AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2019 – 2021

TITLE: Novel Delivery Method for Crop Protection Agents and Insecticidal Proteins

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EXECUTIVE SUMMARY:

The market for biopesticides has the potential to outpace chemical pesticides. We are investigating a delivery method which could provide cheap production, targeted delivery and toxicity, and other desirable properties for biopesticides derived from naturally occurring proteins and peptides. Overcoming current barriers to using proteins for pest control will lead to needed solutions for agriculture in Oregon and around the world. Precision Protein Delivery Systems (PPDS) has developed a tentative manufacturing pipeline of bionanoparticles (aka beads) engineered for multiple applications. Their method takes advantage of a natural process in bacteria that produce biodegradable polyester beads armed with specific selected proteins expressed on their surface. PPDS's approach potentially addresses a very important hurdle to commercializing protein biopesticide products, and we are working out some of the details to bring it to scale while assessing the functionality of this platform in order to develop commercializable pest control agents.

We have refined a process to produce purified bionanoparticles in the lab, and developed versions with Green Fluorescent Protein (GFP), and Snowdrop Lectin protein and GFP. We have observed that these bionanoparticles do not result in toxicity to adult honey bees. We will soon make a bead construct with Snowdrop Lectin, GFP, and a Spider Venom Toxin protein, and begin testing our beads in additional insect species. We hope that by choosing proteins with care, we can develop targeted products that are low in toxicity to beneficial species like bees yet act as control agents for pest insects.

OBJECTIVES:

Using previous ARF funding, we began to examine some of the properties of PPDS beads. We have found that the beads are smaller than particles in many current commercial pesticide formulations. We also discovered that honey bees readily consume the unmodified beads when fed in sugar solution with no apparent toxicity observed. Exposing bees to a dried residue did not result in any changes in behavior or mortality. We concluded that the PPDS beads are ideal for safely delivering proteins or other substances to bees that could help in controlling varroa. There are many remaining questions about producing the bionanoparticles in the lab, and the selection of proteins to attach to them that provide the desired functionality. Our objectives reflect the iterative nature and trial and error we expect in our work as we investigate various protein/bead constructs in multiple insect systems.

Objective 1: Create PPDS beads that are toxic to specific pests. This step involves mining the literature for the most appropriate proteins.

Objective 2: Investigate the toxicity of a PPDS bead customized with an insecticidal protein. We will use a dose response approach to determine how much of a bead solution is needed to cause mortality in various insect models. We will also continue to use the fluorescent properties of our beads to visualize their distribution in insect systems such as hemolymph and other organs, and how long they persist in the insect.

Objective 3: Our expectation is that the bionanoparticles will require continued modifications for optimal functionality and customization for various pest insects and environments. Based on the literature and what we observe in our initial experiments, our third objective is to continue to design and engineer the next iteration of bionanoparticles with improved functionality that ultimately leads toward a family of insecticidal bionanoparticles.

PROCEDURES:

Objective 1. Selection of insecticidal protein. Our budget allows us to develop at least two bead iterations. Previous researchers have found that proteins from many sources are toxic to insects. In one example, Snowdrop Lectin was fused to a spider venom toxin (Erich), and was found to be toxic to multiple orders of insects, but not honey bees. Similarly, we have produced a GFP/Snowdrop Lectin bead construct first, in order to ascertain whether this can act as a carrier to move other proteins from the gut into insect hemolymph. We will later add Spider toxin to this construct for comparison to previous work with fusion proteins. We continue to work on creating a database of proteins with potential pesticidal properties, and we will use this database to select future proteins for our bead constructs. Our priorities are proteins which are not toxic to mammals, but have potential for broad spectrum efficacy in controlling significant pest species. Once selected, the beads will be produced in the laboratory or by PPDS.

Objective 2. Toxicity of custom PPDS beads. We have begun work on examining toxicity in honey bees and varroa mites, and will soon begin work in other model insect species (Fruit Flies, Brown Marmorated Stink Bugs) with two of our bead constructs (GFP beads, GFP/Lectin), and will develop a GFP/Lectin/Spider Toxin bead soon. Using existing bioassay protocols for each species, we will use multiple doses to determine what concentration of beads is needed to observe mortality relative to control. In the case of honey bees, we will also observe mortality of varroa mites. The values that emerge from these dose response experiments will enable us to compare the efficacy of our beads with that of commercially available pesticide active ingredients and extrapolate potential field doses. We will also continue to use fluorescent microscopy and spectroscopy to localize the GFP beads so that we can understand delivery of the beads within the insects.

For application in cropping systems, we expect that bionanoparticle products would be sprayed onto foliage and may dry before being encountered by an insect. This could result in incidental ingestion and mortality. Using a protocol we have developed, we will apply GFP beads to hazelnut leaves, place them

in petri dishes, introduce insects, and observe mortality. If mortality is observed, we will allow the bionanoparticle-treated leaves to weather in the field and analyze how long in days insects exposed to the leaves display mortality. The result of these experiments will determine the potential residual activity of bionanoparticle insecticides and may suggest needed improvements related to their stability or adhesion capabilities, in addition to formulation chemistry that would encourage ingestion.

Objective 3. The next iteration. Up to seven proteins may be added to the beads, and there are many proteins which may be investigated for pesticidal activity. Multiple properties can be added, depending on the proteins selected. Multiple insecticidal proteins could be added to any given bead construct, which is a strategy used to combat resistance. Our database from Objective 1 can help guide us in what direction to turn next:

Oral availability. Delivery of proteins and pesticides from the gut to the target site after oral consumption is a challenge in both insect and human systems. Plant lectins are a family of proteins some of which have been found to enable proteins to pass through the gut or reach other organ systems. We will continue to learn which lectins are best suited for our work. We will also spend time at this stage ascertaining the value of this approach to facilitate delivery of the beads into various insect tissues.

Pesticidal Proteins. A variety of insecticidal peptides and proteins are available to choose from (Whetstone, Kroemer). These compounds derive from a number of sources including the venoms of predatory/parasitoid animals such as spiders and scorpions, arthropod-pathogenic microbes, plant proteins such as lectins, and enzymes and other factors from insects themselves. We are particularly interested in insecticidal proteins that have already show efficacy in other research, and do not show toxicity to honey bees or other beneficial insects, for example (Erich).

Plant Transport. The probability that our bionanoparticles are taken up and transported systemically in plants is unknown. However, there is a great deal of intriguing research about the uptake and transport of different types of nanoparticles (Schwab), and viruses (Lucas). A thorough literature review may reveal a Movement Protein or other factor that can enable the beads to move through the complex architecture of plants, or specific crop plants.

We expect that the process of engineering an insecticidal bionanoparticle will require much thought and multiple iterations. Here we are proposing the next step in a path to a final product, which we also hope provides sufficient preliminary data for additional funding.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

In previous work, we used PPDS beads tagged with GFP to learn that once consumed, the beads do not migrate from the gut into the hemolymph (insect blood) using a fluorescence microscope, although the current beads are difficult to distinguish from background fluorescence. We have now added a Snowdrop Lectin protein that is expected to enable the beads to move from the insect digestive system and into the hemolymph and organs (Erich). This will also enable us to learn whether the beads are consumed by varroa mites once they are in the hemolymph of bees, and whether the oral availability is

also observed in other insects such as Brown Marmorated Stink Bug and fruit flies. We will create additional bead constructs with additional insecticidal protein(s) to the GFP/Lectin beads, and test their efficacy.

Our literature review found that Snowdrop Lectin protein has previously been shown by others to bind to insect gut epithelium and pass into the hemolymph when introduced in the diet. Although snowdrop lectin is being used as a way to deliver other proteins, our approach of attaching proteins to PPDS beads offers many advantages over proteins alone. In addition to PPDS beads tagged with Green Florescent Protein (GFP), we have now created a PPDS bead tagged with Snowdrop Lectin protein and Green Florescent Protein (Lectin/GFP Bead). This work required significant time and effort, as after an *E.coli* strain was developed that produced the bead, we had to redevelop a system to purify and filter the beads from the bacterial culture, as previously used facilities were no longer available. While it can be a struggle to develop such a method in the lab without a large equipment budget, our methods can easily be scaled up, suggesting that industrial quantities of these bionanoparticles could easily be isolated. This is a significant advantage over proteins which are not attached to beads and is likely to greatly reduce production costs of any resulting product.

Like the GFP bead, honey bees readily consume the Lectin/GFP Bead when provided in high concentration in a sugar solution. If this is observed in other insect orders, it is possible that the beads can deliver proteins in bait formulations. Although Snowdrop Lectin protein is toxic to some insects, that has not been observed in honey bees. Similarly, we observed that the GFP beads and Lectin/GFP beads did not result in mortality in adult honey bees. It is our hope that we will find proteins that can control insect pests without harming beneficial insects, which is one reason we chose the snowdrop Lectin to begin our investigations. We are continuing to refine our methods to track the fluorescence of GFP or use other means to investigate the fate of the beads within insect systems.

Fusion proteins connecting Snowdrop Lectin and Spider Venom Toxins have been developed by others as crop protection agents. We believe that we can improve on this technology by attaching this hybrid to our beads, making it much easier to produce protein-based pesticides, and stabilize them in formulation. We are also investigating use of the beads to facilitate the production of other types of proteins used in medicine and other fields.

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ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Funding from Ecampus supported the work of the PI, Dr. Louisa Hooven, who oversaw the project and performed insect bioassays.

Dr. Alan Kadish of PPDS gave freely of his time to develop systems to produce and purify bionanoparticles in the laboratory.

Dr. Eric Altermann of PPDS developed the constructs used to create EColi strains

FUTURE FUNDING POSSIBILITIES:

If our work demonstrates that the bionanoparticles can deliver functional proteins to molecular targets within insect systems, it opens up a myriad of possibilities. Using OSU's Advantage Accelerator resources to move our project to the next step to commercialization is likely our first stop. We have already had several conversations with them.