FELLOWSHIP SUMMARY REPORTS

- Your name:
  - **Valerio Hoyos-Villegas**
- The subject title and theme number of your research fellowship:
  - **"Merging Engineering and Plant Breeding: Bendable Electronics and Sensing Technologies to Improve Plant Productivity"**
- Your host institution:
  - **University of Glasgow/Northeastern University**
- The name of your host collaborator:
  - **Ravinder Dahiya**
- The dates of your fellowship:
  - **2 July 2022 to 24 September 2022 (first half)**
  - **8 September 2023 to 20 October 2023 (second half)**
- Your consent to your report being posted on the Co-operative Research Programme's website, or alternatively, a short paragraph about your fellowship which could be used anonymously.:
  - **I consent**

1. What were the objectives of the research project? Why is the research project important?

Objectives

- To refine the technical specifications of bendable sensors to fit the sensitivity of pH changes due to white mold infection in common bean
- To refine the technical specifications of bendable sensors to fit the surface moisture and texture conditions of pH changes due to white mold infection in common bean
- To conduct preliminary inoculation trials with white mold while simultaneously monitor pH changes due to pathogen infection

2. Were the objectives of the fellowship achieved?
Or are they on the way to being achieved?
If not, for what reasons? (The data or research is still ongoing or being analysed; technical reasons (e.g. equipment not working, adverse weather conditions, unexpected results, etc.; other reasons?)

General comments

This collaboration has led into new insights into the use of bendable sensors for monitoring plant characteristics, such as pH. It is my pleasure to report that our estimation of the objective originally set out in the proposal where largely achieved in the two six-week periods (2022 and 2023) conducted as part of this fellowship.

Materials and methods

Three flexible pH sensors were tested. The first of which consisted of a fabric substrate [67] with a silver (Ag) reference electrode and will henceforth be referred to as the fabric sensors. The second sensor consisted of a polymer substrate with an Ag / silver chloride (AgCl) reference electrode and will be called the silver sensor. Lastly, the third sensor had a polymer substrate with a carbon reference electrode and will be known as the carbon sensor. The sensitive electrode of all three sensors was composed of graphite polyurethane (G-PU).

The sensors were connected to the analog (A0) and ground pins on the Arduino. To test the sensors, pH buffer solutions at pH 4, 7, and 10 () as well as hydroden chloride (HCl) / potassium hydroxide (KOH) solutions of pH 4, 5, 6, 7, 8, 9 were prepared. The pH of these prepared solutions were verified using a pH meter (model/needed).

Hysteresis effects were determined by gradually increasing the pH of an HCl/KOH solution with an initial pH of 4, followed by gradually lowering the pH. Initial voltages readings were compared to final voltage readings. For the

hysteresis experiment, voltage readings in carbon sensor 2 were transformed to account for the backwards wiring. An inverse transformation was performed and scaled up to match the range of carbon sensors 1 and 3.

Flexible pH sensors were then applied to the 8 common bean varieties. The sensors were used to take voltage readings of three separate leaves from each plant, prior (mock-) inoculation and 8 days after (mock-) inoculation. The sensors were held in place with vinyl paper clips, such that the reference and sensitive electrodes would be in contact with the back side of the leaf, where the treatment site was located. Readings were allowed to stabilize for 30 seconds before measurements were taken for 60 seconds. pH sensors were rinsed with distilled water and allowed to dry between measurements.

3. What were the major achievements of the fellowship? (up to three)

Results

Three types of flexible miniaturized pH sensors that differed in base substrate and reference electrode were compared. The three sensors were initially used to measure the voltage of three standard pH buffer solutions. The voltage readings from the carbon sensors ranged from 4.89 mV to 239.49 mV. The voltage readings from the silver sensors had values between 53.76 mV and 171.07 mV. Lastly, the voltage readings from the silver sensors fell in the range of 14.66 mV and 43.99 mV, indicating that it had the lowest sensitivity of the three sensors. Since the carbon sensors were found to have the greatest sensitivity of the three tested sensors, we proceeded to use the carbon sensors moving forward.

The leaf apoplastic pH was determined for the 8 varieties before and after (mock-) inoculation (Fig. 5 – currently labelled Fig 6). For the mock-inoculated group, the pH increased following mock-inoculation for all varieties. For the inoculated group, the pH increased following inoculation for 5 varieties, specifically the three susceptible (Othello, Montrose, and Beryl), one intermediate (Bunsi), and one partially resistant (WM-1) variety. The pH decreased following inoculation for 3 varieties, specifically one intermediate (Eldorado) and two partially resistant (WM-12 and G122) varieties. The analysis of variance indicated that pH between inoculated and mock-inoculated treatments were not statistically significant (Table S1). + need to also include another supplemental table with all the raw data – white mold scores and so forth.

The carbon sensors were used to assess the pH of intact common bean leaves before and after inoculation with Sclerotinia. Since repeated use of the sensors wore them down over time, only carbon sensor 1 was used to measure the voltages. In the mock-inoculated group, half of the varieties showed an increase in voltage following mock-inoculation, while half showed an increase in voltage. More specifically, Montrose, Eldorado, G122, and WM-1 had an increase in voltage, while Othello, Beryl, Bunsi, and WM-12 had a decrease in voltage. There were no predictable trends observed for the susceptibility groupings. In the inoculated group, 4 varieties had an increase in voltage after inoculation, specifically one susceptible (Montrose), two intermediate (Eldorado and Bunsi), and one partially resistant (G122) variety. Meanwhile, 3 varieties had a decrease in voltage, specifically one susceptible (Beryl) and two partially resistant (WM-12 and WM-1) varieties. One susceptible variety, Othello, had no change in voltage following inoculation. The analysis of variance indicated that voltages between inoculated and mock-inoculated treatments were statistically significant (Table S2). Differences in least square means were determined for susceptibility. The mean difference between susceptible and partially resistant varieties was 10.75. The mean difference between susceptible and intermediate varieties was not statistically significant. Lastly, the mean difference between the intermediate and susceptible varieties was 9.71.

Conclusions

This case study is a proof-of-concept with preliminary data for the use of bendable pH sensors for monitoring plant diseases. A minor correlation found between the voltage readings and the straw test score, suggesting that with further refinement, their feasibility to track pH-induced changes due to disease may be possible in the future. Increasing resolution and accuracy in the measurement of pH in plants will provide an avenue for estimating plant health and overall homeostasis, which is desirable in agriculture. Further work and development of accurate, reliable, inexpensive and biodegradable bendable pH sensor units is required in order to bring this technology to the fore.

4. Will there be any follow-up work?

- Is a publication envisaged? Will this be in a journal or a publication? When will it appear?

We are in the process of drafting an initial, proof of concept publication that will be published in the computers and agriculture or sensors journals. We anticipate having this publication ready within the following year As we are in the process of finishing up some validation experiments as well as analyzing the data remaining.

- Is your fellowship likely to be the start of collaboration between your home institution and your host?

As part of this fellowship, I am pleased to inform you that a grant application was approved by the European Consortium CHIST-ERA, which will bolster the capacity for work in the sensors across multiple research groups in Europe and Canada. We are in the process of conducting the expanded research associated with this subject, which started with this fellowship.

- Is your research likely to result in protected intellectual property, novel products or processes?

It is uncertain at this point if the research will produce any new intellectual property, but with the new consortium agreement it is possible that there will be new designs and materials explored and developed in order to bring the applications of sensors as a reality for plant monitoring.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

Potential contribution (As outlined in the proposal document)

Economic aspects: Fewer white mold epidemics and yield losses over time will increase farmer income and will contribute to the economic sustainability of pulse production and agriculture. The information gained from the construction of the sensor and the epidemiological data may create a new opportunity for technology companies to delve into a new area of product manufacturing, such as disease monitoring.
Environmental aspects: Improved management and agronomic decision-making tools will impact on the reduction of fungicide pressure and CO_2 emissions from agricultural machinery, thus contributing towards environmental sustainability goals.

This project will enable a new section of agricultural and environmental to gain insight and resolution on the implementation of policy. New information on the spatial and temporal impact of agricultural policies on the management of plant diseases will enable governments and local organizations to monitor the impacts of practices more closely while gaining real-time data for effective decision-making. Local farming organizations will be capable of deploying practices and prioritizing investment activity to address plant productivity problems in a timely fashion. Over time, real-time knowledge of disease pressure will enable a better understanding of disease foci. This understanding will allow for risk in agricultural production to be minimized by local farming industry and organizations. By reducing risk, the probability of crop losses will be diminished, individual farm profitability will increase and insurance premiums and claims will decline.

6. How was this research relevant to:

- The objectives of the CRP?
- The CRP research theme?

This project is a perfect example of international cooperation among OECD countries. The two institutions (McGill University and University of Glasgow/ Northeastern University) have come together from interdisciplinary areas to bring a solution to a problem around plant health status monitoring. This is an emerging area of Science and engineering but thanks to funding efforts like this it will provide the necessary elements in order to build up

preliminary data in order to bolster it and grow it into higher levels of application and deployment into technologies for farmers and other individuals involved in plant research.

In order to address the growing problems related to global fruit production, greenhouse gas mitigation, labor issues, and related to food prices, it is important to bring new sources of information and technologies that Enable new industries to develop and facilitate the much needed increases in productivity in agriculture. This project has indirect implications into global issues such as food security and climate change in the sense that it provides new avenues for the exploration of plant characteristics in the field, which in turn result in a step change towards understanding the factors necessary in order to minimize plant productivity losses. It also enables plant scientists with a better tool to ask questions around the biochemical and biophysical processes that take place in plants, thus expanding the knowledge and resolution to drive agricultural science forward.

7. Satisfaction

- Did your fellowship conform to your expectations?

Totally. Enabled me to engage in an area of research that I would normally not be involved in, and develop a new collaboration which is already resulting in positive outcomes for the research subject.

- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.

Yes, this fellowship will enable me to gain recognition and disability in the research community around the development of plant sensors for internal plant phenotypes.

- Did you encounter any practical problems?

Yes the COVID 19 pandemic placed major delays in the development of this project, given that it imposed serious restrictions around my ability to travel. Outside of that, I did not encounter any major logistical problems related to carrying out the activities indicated in the proposal.

- Please suggest any improvements in the Fellowship Programme.

None, the program administrators and the program itself involve the necessary elements in order to make it a successful program. Involved a preliminary stage where the proposal and research were outlined, necessarily indicating how the research was impactful. From that point on, the fellowship was initiated smoothly (with the exception of the covid 19 pandemic issues) And without any problems from the administrative side.

One suggestion would be that an increase in the amount of funding available should be considered given that post-covid 19 pandemic cost related increases in travel and lodging costs make it less feasible for researchers among OECD member countries to travel as part of the fellowship.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?

My collaborator at the University of Glasgow informed me of it.

- What would you suggest to make it more “visible”?

More advertisement and promotion to make it more visible among researchers of partner countries, while also indicating the requirements and potential areas of research that may be funded

- Are there any issues you would like to record?

No major issues.