Research Committee Update October 2020 for BCHPA AGM

The research committee, composed of Liz Huxter, Ali McAfee, Gerry McGee and chaired by Heather Higo, met via teleconference in April 2020 to consider ideas for potential projects to make use of current available funding. Due to the Covid situation and the restrictions placed on researchers to access labs and carry out any project work, the decision was made to ask the BCHPA executive to request a deferral of 2020 funding.

Updates on previous projects:

1) Testing of BC queens using fluorescent microscopy – one article on this work has now been published: (McAfee, A., Milone, J., Chapman, A. *et al.* Candidate stress biomarkers for queen failure diagnostics. *BMC Genomics* **21,** 571 (2020). https://doi.org/10.1186/s12864-020-06992-2) to be followed by a news article about this research coming in Jan 2021 in Scientific American. A second research paper has been submitted.

2) Bee Health in Blueberry Pollination (Project Leader: Dr. Marta Guarna, Agriculture and Agri-Food Canada, Beaverlodge, AB). Since our last report, we have completed most of the pathogen analysis and quantified the bacterial etiological agent of EFB, *Melissococcus plutonius*, in adult honey bees collected at different time points from pollinating and non-pollinating colonies. Levels of *M. plutonius* increased over time, and were generally higher in blueberry-pollinating colonies compared to non-pollinating colonies. We also found that *M. plutonius* could be detected in nurse bees prior to the emergence of clinical symptoms. This observation is in agreement with previous studies showing that colonies can have a high level of infection with low levels of disease symptoms.

Completion of honey and bee bread pesticide analysis from samples collected in the field experiment was delayed due to the unexpected covid-19 situation. On September 15, we were able to return to the AAFC laboratories and we have now submitted all honey and bee-bread samples for analysis. If there are no additional unforeseen delays, we expect to have these residue analyses results in the new year.
In collaboration with Patricia Wolf-Veiga of the National Diagnostic Laboratory (NBDC) and with Drs. Sarah Wood, and Elemir Simko of the University of Saskatchewan, we studied bacterial isolates of *M. plutonius* from blueberry field samples and identified an atypical strain which was then used to implement an *in vitro* infection system (see our recent publication: Wood et al. (2020) *In Vitro Effects of Pesticides on European Foulbrood in Honeybee Larvae*. Insects, 11(4), 252. https://doi.org/10.3390/insects11040252). This collaboration continues on a project entitled: *Effects of host, pathogen, and environmental factors on increased incidence of European foulbrood in honey bee colonies pollinating blueberries in North America*. Progress on this project includes the implementation of both *in vitro* and *in vivo* infection systems to evaluate the effect of potential stressors on the expression of EFB symptoms. Potential stressors being tested include pesticides used in blueberry fields as well as pollen collected by colonies during pollination as this pollen may have an unfavorable nutrition composition or chemical residues.

Some results from this project will be presented by Amanda Gregoris at the Entomological Society of America meeting in November 2020 in a poster titled "Investigating Incidences of European Foulbrood and Other Pathogens in Highbush Blueberry-Pollinating Honey Bees"

3) A Novel Compound to Control Varroa (project lead: Dr. Erika Plettner, Simon Fraser University). Work is continuing on this project, with some disruptions as noted.

a) Field tests could not be done this year due to Covid and are being planned for next summer.

b) Some laboratory tests were continued, providing results consistent with the high activity of the leading compound, and helping formulate hypotheses as to how it works. Although the target site is not yet known, a better picture is emerging that helps to focus the search.

c) We have been doing chemical analyses from 2019 field test samples. So far we can say that the compound is indeed a fumigant that we can find on the wax immediately after removal of the device. We are also analyzing the devices pulled from the colonies and found that they were not depleted during the 28-day trial, confirming the dose used was appropriate for the 28 day period. Additional lab work is ongoing, with field trials planned for 2021.

4. Honey Authentication Testing – (Project Leads: Leonard Foster, Peter Awram)

Work is continuing on this project, although some lab work was delayed due to Covid restrictions. Following is a short article summarizing the project:

Spectrometric methods for detecting honey adulteration Peter Awram, Leonard J. Foster

Honey consumption has increased significantly over the last 6 months and with a slowdown in imports, honey stocks in North America have been depleted. This has driven honey prices higher, which then provides additional incentive for people looking to pass a cheaper product off as honey. Thus, the need for improved methods for detecting adulterated honey is still essential. We have recently summarized the current methods, including their pros and cons, in an ABJ article (available early at https://www.apiservices.biz/en/articles/articles-honey-fraud). As we have discussed at previous ABC meetings, and elsewhere, we have been pushing forward with magnetic resonance spectroscopy (MRS) and mass spectrometry (MS) methods for detecting adulteration. MRS in the form of Bruker's FoodScreener is an established technology. The CFIA has used it along with SIRA (C4-testing) to sample a large number of Canadian samples. However their use of MRS has been plagued with issues. A number of beekeepers have been tagged with selling adulterated honey and provided no reasoning. Analysis of the samples on our machines have shown that, while the automated Bruker report diagnoses adulteration, an analysis of the samples indicate that there are high levels of turanose (a sugar that is generated by bees and not plants. In addition there are high levels of raffinose (a tri-saccharide that is not a component of bee feed or rice syrup). Neither of these are indicators of honey adulteration. Raffinose is often associated with the *Brassica* family (Canola being the most notable honey plant, but also cabbage and broccoli). What seems likely is that there are plants that the bees visiting that are not represented at all in the database. We have looked at several sunflower samples and have found a wide

variety of results. The samples seem to distantly resemble the sunflower samples currently in the database, but there seem to be significant differences between the sunflower grown in Alberta and Manitoba (where we have gotten samples) compared to what exists in the database.

Another issue that has arisen recently is the detection of syrup feeding in a number of beekeepers in Alberta. In some cases, this is being claimed on the basis of liquid chromatography/elemental analysis combined with isotope ratio MS (LC/EA-IRMS), which is a modified version of the C4 test designed to detect C3, as well as C4 sugars. However, it is well known to be very hard to reproduce reliable results with this method. In our own experience, we have found that bees will move feed syrup up from the brood chambers in early pulls of honey and this can cause issues with adulteration tests. We feel that there needs to be a proper study to determine what are acceptable levels of feed syrup that allow proper bee management, and how to best detect that.

MS is widely used for diagnostics but is not currently used for detecting honey adulteration, although several companies are moving in this direction. Some companies, notably EuroFins, are starting to develop MS as a competing technology to MRS. Their approach is similar to how we test for performance-enhancing drugs in athletes - very sensitive analyses targeting components known to be in honey or known to come from adulteration processes. While accurate, this approach has two disadvantages: a) the fraudsters know what they need to do to beat it, b) it does not account for unanticipated samples (e.g., new methods of adulteration, previously unknown honeys). This method relies on knowing what the adulterant contains and is simply a more sensitive version of previous testing methodologies. The QSI/Bruker MRS solution uses a library that underrepresents North American honeys. Much of our effort in the last year has focused on collecting honeys from around our region of the world and acquiring MRS spectra of each one. We are now working with Bruker to have these spectra added to their library. Our hope is that, once this is finished, we will have fewer failed diagnoses of real honeys from our collective operations. Currently approximately 500 samples have been submitted to Bruker and we are going through the verification of the samples for entry into version 3 of the database.

MS is a complementary technology to MR in that it measures different properties of the molecules in the sample. We are continuing to develop a fingerprinting approach to distinguish real from fake honey. This does not rely on specific chemicals but rather looks at the overall pattern of chemicals. We have a working system for using MS to distinguish real from fake honey so our focus now is on expanding the library of honeys and seeking funding to develop the whole process to a point where it could meet regulatory requirements. Both MRS and MS depend on, however, a reliable library of real honey samples to compare an unknown sample. A combined system using MRS and MS holds much greater promise of keeping ahead of the adulteration techniques.

5) **NBDC project to continue research on foulbrood diseases of honey bees** (Project lead: Patricia Wolf-Veiga). This project is continuing, although as with other projects, Covid restrictions have halted or delayed much lab work this year. A collaborative research paper was recently published (also mentioned in the blueberry project, above) Wood et al. (2020) *In Vitro Effects of Pesticides on European Foulbrood in Honeybee Larvae*. Insects, 11(4), 252. https://doi.org/10.3390/insects11040252)