potential for integration with other biocontrol agents or with chemical and mechanical removal methods. Melaleuca is still present in several areas within the Greater Everglades Ecosystem, and these stands may create some new small foci of reinvasion, but most areas that received treatment now have healthy populations of native graminoids, shrubs, and forbs. Melaleuca is currently under maintenance management using aerial foliar herbicide application to reduce the stem density of melaleuca on an occasional basis, as needed. Other than prescribed burns, mechanical removal methods such as cutting are rarely used. The last biological control agent approved for release (*L. indentata*) is intended to improve control in the areas where melaleuca has persisted, especially areas where *O. vitiosa* cannot establish.

ACKNOWLEDGMENTS

The list of people who contributed significantly to the sustained control of melaleuca is long. Joe Balciunas (USDA-ARS) and Matthew Purcell and colleagues at the Australian Biological Control Laboratory led the search for melaleuca biological control agents for nearly forty years. Ted Center and Allen Dray (USDA-ARS) and Dan Thayer, Mike Bodle, Amy Ferriter, Leroy Rodgers, and Francois LaRoche (of the South Florida Water Management District) and Bill Zattau and Jon Lane (of the U.S. Army Corps of Engineers) developed conditions that allowed for interagency cooperative management. Paul Pratt developed the Areawide Pest Management Program (TAME Melaleuca). Thai Van, Min Rayamahji, Phil Tipping, and Melissa Martin provided conceptual guidance on evaluation of the biological control insects' impact on melaleuca in the presence and absence of other control techniques. Finally, none of these insects would have made it out of quarantine without the insect rearing skills of Gary Buckingham, Susan Wineriter Wright, Philip Clark, and many technicians.

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CHAPTER 30

Erythrina Gall Wasp Successfully Controlled by the Introduction of a Parasitoid Wasp in Hawaii

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NON-TECHNICAL SUMMARY

The erythrina gall wasp (*Quadrastichus erythrinae*) (Hymenoptera: Eulophidae) invaded Hawaii in 2005. High infestation levels and tree mortality rates were observed on the endemic wiliwili (*Erythrina sandwicensis*) tree and other exotic erythrina species soon after the gall wasp's arrival. A classical biological control program was initiated a few months after detection. A promising biocontrol agent, *Eurytoma erythrinae* (Hymenoptera: Eulophidae) was collected during foreign exploration in Africa and approved for release in 2008. The parasitoid quickly established in Hawaii and significantly reduced leaf infestation levels and tree mortality. Flowering and seed production resumed, but flower-infestation levels are still high at some sites. A second biocontrol agent is currently being considered for release to provide an improved level of control.

HISTORY OF INVASION AND NATURE OF PROBLEM

Quadrastichus erythrinae (Hymenoptera: Eulophidae), also known as the erythrina gall wasp (EGW) (**Fig. 1**), is an invasive species in Hawaii that was recorded for the first time in the Mascarene Islands and Singapore in 2003 (Yang et al., 2004). The gall wasp quickly spread through the islands in the Indian Ocean, in South-East Asia, and islands in the Pacific Ocean (Day et al., 2021). It was first detected on the island of Oahu in Hawaii in 2005, and within a few months it spread through all the main Hawaiian Islands (Heu et al., 2008).

Figure 1. Adult female *Quadrastichus erytrhinae* on a galled leaf. (M. Tremblay, University of Hawaii)

Female gall wasps lay eggs inside young leaves, stems, petioles, flowers, and seedpods of trees in the genus *Erythrina* (Kim et al., 2004; Heu et al., 2008).

Kaufman, L. V. and M. G. Wright. 2022. Erythrina gall wasp successfully controlled by the introduction of a parasitoid wasp in Hawaii, pp. 367–372. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

Figure 2. Galled erythrina leaves, showing severe infestation and malformation. (L. Kaufman, University of Hawaii)

Larval feeding induces swelling (galling) of the infested tissues. Heavy infestations (**Fig. 2**) cause defoliation and can lead to tree mortality (Kim et al., 2004).

In Hawaii, the EGW was first found infesting the exotic tree *Erythrina variegata* in urban and agricultural settings (Heu et al., 2008), and shortly afterwards infestations were detected in forests on the endemic tree *E. sandwicensis*, locally known as wiliwili. *Erythrina variegata* was commonly used in Hawaii as a windbreak in agricultural fields, while the ornamental form of the same species was a common tree in urban areas. The endemic wiliwili is one of the few dominant endemic trees of lowland dry forests in Hawaii, and it has important ecological functions in that ecosystem (Little and Skolmen, 1989; Wagner et al., 1990). Lowland dry forests are considered one the most endangered habitats in the Hawaiian Archipelago (Cabin et al., 2000). The endemic wiliwili also has strong cultural significance for native Hawaiians.

WHY CONTROL THIS INVASIVE SPECIES?

High levels of EGW infestation were observed on exotic and endemic erythrina trees soon after the detection of the invasive wasp in Hawaii, and these quickly caused tree mortality in both urban and natural areas (Kaufman and Yalemar, 2017; Kaufman et al., 2020). The Department of Parks and Recreation of the City and County of Honolulu removed over 1,000 dead *E. variegata* trees within a year of the invasion (Vorsino, 2006). Early methods of control included pruning and removal of infested material, tree removal, and chemical control (Kaufman et al., 2020). None of these methods were found to be sustainable for controlling the gall wasp, especially in remote natural areas. Without effective and self-sustaining control tools, the endemic wiliwili was at risk of extinction, with trees dying and surviving trees not flowering or producing seeds.

THE ECOLOGY OF THE PROBLEM

The windbreak and ornamental forms of the exotic *E. variegata* proved to be highly susceptible to the gall wasp, and most of those trees quickly died. Similarly high mortality rates were observed for the endemic wiliwili, which caused great concern to the conservation community. A seed-banking program was started in 2007 to ensure there would be germplasm available for future restoration efforts (Hollier, 2007).

The EGW was also reported infesting several exotic species of *Erythrina* growing in botanical gardens around the islands, with various degrees of severity. A susceptibility study that examined 71 *Erythrina* species present in Hawaiian botanical gardens determined that *Erythrina* species of African origin were more tolerant or resistant to the gall wasp than species from other regions, suggesting a possible African origin for the invasive wasp (Messing et al., 2009).

Given that no other control method was sustainable or practical as a means to save the endemic wiliwili, a classical biological control of the EGW was started only a few months after the detection of EGW in Hawaii, with the goal of reuniting the invasive pest with natural enemies from its area of origin and restoring balance to the EGW/*Erythrina* tree system in the invaded area.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

In 2005, shortly after the severity of EGW infestations in Hawaii was realized, exploration for natural enemies of EGW began in Africa. Africa was considered the most likely origin of the pest, as many species of *Erythrina* had previously been observed hosting gall insects in South Africa. The spread of EGW also appeared to have started on islands close to the east coast of Africa (Mauritius and Réunion), followed by rapid invasion of other islands in the Indian Ocean, then Pacific Ocean islands, Southeast Asia, and Taiwan. In 2006 and 2007, explorations were carried out in South Africa, Mozambique, Kenya, Tanzania, Benin, and Ghana by entomologists from the Hawaii Department of Agriculture and the University of Hawaii at Manoa. Collections returned to the Hawaii quarantine facility yielded at least 13 species of parasitoids. Of these, two species (*Eurytoma erythrinae* [**Fig. 3**] and *Aprostocitus nitens*) (both Hymenoptera: Eulophidae) were selected for further study because they appeared to be dominant parasitoids and could be reared in the quarantine facility. *Eurytoma erythrinae* was collected from a wide geographic range (Tanzania to South Africa) and from various species of gall wasps attacking *Erythrina* species in Africa. Also, this parasitoid had promising impacts on the target gall wasp under quarantine conditions. Non-target host screening was conducted in quarantine on seven non-target species in 2008. These studies found that *E. erythrinae* was host specific to EGW versus gall-makers found in the Hawaii fauna. The non-target gall-making species used in these host specificity tests included one Hawaiian endemic psyllid, four beneficial galling insects used for weed biological control (three tephritids and one eriococcid), and two immigrant galling wasps (one agaonid and one eulophid). Permission was obtained in December 2008 to release *E. erythrinae* in Hawaii. Batches of 30–60 wasps were released into populations of EGW-infested *Erythrina* trees. The parasitoid established readily after release, and recoveries were made within three to six months at most release sites. Pre-release monitoring had started previously, in early 2008, while post-release monitoring spanned a period of 10 years. Monitoring sites were established in several locations on the four main islands (Kauai, Oahu, Maui and Hawaii Island). High levels of gall wasp mortality were observed within a year of releasing the biological control agent (**Fig. 4**).

Figure 3. Female *Eurytoma erythrinae.* (Hawaii Department of Agriculture)

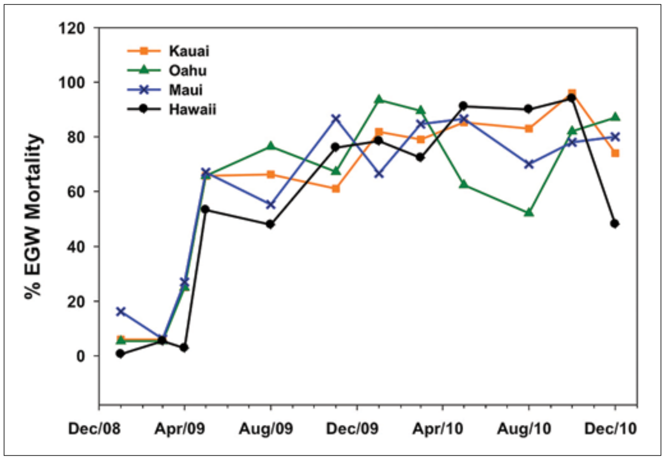

Figure 4. *Quadrastichus erythrinae* mortality attributed to Eurytoma erythrinae in galled Eythrina leaves on four Hawaiian islands over a two-year period following release of the biocontrol agent. (from Kaufman et al., 2020, reprinted with permission from Elsevier)

HOW WELL DID BIOLOGICAL CONTROL WORK

Pre- and post-release monitoring data measuring gall wasp damage levels and *E. erythrinae* persistence over a period of ten years showed that the biocontrol agent *E. erythrinae* was very effective at reducing infestation levels, as well as tree mortality rates (Van Driesche et al., 2016; Kaufman and Yalemar, 2017; Kaufman et al., 2020). Monitoring consisted of rating infestation levels separately in leaves and flowers and using a 4-point scale (scale from 0 to 3). During flowering season, young inflorescences were tagged and inspected monthly until seeds reached maturity. During the pre-release monitoring period, over 70% of young shoots inspected were rated as severely infested (infestation levels 2 or 3) (**Fig. 5**). At the end of the third post-release year, over 80% of the young foliage was free from gall wasp damage (Kaufman et al., 2020). Before the release of the biocontrol agent, about 15% of monitored trees were able to flower, and <3% were able to mature seeds, due to high gall wasp infestations in flowers and seedpods. By the third-year post-release, over 60% of monitoring trees were able to flower and over 20% of the trees were able to mature seeds (Kaufman et al., 2020). Even though flowering and seed production resumed after release of the first biological control agent, infestation levels in inflorescences and seedpods remained high at some sites (Van Driesche et al., 2016). With the goal of further suppressing galling of inflorescences, a second biocontrol agent, *Aprostocitus nitens* (Hymenoptera: Eulophidae), is currently being considered for release. An Environmental Assessment and Cultural Impact Assessment have been completed, and the filing for release permits by the Hawaii

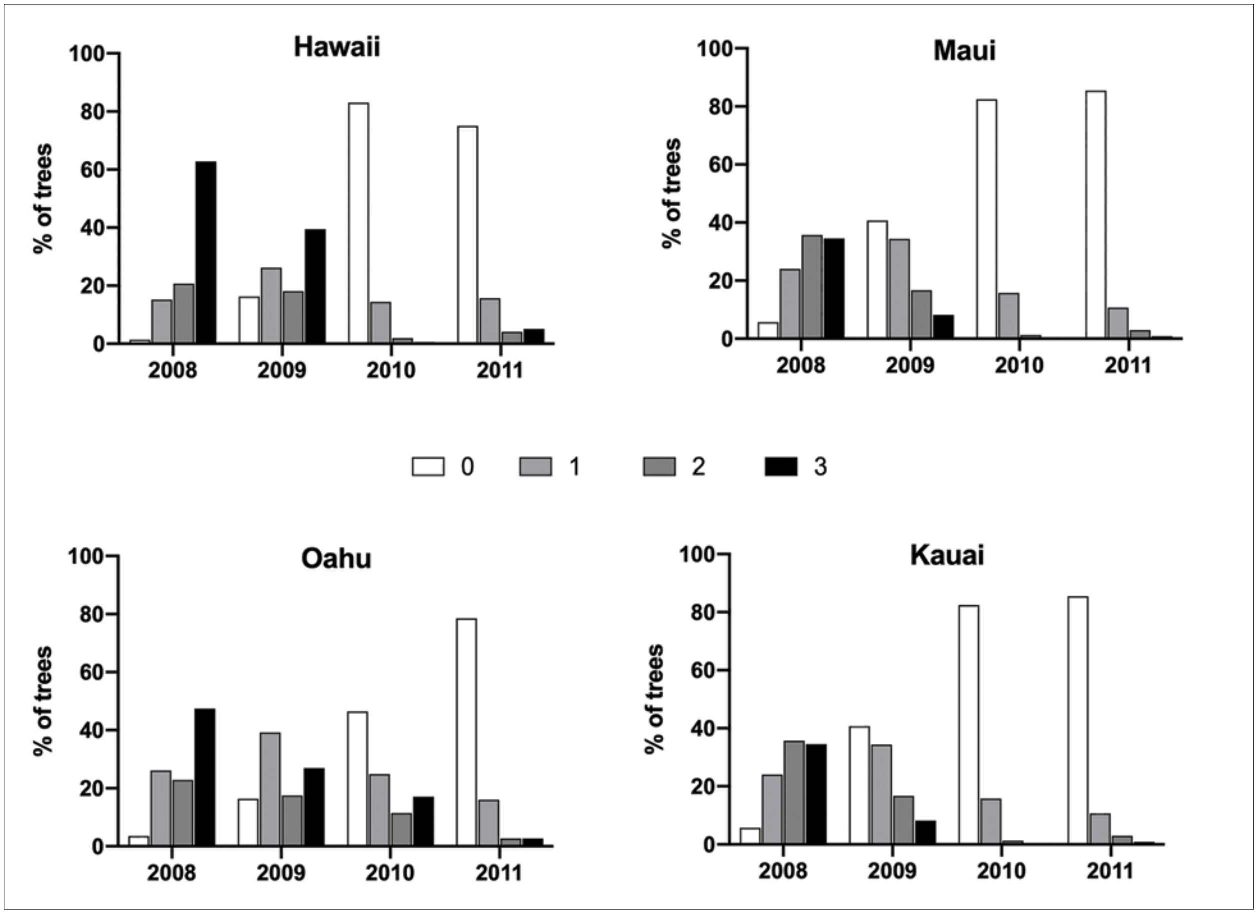

Figure 5. Changes in *Quadrastichus erythrinae* damage levels on a 0–3 scale (0 = zero infestation; 3 = 66–100% infestation) over a three-year period following release of *Eurytoma erythrinae* in 2008, on four Hawaiian islands. (from Kaufman et al., 2020, reprinted with permission from Elsevier)

Department of Agriculture is pending. Pre-release monitoring is currently documenting the status of flower and foliage infestations at several sites. This second biocontrol agent is expected to have different feeding preference and therefore reduce overall infestation levels (Kaufman et al., 2020).

A census of wild wiliwili stands conducted in 2012 found that 30–35% of trees had died due to EGW infestations (Van Driesche et al., 2016). Mortality rates would have been higher if the biocontrol agent was not released in a timely manner.

BENEFITS OF BIOLOGICAL CONTROL OF ERYTHRINA GALL WASP

Classical biological control of EGW in Hawaii is clear proof that the approach can be successfully used for the conservation of native species and ecosystems (Kaufman et al., 2020). EGW posed an imminent threat of extinction to the endemic wiliwili. The timely release of *E. erythrinae* significantly reduced infestation levels and mortality rates of the endemic wiliwili, eliminating that threat to conservation of a critical native tree. Before the parasitoid's release, the only effective method of EGW control was the injection of infested trees with systemic insecticides. Even though chemical control was effective at reducing infestation levels in urban settings, it was not cost-effective or feasible for remote wild tree populations. Data collected after the release of the biological control agent also showed that tree recovery was delayed in sites that received pesticide treatments, suggesting that pesticide residues interfered with the successful establishment of *E. erythrinae*. In non-treated areas, the biological control agent established readily after release and self-dispersed to remote natural areas. The biocontrol agent is also safeguarding many species of exotic erythrina grown as ornamental trees in Hawaii.

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Chapter 31

Biological Control of Arundo, an Invasive Grass Threatening Water Resources and National Security

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NON-TECHNICAL SUMMARY

A giant bamboo-like grass, known as arundo, giant reed, or carrizo cane in the United States, and scientifically as *Arundo donax* (Poaceae), was introduced from Mediterranean Europe for use in fences, roof construction, and erosion control. It has invaded riparian habitats (areas close to water) along rivers, creeks, arroyos, and lakes in the dry southwestern United States. Arundo consumes and wastes billions of gallons of water per year in the Lower Rio Grande Basin of Texas and Mexico, as well as in the Central Valley of California. Arundo also displaces native plants and animals, harbors crop and veterinary pests, blocks flood control systems, and fuels wildfires in the United States, as well as hindering border enforcement in Texas and thus threatening national security. A biological control program directed by the U.S. Department of Agriculture (USDA), Agricultural Research Service has led to the release and establishment of two insects against arundo. The first is called the arundo gall wasp, *Tetramesa romana* (Hymenoptera: Eurytomidae), a small black stingless wasp harmless to humans that lays its eggs in arundo shoot tips, resulting in galls inside which the wasp's larvae develop. Released wasps have established at over 25 sites along the Rio Grande River on the Texas-Mexico border, in central Mexico, and at a few sites in northern California. By 2014 (five years after release), feeding by the arundo gall wasp had reduced live arundo shoot weight an average of 22% across 10 sites, and this impact increased by an additional 32% (total of 54%) at five sites checked again in 2016 (seven years post release), allowing a 1.8-fold increase in plant diversity along the Rio Grande and potentially saving water valued at up to $10 million per year. The other released biological control agent, the arundo armored scale, *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), is a tiny, mostly immobile insect that feeds on plant sap inside the tuber-like rhizomes of arundo. It was first released in 2011, and by 2020 it had established at over 25 sites in Texas and at seven sites in northern California. In 2018 (seven years after release), plots with both arundo scale and arundo gall wasp at two sites along the Rio Grande had live shoot weights that were only 45% that of neighboring plots with only the arundo gall wasp present. In addition to the above two agents, a tiny fly, *Lasioptera donacis* (Diptera: Cecidomyiidae), that mines arundo leaves has been approved for release, and studies on how to mass rear it are underway. The arundo biological control

Moran, P. J. and J. A. Goolsby. 2022. Biological control of arundo, an invasive grass threatening water resources and national security, pp. 373–389. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

program has led to beneficial reductions of arundo reed stands, enhancing protection of water resources and improving visibility and national security along the U.S.-Mexico border. Further increases in arundo control over expanding areas are expected as this project continues.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Arundo, *Arundo donax* (Poaceae: Arundinoideae), is a giant grass that can be easily recognized by its large stature (typically 1–6 m or 3–20 ft; and up to 9 m or 30 ft) (Perdue, 1958) (**Fig. 1a**), its stiff, upright, plume-like flower stalks (inflorescences or panicles) (**Fig. 1b**), and its thick, solid, scaly, tuber-like rhizomes found on or just below the soil surface (**Fig. 1c**). It is native to western Mediterranean Europe, eastward through the Middle East and the Persian Gulf Region, on to India and Nepal (Hardion et al., 2014; Jimenez-Ruiz et al., 2021).

Hollow arundo shoots were and still are used to make woodwind musical instruments, for which the plant is still cultivated in France (Perdue, 1958; Tracy and DeLoach, 1999). As early as the 1500s, arundo was introduced to North America for use in roof construction and fencing (Dunmire, 2004). Arundo was also planted for erosion control on canal and river levees in the United States until the mid-1900s (Perdue, 1958; Frandsen, 1996; Tracy and DeLoach, 1999). This giant grass was already widespread in the Los Angeles Basin in southern California by the early 1800s (Bell, 1997; Dudley, 2000). Arundo spread rapidly from past places of cultivation into natural riparian habitats (areas adjacent to rivers, creeks/arroyos and lakes) and other wetlands in the arid southwestern United States. It now occupies at least 10,756 ha (26,579 acres) in the Lower Rio Grande Basin of Texas and northern Mexico (Yang et al., 2009, 2011) and 4,095 ha (10,120 acres) in California (Cal-IPC, 2020), with additional major infestations in the Upper Rio Grande Basin of New Mexico and along other rivers in New Mexico, Colorado, Arizona, Utah, and Nevada (**Fig. 2**).

The source of most of the invasive arundo in the southwestern United States was the Mediterranean coast of Spain (Tarin et al., 2013). In contrast, arundo in the southeastern United States came from the

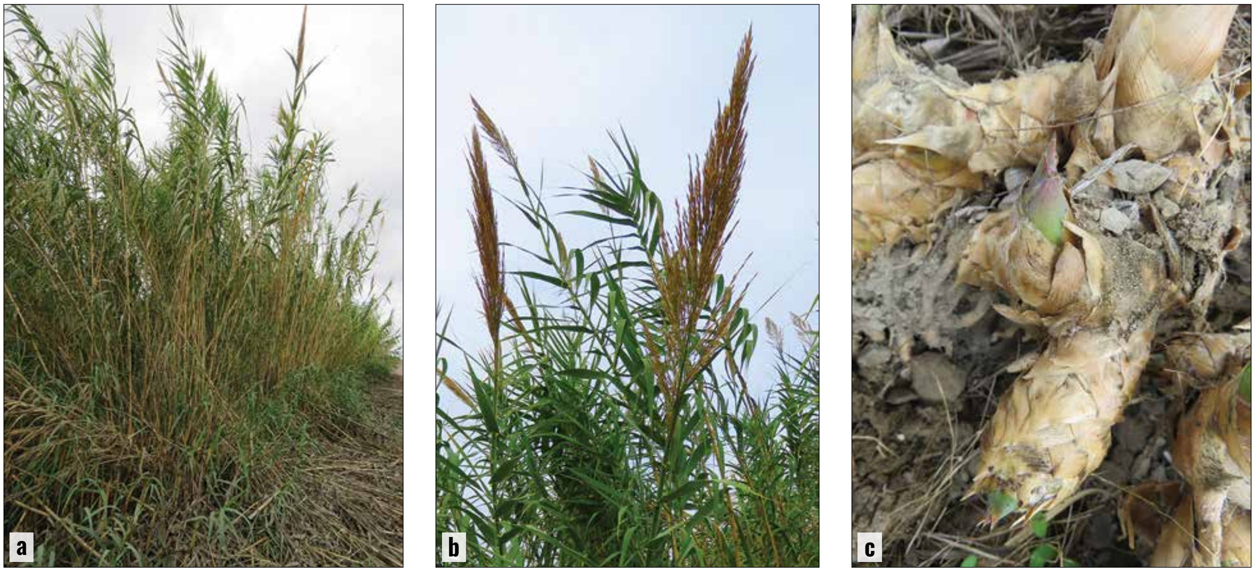

Figure 1. (a) *Arundo donax* stand on a canal bank in California. This species has characteristic (b) upright, plume-like inflorescences (panicles) and (c) thick, solid rhizomes (modified stems) that sit on or just below the soil surface and can be recognized by their dense, leafy, sharp-tipped scales and red/pink shoot buds. (a–c: K. Santa Caruz, USDA-ARS)

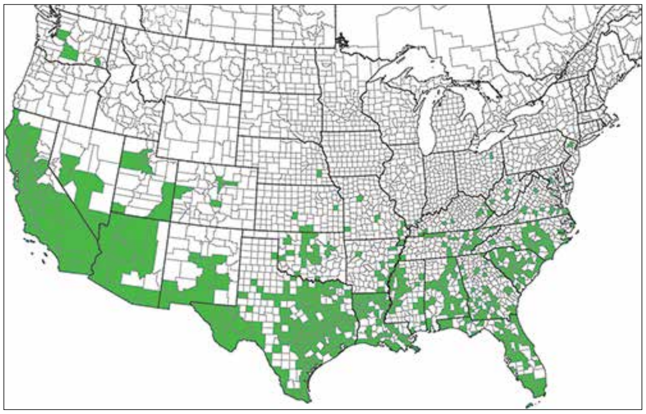

Figure 2. Occurrences of *Arundo donax* in the United States by county, showing also U.S. state boundaries. (EDDMapS, 2022)

Canary Islands (part of Spain) and the Seville area in southwestern Spain (Tarin et al., 2013). Arundo is not as invasive in the southeastern United States as in southern Texas to northern California (Tracy and DeLoach, 1999). Arundo is also found in isolated locations in states with a generally cold temperate climate (EDDMapS, 2022), including New Jersey, Maryland, Ohio, Nebraska, Colorado, Utah, and Washington (**Fig. 2**).

Arundo continues to spread through movement of vegetative propagules by water within watersheds and, between watersheds, as fragments attached to earth moving equipment (Decruyenaere and Holt, 2005; Wijte et al., 2005; Cal-IPC, 2020). Floods dislodge and move rhizomes and broken shoots. Both rhizomes and shoot pieces as short as a few cm (1 in) that bear axillary buds can quickly form roots and new shoots when deposited on moist sand, silt, or clay (Wijte et al., 2005; Boland, 2008; Mann et al., 2013) as arundo can grow in a wide range of soils (Quinn and Holt, 2008; Goolsby et al., 2013a). In the United States and beyond, arundo has been used or considered for use in wastewater treatment to remove heavy metals (Cristaldi et al., 2020) or as a biofuel (Lewandowski et al., 2003; Angelini et al., 2005; Mariani et al., 2010), and attempts to capitalize on such possibilities may contribute to further spread.

Nature of the Problem

Arundo consumes water in arid southwestern U.S. ecosystems, where water is scarce and is expected to become scarcer due to climate change (IPCC, 2021) as a result of decreased precipitation, altered water resource patterns (rainfall to snowpack ratios), and/or increased evaporation from soil and vegetation. Even in winter, arundo in the Lower Rio Grande Basin of Texas consumes and releases water to the air (evapotranspiration) at a level equivalent to corn in the summer in the southern United States (Gowda et al., 2011; Watts and Moore, 2011; Racelis et al., 2022). Indeed, water use by arundo occurs at levels that are higher than ever seen in most plants (Cal-IPC, 2020). Based on water use measured on individual plants, with instruments suspended over arundo patches, or with satellite imagery analysis, arundo has been estimated to consume an estimated 56.2 million m^3 of water (45,540 acre-ft) per year in the Lower Rio Grande Basin of Texas and Mexico (Seawright et al., 2009) and 37.8 million m^3 (30,086 acre-ft) per year in the Central Valley of California (Cal-IPC, 2020). The significant threat posed by arundo stands to water resources is recognized worldwide wherever it is invasive, including Australia (Haddadchi et al., 2013), New Zealand (Virtue et al., 2010), and South Africa (Versfeld et al., 2000; Nkuna et al., 2018).

In addition, arundo has other detrimental effects in the southwestern United States. It exacerbates fire hazards in riparian habitats (Frandsen, 1996), acting as a vertical fuel source and increasing fire intensity (Coffman et al., 2010). In the Central Valley of California, fire frequency in watersheds is positively correlated with the area of arundo, and in coastal watersheds in southern California, fires in arundo are known to promote fire spread to native riparian habitat (Cal-IPC, 2020). Fire aids arundo invasions because this weed recovers quickly, while native plants often recover more slowly (Coffman et al., 2010). Dense, monotypic arundo stands reduce biodiversity of plants (Bell, 1997; Quinn and Holt, 2008; Racelis et al., 2012a; Rubio et al., 2014), insects (Herrera and Dudley, 2003), and birds and reptiles (Tracy and Deloach, 1998; Dudley, 2000; Cal-IPC, 2020), as well as rare fish populations (Tracy and DeLoach, 1999), likely causing the extinction of one endemic Mexican fish species (McGaugh et al., 2006). Arundo facilitates invasion of a major veterinary pest, the cattle fever tick (*Rhipicephalus microplus*), along the Rio Grande (Racelis et al., 2012b). Arundo is considered an 'ecological transformer' species (Quinn and Holt, 2008; Racelis et al., 2012a) because it invades both human-disturbed and some undisturbed riparian habitats and converts them into habitats favoring its own further invasion. Arundo invasion alters stream geomorphology, converting shallow sandy, rocky, or silty emergent riparian floodplain habitats into narrow, deep channels with monotypic stands of arundo along their banks (Cal-IPC, 2020) (**Fig. 3**). When this process takes place in flood control channels, their ability to contain floodwaters can be reduced (Spencer et al., 2013). Arundo also harbors pathogenic fungi and viruses that can infect crop plants (Tracy and DeLoach, 1999). Finally, arundo interferes with law enforcement activities by reducing visibility along the international border between the United States and Mexico (Goolsby et al., 2017a), and it screens transient occupancy and other illegal activities along rivers in the Central Valley of California, exacerbating fire hazards (Cal-IPC, 2020).

Figure 3. Prior to biocontrol, dense *Arundo donax* stands converted sandy and silty floodplains along the Lower Rio Grande on the U.S.-Mexico border to narrow deep channels, depriving native plants and animals of habitat. (J. Goolsby, USDA-ARS)

WHY CONTROL THIS INVASIVE SPECIES?

Arundo is a deep-rooted perennial grass that remains green year-round in subtropical habitats such as those in the Rio Grande Valley of South Texas (Gowda et al., 2011), while senescing (leaves turning brown) in the winter in more temperate parts of its range, as in northern California (Spencer et al., 2005; Thornby et al., 2007; Spencer, 2012). Either with or without winter browning, first-year canes without branches sprout many lateral branches beginning in winter or early spring and throughout the second and third years. Young shoots can grow as fast as 10 cm (4 in) a day, among the fastest growth rates known in plants (Perdue, 1958; Bell, 1997). Dead canes remain standing for years, contributing further to the thickness of arundo stands. The most common methods used to control arundo are herbicide application, mechanical removal, mowing, and fire. Glyphosate and imazapyr, applied alone or in combination from the ground or air, can kill 90% or more of shoots and be temporarily effective, but regrowth occurs, requiring follow-up treatment (Spencer et al., 2009, 2011; Bell, 2011; DiTomaso et al., 2013). Reductions in arundo after chemical control can be sustained for three or more years after treatment (Lawson et al., 2005; SEC, 2019), but this method is usually cost-prohibitive for large areas. Mechanical removal and mowing also provide only temporary benefits, as they must be done repeatedly (e.g., every year for five or more years), often in hazardous, steep terrain

(Bell, 1997; Goolsby et al., 2019a). Physical control with fire is ineffective because arundo uses nutrients released by fire and re-grows rapidly (Coffman et al., 2010). None of these control methods are selective for arundo; all threaten native plants and the animals that depend on them. These methods are also very costly; over $100 million has been spent in California for control of arundo, and the expected total cost to control arundo chemically in the Central Valley is $70 million (Cal-IPC, 2020). Large-scale programs in Texas have incurred similar costs (Seawright et al., 2009; J. Goolsby, pers. obs.). Sustainable control is urgently needed; the expected benefits to water, improved riparian habitat, fire prevention, and protection of rare, threatened, or endangered species in California's Central Valley are estimated at $115 million (Cal-IPC et al., 2020).

Given the low sustainability of large-scale chemical or mechanical control activities, a biological control program against arundo was developed (Goolsby and Moran, 2009; Goolsby et al., 2009a). Arundo is the first grass species in the world targeted for classical biological control for which actual field releases of non-native, introduced insects have been made (Sutton et al., 2019). Grasses had long been thought of as being unsuitable for biological control because their 'simple' architecture would likely host few host-specific herbivorous insects (Evans, 1991). However, in recent decades this assumption has been proven wrong, based on studies of shoot-feeding insects (Tscharntke and Greiler, 1995; Tewksbury et al., 2002; Sutton et al., 2019, 2021).

Successful biological control of arundo was projected to potentially save water valued at $3.5–5 million per year in the Lower Rio Grande Basin of Texas, in 2009 dollars (Seawright et al., 2009), and successful control using chemical and mechanical methods in the Central Valley of California was projected to save at least 46,700 m^3 of water per ha (or 15.4 acre-ft per year per acre of arundo). The goal of this biological control project was to reduce the ability of new arundo shoot tips to survive, thus reducing stand density, and to reduce the ability of rhizomes to spread the plant vegetatively by producing new shoots. A key anticipated outcome was reduction of arundo stands to facilitate recovery of native vegetation, as studies near Laredo, Texas that simulated live shoot reduction in plots by hand removal, led to the emergence of native plants from seed banks (Racelis et al., 2012a; Rubio et al., 2014). Additional desired outcomes included reduction of water use by arundo and improved visibility along the U.S-Mexico border for law enforcement.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Efforts to control arundo biologically began in the 1990s with a review of its known herbivores (Tracy and DeLoach, 1999). Tall perennial grasses like arundo are often hosts for many internal leaf-mining, shoot-boring, or root-boring flies, sawflies, gall wasps, or beetles. Known external feeders include leaf- or stem-chewing or sucking insects such as aphids, scales, and true bugs (Hemiptera), as well as various beetles and moths (Tewksbury et al., 2002; Lambert et al., 2010). Surveys of arundo in the United States found very few herbivores (Tracy and DeLoach, 1999). An adventive (meaning self-introduced at an unknown time; in this case, probably accidentally-introduced) aphid, *Melanaphis donacis* (Hemiptera: Aphididae), is present in California (Dudley et al., 2008; Moran, 2020), but not in the Lower Rio Grande Basin. It is unsuitable for biological control, as it is known to feed on common reed, *Phragmites australis*, in its native range (Tracy and DeLoach, 1999) as well as plants in the rose family (Blackman and Eastop, 2018). It is widely distributed in its native range (Amin et al., 2019) and in other areas invaded by arundo, including Argentina (Ortego et al., 2004) and Chile (Undurraga et al., 2020). Shoot tip-borers have been observed in California (Dudley et al., 2008), and the native fly *Chaetopsis massyla* (Diptera: Ulidiidae) was observed in both greenhouses and less commonly in the field in Texas (Goolsby and Mangan, 2010).

For the purposes of this project, the native range of arundo was considered to run from India and Nepal through the Persian Gulf and Middle East to western Mediterranean Europe and the northern coast of Africa (Moran and Goolsby, 2009). Some authors, based on genetic evidence, restrict the native range to India, Nepal, Pakistan, and Afghanistan (Jimenez-Ruiz et al., 2021), but we found insect diversity on arundo to be highest in the Mediterranean region. A literature survey (Tracy and DeLoach, 1999) found only one potentially host-specific (monophagous) insect feeding on arundo in its native range, the shoot-tip-galling wasp *Tetramesa romana* (Hymenoptera: Eurytomidae), which had been reported from Europe. Field surveys in India and Nepal were conducted in 2000 (Stelljes, 2001) by R. Carruthers, A. Kirk, and T. Widmer, of the USDA and T. Dudley of the University of California, Santa Barbara. Kirk surveyed many other areas in both the native range and invaded Asian, South African, and Australian ranges. Climate match modeling indicated that the climate of Valencia in Mediterranean Spain showed a strong match to the climate of the Lower Rio Grande Basin (Goolsby and Moran, 2009), and genetic analyses indicated that the origin of most southwestern U.S. populations of arundo was the southern or eastern coast of Spain (Tarin et al., 2013). Intensive surveys were, therefore, carried out in this region, which led to the importation and testing of several candidate biological control agents in quarantine laboratories in the United States between 2007 and 2013.

Tetramesa romana, a Shoot Tip-Galling Wasp

Figure 4. The arundo wasp (*Tetramesa romana*) adult female injects eggs into an arundo shoot tip. (J. Goolsby, USDA-ARS)

Tetramesa romana (Hymenoptera: Eurytomidae) is a shoot-tip galling wasp on arundo, and it is referred to here as the arundo wasp. This wasp is a small (5–7 mm or ⅛–¼ in) (Moran and Goolsby, 2009) insect (**Fig. 4**). The arundo wasp was found in Spain, southern France, Italy (including Sicily), Morocco, Egypt, Greece (including Crete), Turkey, and Bulgaria in the native range (Goolsby and Moran, 2009). Adventive arundo wasps were also found as localized populations in Laredo, Texas along the Rio Grande (Racelis et al., 2009); Austin, in central Texas (Goolsby et al., 2009a); southern California (Dudley et al., 2008); South Africa (Canavan et al., 2019); and China (Goolsby and Moran, 2009). The genus *Tetramesa* includes over 200 species in Europe, Asia, Africa, Australia, and the Americas, most (or all) of which feed on one or just a few grass species (Claridge, 1961; Al-Barrak, 2006). The adventive species *Tetramesa phragmitidis* is present in the United States on common reed and appears to feed only on this grass (Tewksbury et al., 2002). Two *Tetramesa* species are under evaluation in South Africa for control of giant rat's tail grass (*Sporobulus pyramidalis*) in Australia (Sutton et al., 2021), and a new *Tetramesa* species was recently described from medusahead (*Taeniatherum caput-medusae*) in Greece (Lotfalizadeh et al., 2021).

Tetramesa romana females emerge with developed eggs which are deposited into shoot tips, and so they can reproduce without mating (Goolsby et al., 2014). Each female can produce an average of 12–25 offspring on arundo (Goolsby et al., 2009; Moran and Goolsby, 2009). Limited reproduction (7–14% of the level found on arundo) occurs on *Arundo formosana* (a non-native species used rarely as an ornamental plant in the United States). The arundo wasp does not develop on common reed or 26 other grasses and 8 non-grass, habitat-associate plants tested (Goolsby et al., 2009a). After release in Texas and Mexico, no wasp exit holes were found on common reed, 10 other wild grasses (both native and non-native species), or two economic grasses (corn, sugarcane) growing adjacent to arundo (Goolsby et al., 2020).

Gall-forming insects insert their eggs into suitable plant tissues, such as shoot tips, and either adult females or eggs release plant hormones that cause the plant to produce an abnormal tumor-like growth, inside which the next generation of immature gall-makers feed (Raman et al., 2005). Gall-formers are known for their high host specificity and hence have high utility in biological weed control (Muniappan and McFadyen, 2005). Several shoot-galling insects have been found to be effective biocontrol agents (Dhileepan, 2004; Aigbedion-Atalor et al., 2019).

Female arundo wasps insert eggs into the tips of young stems, and each egg forms its own gall. Galls are often clustered near the shoot tip, resembling one large gall (**Fig. 5a**). Galled main shoots become bent and distorted (**Fig. 5b**). Galls are most often found on lateral shoots on mature main shoots. Larvae feed, develop, and pupate inside the gall. Adult emergence holes (**Fig. 5c**) on green or brown shoots allow observers to spot wasp presence, and by counting them it is possible to estimate wasp density (Marshall et al., 2018a), as each wasp makes its own exit hole (Moran and Goolsby, 2009). Wasps enter a quiescent (hibernating) state as final-stage larvae or as pupae under cold winter conditions or drought (P. Moran and J. Goolsby, pers. obs.), but otherwise can complete several generations per year (Racelis et al., 2010; Goolsby et al., 2014). Abundant sunlight, water, and nitrogen enhance the population growth rate of the arundo wasp by increasing the output per female and/or shortening total development time (Moran et al., 2014; Moran and Goolsby, 2014: Moran, 2015).

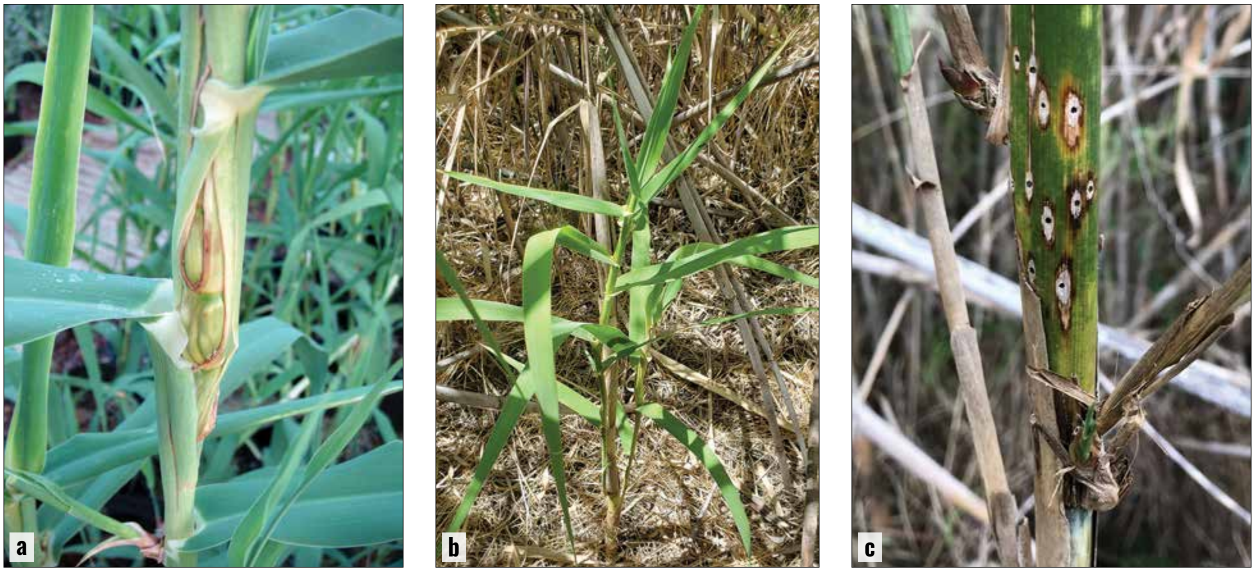

Figure 5. (a) Galls made by the arundo wasp on a young main shoot; (b) young main shoot of *Arundo donax* galled by the arundo wasp (note the characteristic bent and distorted appearance of the shoot; galling increases the likelihood of shoot breakage and death); (c) exit holes made by emerging arundo wasps. (a,b: P. Moran, USDA-ARS; c: J. Goolsby, USDA-ARS)

Arundo wasps from eastern coastal Spain and France were first released in 2009 in the Lower Rio Grande Basin, specifically the far southern tip of Texas along the Rio Grande. Over 1.2 million total arundo wasps, from three Spanish and three French locations, and from the adventive Laredo, Texas population, were mass-reared between 2009 and 2011 (Moran et al., 2014). They were released from the ground or, in remote areas, dropped from airplanes (with wasps inside custom-designed cardboard boxes) (Racelis et al., 2010), at 20 sites between Brownsville and Del Rio, Texas. Surveys in 2013 found established *T. romana* populations at 25 sites in this region (Goolsby et al., 2014). Dispersal occurred to sites 32 km (20 mi) from the nearest release area. Genetic marker analysis indicated that 390 of 409 arundo wasps were of a genetic form (haplotype) that matched the three Spanish populations but was mixed with, and indistinguishable from, the Laredo population at the Rio Grande sites; the Spanish populations were present separately at a few sites; and the French wasps did not establish (Goolsby et al., 2014). In subsequent years, wasp galls from the Rio Grande sites were reared in plots of arundo at a Texas A & M University research farm in Weslaco, Texas, and cut stems or galls were placed at field sites in the spring, summer, or fall to further distribute this gall wasp. Mowing or 'topping' of dense arundo patches before release to induce prolific side shoot regrowth was found to increase subsequent arundo wasp density after release (Racelis et al., 2012c). This release technique led to establishment in Big Bend National Park, much further west on the Rio Grande (Goolsby et al., 2019a). The arundo wasp was also released and established in Mexico in Nuevo Laredo in the state of Tamaulipas on the Rio Grande, and near Jiutepec in the state of Morelos in central Mexico (Martínez Jiménez et al., 2017). In California, galls from Texas were held in cardboard barrels (Moran et al., 2014) for adult emergence. Approximately 12,000 adult wasps were released at nine field sites between the northern Sacramento River watershed to the southern San Joaquin River watershed between 2013 and 2017, and the arundo wasp established at two sites, one in each watershed (Moran, 2020). Reduced climate suitability may be the reason why establishment has not been as successful, in terms of the number of sites, in dry, temperate northern California as in the humid, subtropical Lower Rio Grande Basin of Texas, where wasp populations are 10 to 39-fold higher than in the wasp's native Mediterranean range (Marshall et al., 2018a).

Rhizaspidiotus donacis, a Rhizome-Feeding Armored Scale

The armored scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae) is referred to here as the arundo scale. It is the first and, to date, only armored scale in the world to be intentionally released for biological weed control (Winston et al., 2021). The scale occurs in France, Spain, Italy, Turkey, and Algeria (Goolsby et al., 2009a). Insects for host-range testing were collected in southwestern France and the eastern Mediterranean coast of Spain, and additional collections were later made in Greece and Italy to examine the scale's occurrence in a broader range of habitats (Goolsby et al., 2013a). The scale's life cycle begins with the birth of live 'crawlers' and lasts two months until the production of short-lived winged adult males that leave their scale covers behind, or six months when immobile females, which are black or brown and measure about 1.2 mm (¹⁄₂₀ in), mature (**Fig. 6a,b**) (Moran and Goolsby, 2010). In the field in Spain (Cortés et al., 2012) and in southern California in an adventive population (Braman et al., 2021), there is one scale generation per year, and possibly two in subtropical field settings such as the Lower Rio Grande Basin (J. Goolsby, pers. obs.). In laboratory tests with 200 crawlers released per plant, 21 ± 4 females were produced per arundo plant across 29 plants tested, and only 14% as many adult females were produced on the related, non-native plant species *A. formosana* (3 ± 2 females across three plants tested). The scale produced 3 or 0 females on two specimens of native sprangletop grass (*Leptochloa virgata*). No adult females were produced on any of 32 other grass species or 9 non-grass plants, including species that often grow close to arundo (Goolsby et al.,

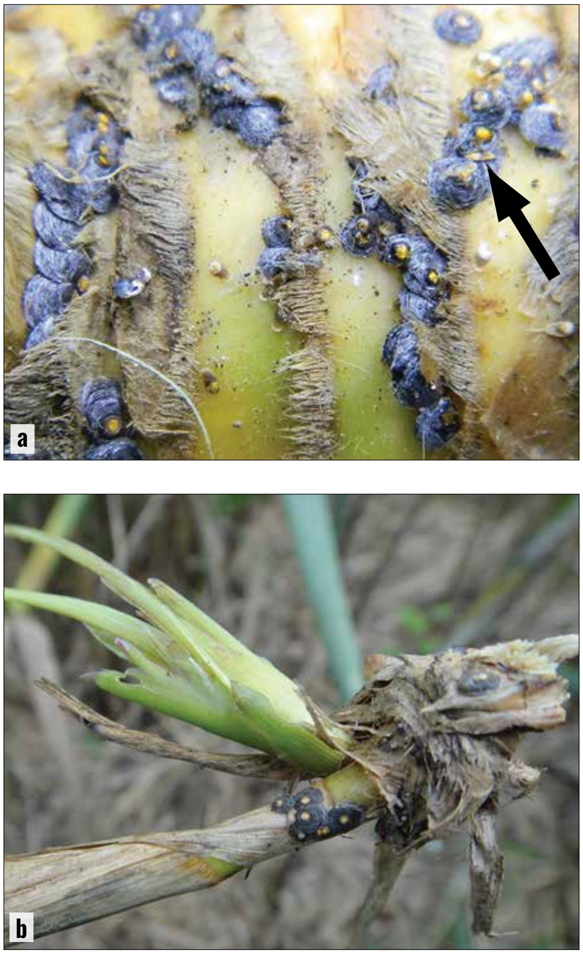

Figure 6. The arundo armored scale, *Rhizaspidiotus donacis*, (a) showing both black, round adult females and much smaller brown, oyster-shaped adult male scale cover (arrow), on arundo rhizomes in Greece; (b) adult females on a side shoot of arundo near Brownsville, Texas, showing 'witches' broom' shoot distortion. (a,b: J. Goolsby, USDA-ARS)

2009b). In follow-up laboratory tests with 1,000 crawlers per plant, 130 adult females were produced per plant on *A. donax*, 5 per plant on *L. virgata*, and 1 or less on smooth cordgrass, *Spartina alterniflora*. Field surveys in the native range in Spain found no infestation by this scale on *Leptochloa* or *Spartina* species (Goolsby et al., 2009b). Post-release surveys in Texas found the arundo scale only on arundo, and it was not found on common reed or other neighboring grasses (Goolsby et al., 2020). In greenhouse tests, arundo scales from an adventive southern California population (and a French population used for comparison) were able to produce adult female offspring on native accessions of common reed, but at levels of only 1–2% (1–6 females) of that produced on arundo (105–221 females) (Braman et al., 2021). The arundo armored scale poses little to no risk to plants other than arundo in the field.

Greenhouse studies confirmed that *R. donacis* from eastern and southern Spain can produce large numbers of progeny on arundo from the Rio Grande Basin (Goolsby et al., 2013b). The arundo scale was first released in January 2011 at two sites: an experimental plot of arundo at the USDA-APHIS facility in Edinburg, Texas (147,000 crawlers) and in a patch of arundo in Del Rio, Texas on the Rio Grande River (635,000 crawlers). By July 2011, a new generation of settled scales was observed at both sites, indicating establishment (Goolsby et al., 2011). Methods were developed to rear the arundo armored scale on potted arundo 'microplants' (Villarreal et al., 2016) derived from rooted shoot cuttings. Five and a half months after infesting microplants with scales, they were examined to verify the presence of mature females. Plants were removed from pots and planted in holes drilled into rhizome beds in dense arundo patches. Use of this technique resulted in the arundo scale's establishment at all 48 locations where they were released along the Rio Grande River from Brownsville to Del Rio, Texas (Villarreal et al., 2016; Goolsby and Moran, 2019). In northern California, releases were made at nine sites in the San Joaquin and Sacramento River watersheds, and establishment was confirmed at seven sites two years later (Moran, 2020).

HOW WELL DID IT WORK

Before release of the arundo wasp, its likely impact was estimated in greenhouse cage studies in which 6 wasps per stem per week were applied for 12 weeks to potted arundo plants. This treatment reduced shoot growth by 92% over the trial period and induced the formation of lateral shoots (Goolsby et al., 2009c). Addition of the arundo scale to the treatment did not further reduce plant growth, due to the scale's much longer life cycle. In separate greenhouse trials, infestation of arundo plants by *T. romana* at similar high densities (as above) for 12 weeks reduced photosynthetic carbon fixation by 32%, and *R. donacis* infestation for one generation reduced photosynthetic carbon assimilation by over 67% (either with or without the arundo wasp as a co-treatment) (Moore et al., 2010). Pre-release evaluations of the arundo scale in Spain, made by comparing side shoot growth on insecticide-protected and unprotected plants over one year, indicated that the presence of the scale reduced average side shoot growth by 61% (mean ± standard error, 2.2 ± 0.4 cm [0.9 in] compared to 5.7 ± 0.6 cm [2.2 in] of growth on protected plants) (Cortés et al., 2011a). In a comparison of sites with or without the scale in Spain, average rhizome biomass was 46% lower at nine sites with arundo scale (388.0 ± 16.5 g [0.9 lbs]) than at nine sites without the scale (716.7 ± 30.9 g [1.6 lbs]) (Cortés et al., 2011b).

Post-release evaluations of the arundo wasp have been conducted in the Lower Rio Grande Basin in south Texas (**Table 1**). Five years after first release of the arundo wasp in 2009, live aboveground biomass (main shoots + side shoots + leaves) per m^2, as inferred from live main shoot length (Spencer et al., 2006), was 22% lower after releases (mean ± standard error, 86.9 ± 12.6 kg/m^2 [17.8 lb/ft^2]) compared to before releases (111.0 ± 14.2 kg/m^2 [22.7 lb/ft^2]), and the proportion of main shoots that were alive (per 9 m^2 [97 ft^2] plot) was 18% lower than before the release program began (Goolsby et al., 2016). Across 11,403 side shoots examined, 2,010 had galls and 1,336 (66%) of those were dead, while among 9,393 ungalled side shoots, 4,610 (49%) were dead; galling by the arundo wasp thus increased the likelihood of side shoot death by 17% (**Table 1**). Seven years after agents were released (2016), estimated aboveground biomass had decreased to 54% of the level before biological control, to 61.3 kg/m^2 (12.6 lb/ft^2) across five sites (Moran et al., 2017). These findings indicate that *T. romana* reduced the ability of arundo shoots to grow and survive, a key objective of the project.

Because the arundo scale female is immobile for most of its life, impact studies have been limited to release plots. In a 2019 study at two sites, eight years after initiation of crawler releases and out-planting of scale-infested microplants, arundo aboveground live biomass was 55% lower in plots with both the arundo scale and arundo wasp (26.4 ± 6.1 kg/m^2 [5.4 lb/ft^2]), compared to plots with the wasp alone (58.7 ± 11.6 kg/m^2 [12.0 lb/ft^2], **Table 1**) (Goolsby and Moran, 2019). At this point in time, only about half (52%) of the side shoots of arundo in the scale plots had scales at their bases, compared to 82–100% in Spain (Cortes et al., 2011b), suggesting that arundo scale populations were still increasing, and so their full impact had not yet become apparent.

Table 1. Key measures of impact of the arundo biological control program in the Lower Rio Grande Basin of south Texas.

BIOLOGICAL CONTROL AGENT	MEASURE	YEARS POST-RELEASE[1]	IMPACT (CHANGE FROM PRE-BIOLOGICAL CONTROL)	REFERENCE
Arundo wasp	Live aboveground biomass	5	22% decrease[2]	Goolsby et al., 2016
	Proportion live shoots per plot	5	18% decrease	
	Proportion of dead side shoots	5	17% increase	
	Live aboveground biomass	7	32% decrease from 2014[3]	Moran et al., 2017
	Plant diversity	7	1.8-fold increase[4]	
Arundo armored scale + Arundo wasp	Live aboveground biomass	8	55% decrease compared to arundo wasp alone[5]	Goolsby and Moran, 2019

[1] From the start of release program for the agent indicated.

[2] Average decrease across 10 sites.

[3] Additional decline over two years at five sites measured both five (2014) and seven (2016) years after arundo wasp release.

[4] Relative to dense stand of arundo with no other species in plot (Simpson's D = 1).

[5] Average across two sites, total of five pairs of plots with scale + wasp compared to wasp alone.

BENEFITS OF BIOLOGICAL CONTROL OF ARUNDO

A key benefit expected from a reduction of arundo's live aboveground biomass was reduction of water consumption. On the U.S. side (southern- and eastern-most portion) of the Lower Rio Grande Valley (LRGV), a 22% reduction in live arundo biomass five years after release of the arundo wasp resulted in annual savings estimated at 269 ha-m (2,183 acre-ft) of water per year (Goolsby et al., 2016), based on models developed in Seawright et al. (2009). The estimated value of the water saved was $303,000 in 2016 at a water value per acre-ft of $139 for water across both sides of the U.S.-Mexico border, or up to $4.4 million when the water value was estimated at $2,000 per acre-ft for use in irrigated crops on the U.S. side of the LRGV. Assuming a further 32% decrease in biomass (i.e., total of 54% after seven years, Moran et al., 2017) an additional 391 ha-m (3,175 acre-ft) was expected to be saved in the U.S. portion of the LRGV annually, with a value (depending on the price of water) of $441,000 to $6.4 million. The total water savings were thus valued at up to $10.8 million per year by 2017. The projected benefit-to-cost ratio of the arundo biological control program in the Lower Rio Grande Basin is 4.3 dollars of water saved for each dollar spent on research, agent release, and monitoring (Seawright et al., 2009). In the first post-release study, a 24% decrease in water use between 2014 and 2015 coincided with increases of up to 53% in wasp density (Racelis et al., 2022). The arundo scale has not yet dispersed sufficiently to add its impact to the estimates of water savings attributed to the release of the arundo wasp.

A key realized ecological benefit of biological control of arundo in the Lower Rio Grande Basin of Texas is increased plant biodiversity. Seven years after arundo wasp release, 44 total plant species were found across 21 wasp-infested arundo plots examined (three plots per site at seven sites) in the Rio Grande Valley, including 38 native species (1 grass, 19 forbs or herbs, 8 vines, 4 shrubs, and 6 trees). Overall plant diversity (Simpson's D) per plot increased 1.8-fold relative to the baseline condition of dense arundo with no other plants (D = 1) (Moran et al., 2017). These results are consistent with pre-biocontrol studies near Laredo, Texas that found that cutting of arundo shoots to simulate biological control led to emergence of diverse plant species from the soil seedbank (Racelis et al., 2012a; Rubio et al., 2014). Studies on a U.S. Fish and Wildlife Refuge in this region suggested that mechanical topping followed by biological control further increased plant diversity by allowing sunlight to penetrate the arundo canopy more quickly than biological control alone (Goolsby et al., 2019b).

Stakeholders along the U.S. side of the Rio Grande are reporting reduced efforts and cost to control arundo locally with herbicides and mowing (J. Goolsby, pers. obs.). Law enforcement personnel now have greater visibility (>30 m or 98 ft) and ease of access into arundo-infested areas along the Rio Grande (Goolsby et al., 2017a).

REMAINING WORK

The arundo biocontrol project is not yet over, as the following evaluations or introductions would be useful:

(1) Additional post-release field assessments of arundo water use in the Lower Rio Grande Basin are needed to verify the expected water savings noted above.

(2) An assessment of the impact of the arundo wasp on arundo in northern California will begin in 2022, five years after wasp release.

(3) The leaf-mining midge *Lasioptera donacis* (Diptera: Cecidomyiidae) was found in France, Spain, Italy, and Greece, mining the leaf sheaths of arundo. The leaf sheaths rapidly turn brown or black due to the presence of a fungus (Bon et al., 2018), and the larvae feed on both plant and fungal material (Botti et al., 2019). The presence of the leafminer accelerated leaf death in the field in Greece (Marshall et al., 2018b). This midge can infest only arundo (Goolsby et al., 2017b), and was permitted for release in the U.S. in December 2016. Additional research is needed to develop practical rearing and field release procedures.

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CHAPTER 32

Classical Biological Control of Air Potato Vine, *Dioscorea bulbifera*, Infestations in Florida

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NON-TECHNICAL SUMMARY

The air potato vine (or air yam), *Dioscorea bulbifera*, a member of the Old-World yam family (Dioscoreales: Dioscoreaceae), was introduced to the United States over two centuries ago and has become one of the most serious exotic invasive weeds in Florida. It vigorously invades disturbed and undisturbed habitats under public and private ownership across Florida, southern Georgia, Alabama, Mississippi, Louisiana, and Texas. Research shows the vine grows up to 25 cm (9 in) per day and branches profusely. These attributes enable air potato to climb up and over other vegetation, smothering trees, shrubs, and understory plants. It produces numerous aerial tubers (known as bulbils) as it grows, which fall to the ground when the vines die back in winter. The bulbils and underground tubers sprout in spring and repeat the seasonal growth cycle. Herbicidal, mechanical, and cultural methods used by land managers are costly, provide only temporary relief, and can cause damage to non-target plants in the area. In contrast to chemical controls, biological control agents offer a self-sustaining, environmentally friendly, and cost-efficient method for managing this weed. A biological control program against air potato was started in 2002 after a leaf feeding beetle, *Lilioceris cheni* (Coleoptera: Chrysomelidae), from air potato's native range in Nepal was accidentally discovered. Later, the same beetle was discovered in China. Extensive testing at the USDA-ARS Invasive Plant Research Laboratory in Fort Lauderdale, Florida showed that Nepalese and Chinese beetles are highly specific, so federal and state regulatory agencies approved their release in the United States. Adults and larvae feed on air potato leaves, ultimately causing vines to die early and significantly reducing bulbil production. The beetles are relatively less effective at controlling air potato vines in urban and suburban areas, where mosquito spraying programs interfere with the beetles' life cycle. Still, they are very effective in rural areas, federal, state, and local parks, and other natural areas with no or limited spraying.

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HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Dioscorea bulbifera (air potato, air yam; Dioscoreales: Dioscoreaceae) is an herbaceous vine (**Fig. 1**) of Afro-Asian origin (Burkill, 1960; Martin, 1974; Tindall, 1993). The first recorded introduction was in Florida in 1905, although there is evidence of *D. bulbifera* vines in a garden in Mobile, Alabama in 1777 (Bartram, 1998). Introduced into the United States as potential resources for food and medicine, several species of *Dioscorea* have escaped cultivation and now grow in the wild. Ironically, the air potato is the most widespread and best known of these, but it is also the only species whose extremely bitter foliage and tubers (Bhandari and Kawabata, 2005) render it largely inedible. Nehrling (1944, and see also Morton, 1976), an early Florida horticulturist, worried about the vine's potential to become invasive, as proved to be the case. Over time, *D. bulbifera* has become one of the most aggressive noxious weeds in the southeastern United States, particularly in Florida (Hammer, 1998). It has also become established in Hawaii, the West Indies, and Meso-America (Schultz, 1993; Gordon, 1998; Nesom and Brown, 1998; Center et al., 2013; Overholt et al., 2016; EDDMapS, 2020).

Figure 1. Typical *Dioscorea bulbifera* vine mats affecting natural areas with (a) relatively old dark green vines trellising and blanketing tree canopies, and (b) showing relatively young growth bearing vigorously growing foliage with expanding leaves and succulent stem-tips, smothering shrubby vegetation. (a: M. Rayamajhi, USDA-ARS; b: Jorge Leidi)

Burkill (1960) recognized two types of *D. bulbifera* (African and Asian-Polynesian), whose ranges may have become disjunct due to the desiccation of southwestern Asia about 10 million years ago. Martin (1974) also recognized two types, noting that edible varieties common in Africa produced angular bulbils (vegetative propagules produced on leaf axils). In contrast, the globular or spherical bulbils of Asia/Oceania were very bitter (Bhandari and Kawabata, 2005). Terauchi et al. (1991), using RFLP analysis, identified nine discrete chloroplast genomes that clustered into the same African vs. Asian/Oceania dichotomy. RAPD analysis (Ramser et al., 1996) further supports this dichotomy. Originally, *D. bulbifera* vines from Florida were thought to be African in origin (Overholt et al., 2003), and there are reports of an angular variety in the state. However, Florida vines primarily produce two varieties with globular/spherical bulbils: one with brown-colored, rough skin and another with tan-colored, smooth skin (**Fig. 2**; see also Fig. 1 in Terauchi et al., 1991). More recent chloroplast studies by Croxton et al. (2011) confirmed that both common morphotypes in Florida are of Asian origin, as their bulbil shapes suggest. Common garden studies showed these two bulbil types produce indistinguishable vines in terms of leaf morphology and growth phenology, but the bulbils produced on these vines remain true to their parental morphotypes (Rayamajhi et al., 2016, 2021).

Figure 2. Vegetative propagules (bulbils) of *Dioscorea bulbifera* (left) brown and (right) tan morphotypes. (M. Rayamajhi, USDA-ARS)

Dioscorea bulbifera plants are dioecious but produce only pistillate (female) inflorescences in Florida and thus do not produce seeds (Gordon, 1998). Flowering is rare and occurs towards the end of the growing season. The lack of sexual reproduction in North America means that its spread occurs exclusively via the aerial tubers (i.e., bulbils) vegetatively produced in leaf axils. These bulbils weigh from <1 g to >1 kg (<0.035 oz to 2.2 lb) and are produced from summer through early fall (Langeland et al., 2008; Overholt et al., 2016; Rayamajhi et al., 2016).

In the continental United States, air potato vines die during autumn when the leaves and most bulbils drop to the ground (Coursey, 1967; Center et al., 2013; Overholt et al., 2014, 2016; Rayamajhi et al., 2016, 2019, 2021). New shoots emerge from those bulbils and perennial subterranean tubers during the following spring.

Nature of the Problem

Dioscorea bulbifera is aggressive, forming impenetrable mats of vines that can blanket trees, shrubs, and surrounding vegetation (Nehrling, 1944; Morisawa, 1999; Air potato Task Force, 2008; Rayamajhi et al., 2019) (see **Fig. 1**). Recent research shows that the growth rate of *D. bulbifera* vines depends on propagule size; vines generated by larger bulbils grow faster than those from smaller bulbils (Rayamajhi et al., 2016). This weed is prolific, and in southern Florida bulbils weighing 1.0–372.6 g (<0.035–13.1 oz) can produce vines measuring 23–51 m (75–167 ft) long, growing 0.2–25 cm (0.08–9.8 in) per day, and bearing 0–365 bulbils in the leaf axils (Rayamajhi et al., 2016). *Dioscorea bulbifera* vines can be confused with those of edible yam species. However, *D. bulbifera* is easily differentiated from edible species by their cylindrical stems (Overholt et al., 2016) and bitter-tasting foliage and bulbils (Bhandari and Kawabata, 2005).

In North America, *D. bulbifera* spreads mainly through bulbils disseminated by anthropogenic means (Schultz, 1993; Rayamajhi et al., 2016), although hurricane winds can also disperse bulbils (Horvitz et al., 1998). *Dioscorea bulbifera* vines exploit and colonize disturbed sites, including hurricane-damaged natural areas, and such invasions interfere with recovery by native vegetation (Gordon, 1998; Horvitz et al., 1998; Horvitz and Koop, 2001). Vines in older infestations form solid canopies that significantly reduce light penetration, weigh down and collapse supporting vegetation, and ultimately kill plants underneath mats

(Schmitz et al., 1997; Center et al., 2013). *Dioscorea bulbifera* infestations that begin with a single vine can eventually develop into a contiguous patch of innumerable vines covering several hectares (Rayamajhi et al., 2019). Currently, *D. bulbifera* infestations occur in all 67 Florida counties and in southern parts of other Gulf Coast states (Croxton et al., 2011; Overholt et al., 2016; Rayamajhi et al., 2021).

WHY CONTROL THIS INVASIVE?

Air potato is capable of causing both ecological and economic damage in an invaded ecosystem. Unchecked rapid growth by this invader, with its ability to smother canopy and understory vegetation, can convert large species-rich expanses of natural areas into monotypic stands that continue to expand through bulbil production (Rayamajhi et al., 2016). Equipped with these invasive attributes, *D. bulbifera* has become a transformer species that diminishes plant communities by displacing native flora and altering community structure, ultimately disrupting ecological processes and functions (Gordon, 1998; Overholt et al., 2014), as predicted by Nehrling (1944). Introduced primarily during the early 1900s, by the 1980s *D. bulbifera* vines had already smothered hedges, ornamental plants, fences, and vacant properties (Bell and Taylor, 1982), and covered electric poles and transformers (M. Rayamajhi, pers. obs.).

Traditionally, land managers and private property owners used various cultural, mechanical, or herbicidal control methods to temporarily suppress air potato patches on their properties (Overholt et al., 2014). However, treating air potato vines with herbicides is relatively ineffective and can harm non-target plants (Overholt et al., 2014). With or without herbicide treatment, air potato foliage dies back at the end of each growing season, but the perennial subterranean tubers and seasonally produced bulbils, which are unaffected by herbicide treatments, remain to resprout the following year (Overholt et al., 2014; Rayamajhi et al., 2016, 2019). Mechanical methods involving the complete removal of *D. bulbifera*, including extraction of subterranean tubers, may cost several thousand dollars per hectare (Miami Dade County Natural Areas Management, pers. comm.). It is difficult to get an accurate estimate of the cost of air potato eradication because contractors may remove a range of other invasive species during a control operation without differentiating the proportions each species contributed to the overall job. For example, complete removal of several exotic invasive plants including *D. bulbifera* from an 0.809 ha (2 acre) patch in the Everglades National Park was estimated to cost $30,000 (Overholt et al., 2014), and in Fern Forest (Broward County, Florida), the cost of removing *D. bulbifera* and other invasive plants was estimated at $1,750/ha/yr ($709/acre/yr) (Wheeler et al., 2007).

One of the most popular cultural methods of reducing the spread of *D. bulbifera* was through annual bulbil collection and disposal events, popularly known as “air potato roundups” (Weaver, 2008), conducted by students and volunteer groups. These activities intensified public attention on damage from *D. bulbifera* infestations in Florida and helped remove potential sources of new (or re-) infestations (Overholt et al., 2014). Three practical reasons have been cited for the popularity of these programs: 1) *D. bulbifera*’s high prevalence and distinctiveness helped volunteers easily recognize the plant, which was described as a menace to natural areas, public parks, and private lands; 2) picking up bulbils resembling potatoes required little training regardless of volunteer ages and abilities, and required no specialized tools (only buckets); and 3) events could be scheduled during winter, when most of the bulbils have fallen to the ground, and those that have not dropped are easy to see on the dead vines.

However, these methods alone are inadequate and cost-inefficient for long-term suppression of this weedy vine (Wheeler et al., 2007). Rayamajhi et. al. (2016) argued that only self-sustaining control methods that could reduce growth rate and propagule (bulbil) production would mitigate the further spread and smothering impact of *D. bulbifera* vines on native plants.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Air potato in the United States has only a few close relatives and no known specialist natural enemies, and was therefore considered an appropriate target for biological control (Pemberton and Witkus, 2010). Initially, its native range was thought to be on the African continent, and surveys for biological control agents were begun in Ghana and Uganda in June of 2003 (Overholt et al., 2003; Wheeler et al., 2007). However, no host-specific agents were discovered during these surveys (Overholt et al., 2014). In 2002, a chrysomelid beetle was discovered by USDA scientists R. Pemberton and M. Rayamajhi while conducting a survey in and around the Kathmandu Valley for biological control agents of different invasive weeds (*Lygodium microphyllum* and *Paederia foetida*). This beetle's adults and larvae were observed skeletonizing leaves and scraping young bulbils on a few vines that were climbing on native shrubs (Pemberton and Witkus, 2010). These beetles were initially identified as *Lilioceris* sp. nr. *impressa* (Pemberton and Witkus, 2010).

Chrysomelid beetles that have been used as biocontrol agents on other systems have generally been effective (Van Driesche et al., 2008). Consequently, the Nepalese beetles were imported into the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, Florida in 2004 for further study, to potentially initiate an air potato biological control program (Pemberton and Witkus, 2010). These beetles were later identified as *Lilioceris cheni* (**Fig. 3**) based on morphological and molecular studies (Tishechkin et al., 2011; Center et al., 2013).

An extensive host testing process was done of the beetle's feeding behavior, reproductive potential, and overall life cycle on various *Dioscorea* species from the Caribbean, Meso-America and North America, as well as 41 other plant species of economic or environmental importance. These studies demonstrated that *L. cheni* (hereafter, the air potato foliage-feeding beetle or APFB) from Nepal had a very narrow host range, feeding and completing its life cycle almost exclusively on *D. bulbifera* foliage (Pemberton and Witkus, 2010). Later, the beetles were occasionally observed feeding on tender bulbils of this vine at the end of the growing season when green foliage in the field was scarce and the adults were about to enter a quiescent winter period (Center et al., 2013; Rayamajhi et al., 2019). Permission for field releases of the Nepalese biotype of APFB was obtained from the USDA Animal and Plant Health Inspection Services (APHIS) in 2009, but unfortunately by that time the quarantine colonies had died out.

Surveys in southwestern China during 2010–11 located a Chinese biotype of APFB with red elytra (in contrast to the orange Nepalese APFB; **Fig. 3d**). During the 2011 surveys, a second chrysomelid beetle was discovered by F.A. Dray and G. Witkus that seemed to feed in the bulbils. This beetle, later identified as *Lilioceris egena* (Center et al., 2013), and the APFB were imported into IPRL for further testing. Studies on APFB confirmed that the Chinese beetles were the same species and had the same host specificity

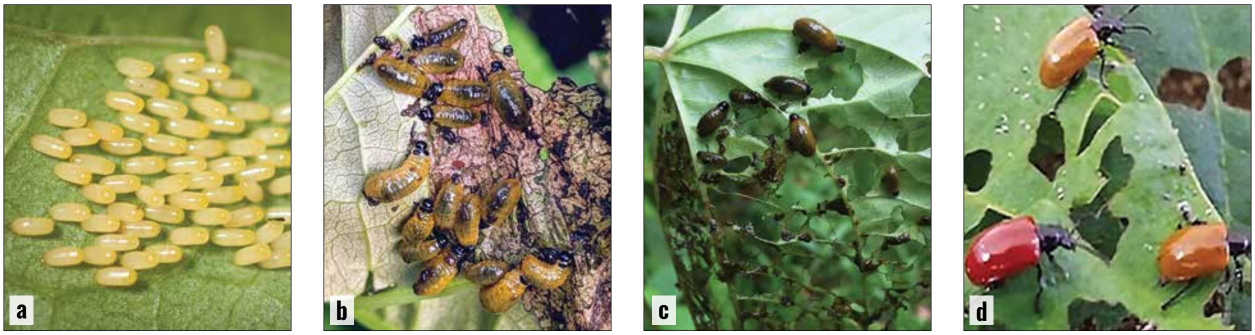

Figure 3. *Lilioceris cheni* (a) egg mass; (b,c) aggregated larvae gregariously feeding on the undersurface of succulent *D. bulbifera* leaves, (b)1st and 2nd-instar larvae feed from lower surface and skeletonize leaves by leaving upper epidermis intact while (c) 3rd and 4th-instar feed through and perforate leaves like the adults do; (d) Chinese (red) and Nepalese (orange) biotypes also feeding and creating holes on the lower surface of *D. bulbifera* leaves. (a: Melissa Smith, USDA-ARS; b: Irvin Louque, iNaturalist.org CC BY-NC-ND 4.0; c: Carrie Seltzer, iNaturalist.org CC BY-NC 4.0; d: Jenna Owens, USDA-ARS)

(based on 10 *Dioscorea* spp.) as the Nepalese beetles, so field tests began in November 2011 (Center et al., 2013). Additional shipments of originally approved Nepalese APFB adults were imported during 2012 and incorporated into the field-release program.

Samples of both the Nepalese and Chinese APFB biotypes were shipped to collaborators at the Florida Department of Agriculture and Consumer Services (FDACS) in Gainesville, Florida, and the University of Florida (UF), Indian River Research and Education Center in Fort Pierce as part of genomic technology transfer activities in 2012–2013. The USDA and collaborating agencies subsequently reared and field released beetles in nearly all 67 counties in Florida (Rayamajhi et al., 2019, 2021; Kraus et al., 2022). Later, a few hundred beetles of both biotypes were shipped to Drs. Rodrigo Diaz (Louisiana State University) and Veronica Manrique (Southern University and Agriculture and Mechanical Engineering College) in Baton Rouge, Louisiana. Drs. Diaz and Manrique, along with FDACS scientists, have released additional beetles at various locations in the southern parts of Alabama, Louisiana, and Texas.

Mass rearing of APFB required abundant, healthy (insect pest- and disease-free) *D. bulbifera* plants, which was most easily achieved by starting with the vegetative propagules (bulbils). Each year, from November through February, our laboratory acquired a few hundred kg (>100 lb) of bulbils from the previous growing season, both via our own direct collections and donations from various parks and volunteer organizations. These bulbils were stored in the dark at 10°C (50°F) to inhibit sprouting until they were needed to produce vines for foliage to feed APFB. We maintained over 1,000 potted plants on benches in screenhouses that provided partial shade and daily watering via overhead sprinklers. These plants were routinely checked for unwanted pests and pathogens and were promptly subject to remedial actions as appropriate.

We initially reared APFB using a slight modification of the techniques developed by Pemberton and Witkus (2010) for maintaining colonies in the IPRL biocontainment. Several adult beetles were placed in 81.5 x 39.5 x 39.5 cm (32 x 15.5 x 15.5 in) acrylic boxes, each holding 1 to 2 small plants in 3.8 L pots. Adults were allowed to oviposit for several days, and then moved to another cage, thereby allowing the eggs (**Fig. 3a**) to hatch and larvae to develop in the original cages. However, these methods were best suited to produce the relatively small numbers of insects needed for host range trials, and so we adapted our approach to facilitate production of larger numbers of insects for a mass-rearing program. We reared APFB in 1.8 x 1.8 x 1.8 m (6 x 6 x 6 ft) Lumite® mesh cages, each containing six air potato plants trellised over bamboo hoops inserted in 11.4 L pots (Center et al., 2013; Halbritter et al., 2021). One side of each cage was fitted with a 1.8 m (6 ft) long metallic zipper to allow for closing the cage to keep beetles from escaping, and opening to allow people to walk in and out to inoculate plants with beetles and hand water plants as needed. Each cage was inoculated with 5–7-day-old adults, including gravid females that oviposited on the lower surfaces of the young tender leaves. Eggs hatched within 5 to 7 days, and larvae (**Fig. 3b**) gregariously fed on leaves and growing tips, becoming 3rd or 4th instars (**Fig. 3c**) within a week. These larvae were removed from the caged plants and transferred onto fresh *D. bulbifera* leaves placed on a ca 2.5-cm (1 in) layer of slightly moist, inert commercial media (vermiculite) in a plastic bin with ventilated cover. After feeding briefly, the larvae burrowed into the media, developed cocoons, and emerged as adults (**Fig. 3d**) in 14–16 days. In most cases, this beetle completed its life cycle in 28–35 days. New adults were allowed to mate for ca 5–7 days and used for experimental purposes or field releases.

Usually, *L. cheni* beetles overwinter from December through early March in southern and central Florida (Rayamajhi et al., 2021); this overwintering period may be slightly longer in the more temperate climates of southern Georgia, Alabama, Mississippi, Louisiana, and Texas. Factors that may affect the length of the beetle's overwintering period include the availability of green vines (e.g., frost-protected areas could have foliage available later in the growing season) as well as soil organic matter content and moisture. Knowledge of these factors permitted us to produce *D. bulbifera* plants year-round by growing under controlled indoor conditions with desired day lengths. This method allowed us to prevent beetle colonies from entering diapause (the inactive period during winter) and reproduce as if in summer. Maintaining active indoor colonies during winter permitted us to have beetles ready for field-release as soon as *D. bulbifera* plants in the field began to sprout and grow.

Dispersal techniques varied somewhat among agencies. USDA scientists packed 10–100 adults into sandwich- to shoebox-sized plastic containers with freshly collected air potato leaves to allow feeding while adults were transported to release sites. Property owners and land managers were contacted before release events, beetles were delivered in person, and questions or concerns about the *D. bulbifera* biological control program were discussed and answered at the time of the releases. The approaches used by FDACS and UF were similar, except that boxes of beetles were shipped to landowners and extension agents upon request, relying upon them to conduct the actual releases (Kraus et al., 2022). Over the first six years of this project, over 600,000 beetles (both biotypes combined) were released (USDA: 343,326; FDACS: 246,747; and UF: 41,224) at more than 6,000 sites throughout Florida. Our collaborators, especially FDACS and LSU scientists, have continued to spread beetles to new locations since 2018, both in Florida and other states (see Kraus et al., 2022 for further information on more recent Florida releases).

While APFB was being dispersed throughout the southeastern United States, the second beetle (*L. egena*) was undergoing host range testing. A total of 82 plant species (including 15 species of *Dioscorea*) were used in the vetting process, but only *D. bulbifera* proved to be an acceptable developmental host (Dray, 2017). These studies also confirmed that this beetle can complete development on air potato leaves but prefers the bulbils. Larvae from a single 12–18 egg cohort can devastate a bulbil before pupating. Results of the host trials were submitted to federal and state regulatory agencies (Dray, 2017), and in March 2021 the USDA-APHIS issued a permit for release of *L. egena* in the United States. Initial releases were conducted in cages at IPRL during August of 2021 as part of a study examining whether the beetles would attack bulbils on the vine as well as those on the ground (F.A. Dray and M. Rayamajhi, unpub. data). Field releases began in October 2021 at two county parks in Broward County, Florida and are continuing.

HOW WELL DID IT WORK?

The ability of *D. bulbifera* to exploit natural and disturbed habitats in rural and urban landscapes made air potato a threat to diverse ecosystems across invaded areas. In its adventive range, the major invasive attribute of *D. bulbifera* is its high growth rate, which in the United States is second only to kudzu (*Pueraria montana* var. *lobata*). Air potato's ability to grow both vertically and horizontally by climbing up and over shrubs and trees to form dense mats, and its prolific vegetative reproduction, made this vine a high-impact invader in both native vegetation and urban landscapes (Rayamajhi et al., 2016). Trees as tall as 5–10 m (16–33 ft) in our experimental plots were observed to be smothered and killed within four years by *D. bulbifera* vines.

In our test plots, *L. cheni* was multivoltine, completing its life cycle in 28–40 days, depending upon food quality and environmental conditions (Center, 2013; Manrique et al., 2017). The APFB proved to be relatively easy to rear and was found to reproduce and spread quickly in the field, provided there were plenty of *D. bulbifera* vines with tender foliage for oviposition and larval feeding at the release site. Both biotypes of APFB are well adapted across the latitude of *D. bulbifera*'s known distribution range in the United States, but the Nepalese biotype may be better suited to the more northern part of the weed's invaded U.S. range due to high fecundity early in the growing season as compared to the Chinese biotype, whose fecundity is more evenly distributed throughout the growing season (Smith et al., 2018). As few as ten beetles can establish a self-sustaining population, but larger release sizes of 50 or more accelerates *D. bulbifera* vine damage and promotes quicker establishment at the field sites (Lake et al., 2018).

Successful biological control of an invasive plant in its adventive range is possible only when the phenology of the target host and biological control agent remain in synchrony. In the air potato-APFB system, the weed and its biological control agent have retained synchrony after being reunited in Florida, despite the vine having experienced more than a century of freedom from suppressive agents (Rayamajhi et al., 2021). The APFB is environmentally plastic in Florida (Smith et al., 2018), and this attribute, coupled with its broad distribution both latitudinally and altitudinally in Asia, suggests APFB appears to have the ability to keep up with its host if *D. bulbifera* extends its U.S. range northward in response to climate changes.

Dioscorea bulbifera vines in Florida sprout during March from perennial underground tubers and the aerial bulbils produced during the previous growing season; new bulbils begin to develop around June in Florida (Rayamajhi et al., 2021). This general pattern varies by up to a month depending upon differences in microenvironment and the latitude of the infestation. There appears to be a 3-month delay between vine appearance and bulbil development in Florida. Similarly, *L. cheni* beetles exit their winter diapause (inactive period) and become more active (and thus detectable) by April in southern Florida and by May in the north-central part of the state. Although APFB feeding reduces bulbil production (see the following discussion) it does not eliminate these vegetative propagules entirely. To address this problem, USDA has recently initiated releases of another biocontrol agent, the bulbil-feeding beetle *L. egena*. However, it is too early to evaluate how successful this agent will be at reducing bulbil production and viability.

To evaluate the impact of the first agent released against air potato, a 5-year project was carried out to measure APFB's ability to reduce bulbil production by *D. bulbifera* in the field (Rayamajhi et al., 2019). At release sites, APFB demonstrated a J-shape (accelerating) population growth curve when foliage was abundant at the time of release (Rayamajhi et al., 2019). At field research sites located in Alachua, Manatee, Broward, and Miami Dade counties, APFB damage lowered mean *D. bulbifera* coverage from 73% in 2012 to less than 23% in 2016 (Rayamajhi et al., 2019). Vines died within a month or so once 40% or more of the vine area had been damaged by skeletonization or perforation of leaves by larval and adult feeding (**Fig. 4**), which eliminated further bulbil development and growth. Lower levels of feeding damage resulted in smaller bulbils and longer times to plant death (M. Rayamajhi, pers. obs.). Reductions in vine cover

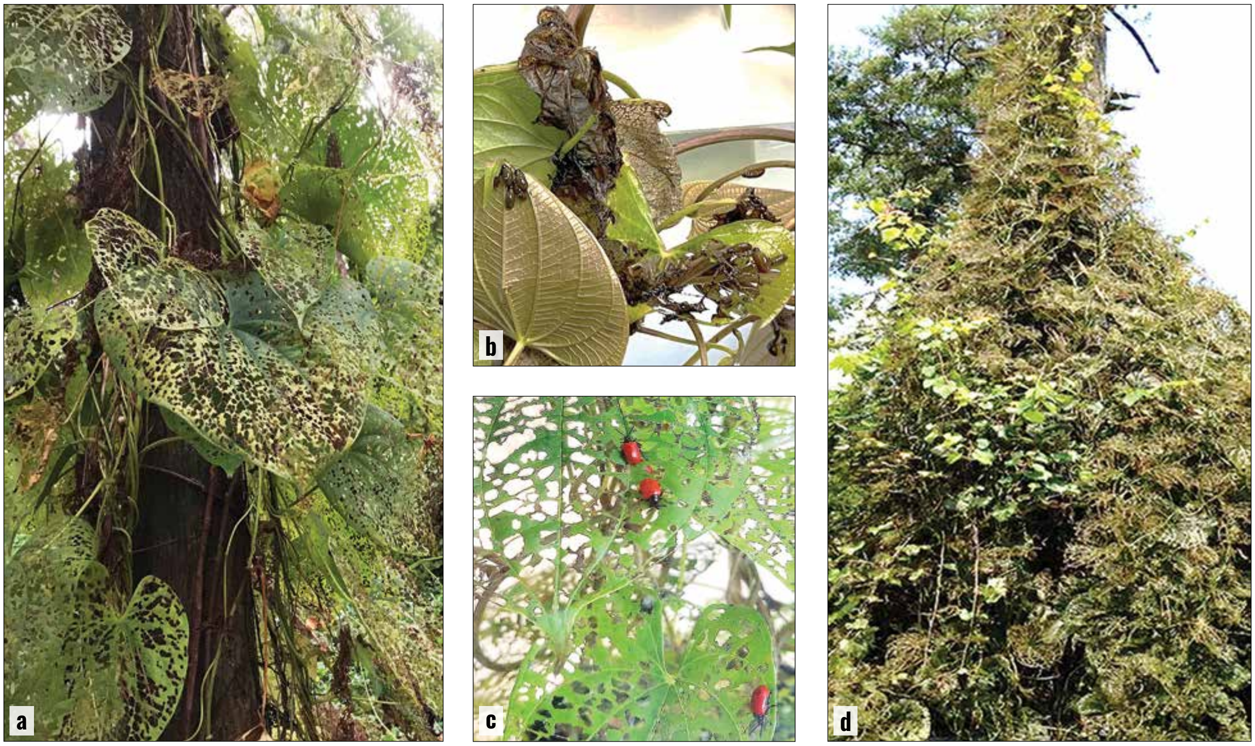

Figure 4. Typical *Lilioceris cheni* adult damage on *D. bulbifera*: (a) leaves of all ages with numerous feeding holes which stopped the vines from branching and blanketing trees and forming new bulbils; (b) expanding tender leaves colonized and gregariously fed upon and partially skeletonized by 2nd and 3rd-instar larvae; (c) three adults of the Chinese biotype feeding and creating holes on mature leaves; (d) severe defoliation of the *D. bulbifera* vines engulfing the bottom portion of a large tree; note emergence of new growth of *Vitis* species following severe defoliation of air potato by beetle larvae and adults. (a; Dale Halbritter; b,c: Jenna Owens; d: M. Rayamajhi; a–d: USDA-ARS)

released smothered vegetation from air potato competition, allowed better growth of previously suppressed perennial plants, and improved species richness (Rayamajhi et al., unpub. data).

Propagule densities in research plots (measured as the number of bulbils on the ground after vine senescence at the end of the growing season) were reduced from 20–42/m^2 (2–4/ft^2) at the onset of the experiment in 2012 to <1/m^2 (<0.09/ft^2) in 2016 in the beetle-treatment plots vs. from 12–56/m^2 (1–5/ft^2) in 2012 to 1–5/m^2 (0.09–0.5/ft^2) in 2016 in control plots (which were treated with granular systemic insecticide at 4-month intervals); both plots were located in the same general area (Rayamajhi et al., 2019). APFB damage to the foliage in control areas became quite high, causing early vine death. As the massive numbers of beetles attempted to feed on pesticide-poisoned vines in the control plots (when healthy foliage at the site became scarce) towards the last quarter of each growing season during the 5-year experimental period, they died within hours after feeding; nevertheless beetles damaged the vines (Rayamajhi et al., 2019). Bulbil biomass in control plots, however, remained unchanged over time because plots were generally free of beetles for three quarters of the growing season, by which time most bulbils had already developed to their mature size. Overall reductions in bulbil density and size at APFB-treated sites explains why many parks and recreational areas in Florida (e.g., Alachua County) stopped annual bulbil roundup programs several years into the biological control program (Jester, 2015; Overholt et. al., 2016).

APFB impacts, in terms of beetle population increase and subsequent reduction of *D. bulbifera* vines, varied between natural areas and urban or suburban areas where mosquito control programs were carried out against malaria and the Zika virus vectors. In areas sprayed for mosquitoes, all life stages of the beetle (egg clutches, larvae, and adults) would be detectable for 2–6 weeks following an APFB release, and vine damage often appeared impressive at first. Soon thereafter, however, all traces of the beetle's population would disappear. This prompted us to test the acute toxicity of two commonly used mosquito-control pesticides (naled and permethrin). Both pesticides caused mortality to the beetle, which was especially sensitive to permethrin (Wheeler et al., 2020). The APFB will likely remain relatively less effective in areas with active mosquito control programs. It is hoped that the second agent, the bulbil-feeding beetle, will be less susceptible to mosquito-control pesticides because the larvae feed inside the bulbils and seal their external openings to their feeding chambers with their own frass (F.A. Dray, pers. obs.).

In non-sprayed natural areas, APFB populations were observed to frequently and quickly infest and damage large swaths of *D. bulbifera* vines by the middle of the growing season, resulting in heavy damage (**Fig. 4**) and large adult and larval populations. Adults then dispersed from such populations to other sites in search of food to produce another generation of beetles. This cycle of destruction of air potato plants, followed by beetle dispersal, was repeated until the end of the growing season (October–December) when vines begin to senesce naturally, forcing adults to overwinter at their last location. The life span of adult beetles in the field varies from three to six months (M. Rayamajhi, pers. obs.), although the average laboratory lifespan was five months (Pemberton and Witkus, 2010). Many adults die while overwintering. Young adults that emerge at the end of the growing season successfully overwinter in dried-up *D. bulbifera* leaves that remain on the vines, under tree bark, or in the duff on the ground. These adults emerge the following growing season with depleted fat reserves and may take over a month of feeding on freshly regenerated *D. bulbifera* foliage before they mate, reproduce, and become abundant enough to be readily detectable by the general public. During this lag period between winter beetle quiescence and high population abundance, many land managers and property owners become impatient because, during the same time, vines are growing rapidly and the beetles that were present and so devastating the previous year seem absent. This spring lag can induce some landowners to turn to herbicides, further delaying APFB build-up on their properties. Through various forestry and agriculture extension programs and *D. bulbifera* working groups, we (USDA, FDACS, UF) have been working to provide clients and stakeholders with a proper understanding of the APFB's population ecology and life-stage synchrony with its host air potato (Rayamajhi et al., 2021) to reduce concerns about the delayed arrival at their property.

BENEFITS OF BIOLOGICAL CONTROL OF AIR POTATO

In summary, *L. cheni* has shown dramatic population increases, caused highly visible, substantial damage to *D. bulbifera* infestations, and reduced this weed's impact on native vegetation in Florida and beyond. Indirect but positive side effects include (1) increased plant diversity due to recovery of native species in places once occupied by *D. bulbifera*; (2) reduced use of herbicides against air potato and associated damage on non-target vegetation; and (3) reduced need for *D. bulbifera* roundup events (Weaver, 2008), permitting environment enthusiasts and students to redirect their attention to control activities for other invasive plants (Jester, 2015, Overholt et al., 2016). Certain hemipteran predators and spiders have occasionally been observed preying upon APFB larvae and adults. However, an increase of predators from the addition of a new food resource (APFB) has not been observed.

UNFINISHED BUSINESS WITH AIR POTATO BIOLOGICAL CONTROL

As mentioned earlier, damage by APFB significantly reduces the size and density of bulbils produced in *D. bulbifera* infestations. However, the bulbils produced during a growing season in areas with either mosquito pesticide sprays or delayed arrival of the leaf-feeding beetle contribute substantially to replenishing infestation levels in subsequent years. Thus, the USDA initiated a project investigating the bulbil-feeding beetle, *L. egena*, as a potential addition to the air potato biological control program. Laboratory studies show that damage by this beetle (the air potato bulbil beetle, or APBB) can prevent bulbils from sprouting. Early field trials show that the APBB attacks young bulbils on the vine as well as mature bulbils on the ground (Dray & Rayamajhi, unpub.). It is too early to predict the outcome of these releases, but by attacking different plant parts, APFB and APBB together should remove the competitive advantages that air potato vines have had in Florida's landscape, promoting natural restoration of native species.

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