BIOAg Project Report

Report Type: Progress

Title: Identifying biologically-based paths reducing insecticide resistance in codling moth

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Abstract:

Codling moth is the key pest in Washington apples, a $2 billion industry. WSU research suggests that reducing insecticide rates by 90% in conventional farms can provide control equivalent to full rates by improving predator conservation that supplements chemical control. However, fears of promoting insecticide resistance has prevented industry adoption. In organic blocks, codling moth is showing signs of insecticide resistance to codling moth granulovirus, a key codling moth organic insecticide. I propose a student theoretical modeling project to provide paths forward for insecticide resistance management in each, organic and conventional systems. First, we will experimentally evaluate the potential of two codling moth pathogens, granulovirus and nematodes, to provide synergistic control by overwhelming the insects' immune system. Next, we are using arms race models to evaluate methods of adapting granulovirus to resistant populations. Finally, for conventional production we will evaluate potential for predator conservation to inhibit evolution of prey insecticide resistance.

Project Description:

Introduction (from proposal)

Codling moth is the key pest in Washington apple and a key pest for pear production. In addition to directly reducing fruit quality through larval feeding, insecticide treatments often disrupt natural enemies, leading to outbreaks of secondary pests. In Washington growers that do not rely heavily on natural enemies spend $930 and $1722 per hectare on pest management in apples and pears, respectively with a large portion spent on codling moth in each case. Recently, a large-scale field, three-year experiment led by Vince Jones showed that reducing typical insecticide (spinetoram) rates by 90% can provide control comparable to full rates of codling moth, leafroller, woolly apple aphid, green apple aphid, rosy apple aphid, or San Jose Scale. These findings suggest excellent potential for dramatic reduction in insecticide use, with associated reductions in grower cost and environmental impacts. Despite this potential cost savings, grower adoption of low rates has been inhibited by fears of promoting insecticide resistance. However, Jones and colleagues found that reducing insecticide rates also increased predator densities, providing a separate selection pressure on pests that may slow evolution of pesticide resistance. Resistance to the codling moth granulovirus relied on in organic apple orchards is already becoming commonplace in European codling moth populations and is likely occurring in Washington as well. Therefore, a game plan is needed to reduce resistance to this key method of organic codling moth control. Here, we will examine theory describing adaptation of pathogens like granulovirus to populations that are developing resistance. In addition, we will evaluate the potential for combining granulovirus with *Steinernema carpocapsae* nematodes that are commercially available for organic management. Research on beetles suggests that combining nematodes with other pathogens can improve pest control. Finally, we will evaluate granulovirus exposure as final instar larvae emerge from fruits to pupate, followed by nematode infection.

Granulovirus may weaken the immune system, allowing greater infection by nematodes.

Procedures (from proposal)
Modeling work will build on coevolutionary models that I have developed with collaborators. I have used two common types of approaches for modeling evolution. First, I have used an adaptive dynamics approach in which clones reproduce asexually and population trait change occurs through the mutation of traits and subsequent growth of mutant clones. This approach is most relevant to primarily asexually reproducing insects like aphids. The other, quantitative genetics, doesn't assume clonal reproduction and tracks mean population traits. This quantitative genetics approach will be used to evaluate paths to develop counter-resistance in biopesticides in lab colonies, and also demonstrate that if possible, conserving natural populations of pathogens will allow for evolution in natural areas. To simulate how pathogens are currently used, we will use consumer-resource models developed by Northfield and Ives to simulate pest growth and evolution with repeated introduction of the pathogen. In our initial simulations, we will assume high pathogen mortality rates, so that reintroduction is necessary, describing the current scenario. We will start with little resistance to the pathogen in the pest, due to low exposure history. For example, the codling moth granulovirus originally came from Mexico and codling moths invaded from Europe with little exposure. Next, we will allow the pest to develop resistance over time and monitor the impacts of repeated pathogen introduction on the evolution of resistance. This process is similar to what has occurred in European populations, and is likely occurring in the Pacific Northwest, where the virus is typically used weekly in organic production. Next, we will simulate our proposed method of evolving counter resistance. Here, we will simulate bringing the resistant pests into the lab and allow the pathogen to evolve alongside the resistant pest population. Here, we will take pest traits representative of the resistant population in the modeling exercise described above and introduce the pathogen with low mortality rates, as might be expected in a laboratory colony. We will evaluate the coevolution for 10 pest generations, to allow the pathogen to adapt to the resistant population. We will also consider the impacts of varying the number of pest generations of exposure to see how long it takes to adapt to the resistant pests. We will then simulate field deployment by repeatedly introducing the pathogen to simulated pest populations, with high pathogen mortality, which necessitates reintroduction. Finally, we will compare the scenarios above with a simulated field scenario where the pathogen is repeatedly introduced to the pest population, but mortality is low (simulating pathogen conservation) and allowed to coevolve with the pest population. This will be to highlight a potential alternative approach of improving pathogen conservation in the field, when possible.

To evaluate the effects of dose and frequency of chemical insecticides, we will use models developed by Tabashnik, informed by pest models used in WSU's DAS, along with the experimental results from work by Jones, and colleagues at WSU to inform our models and create realistic pest control scenarios. As Tabashnik did, we will alter the dose of application, and frequency of application. We will consider two pests, 1) codling moths, and 2) aphids to evaluate two representatives of the range of types of pests infesting Washington tree fruits. We will then evaluate the effects of each dose and frequency on the evolution of resistance in each the pests and predators. We will consider a range of predators, but green lacewings have the most phenological information known (Jones et al. 2016) so this will be one option to provide the most detailed predictions for Washington agriculture.

Finally, to evaluate the effects of codling moth granulovirus and nematodes, we will conduct field and lab experiments. The work will capitalize on a small untreated apple block at Wenatchee TFREC, which has very high codling moth densities. In the field we will place cardboard bands around apple trunks, so that codling moth larvae crawl into the bands to pupate. While both *S. carpocapsae* and S*. feltiae* are

effective against codling moth, we will focus on S. carpocapsae, for which a particular strain is being marketed commercially for codling moth control (e.g., Nemasys C, BASF). The bands with pupae will be brought back to the lab and treated with one of four treatments: 1) water, 2) virus, 3) nematodes, or 4) granulovirus and nematodes. We will then monitor the pupae for survivorship and use a generalized linear model to evaluate the effects of granulovirus, nematodes, and their interaction on pupal survival. This approach will simulate granulovirus applied just before larvae emerge from apples to pupate, so that they will be exposed to the virus just prior to being exposed to nematodes.

Outputs

Overview of work completed and in progress

In 2021 we conducted an experiment evaluating the effects of granulovirus applied to last instar larvae (as opposed to typical application of first instar larvae), along with *Steinernema feltiae* nematodes, which was often the recommended species by Lacey and colleagues for codling moths. Neither pathogen provided control of codling moth larvae, either alone or together in our laboratory experiments, when they were sprayed directly onto last instar larvae just prior to pupation. We also developed and analyzed models to better understand evolution between pests and pathogens, to inform biopesticide resistance management. When transmission is linked to mortality, such as nematodes that kill hosts before transmitting, incorporating resistant individuals is expected to improve the biopesticide activity against the resistant pests. However, our preliminary research suggests that when pathogen transmission typically occurs between living hosts, pesticide resistance may consist of low virulence (pathogen-induced mortality), rather than low infection rates. In this case, introducing resistant pests to the pathogen rearing methods may not improve the biopesticide, as low host mortality does not negatively impact pathogen fitness or selection pressure. Thus, the models suggest the best strategy for overcoming pest resistance to biopesticides depends greatly on the mode of pathogen transmission, and the type of resistance provided (lower infection rates, or lower rates of pathogen-induced mortality).

Methods used in 2021

Nematode and virus evaluation. We conducted an experiment to evaluate the potential synergistic effects of sublethal exposure of codling moth granulovirus and nematodes in laboratory experiments. To conduct the experiment, we collected 480 last instar codling moth larvae by placing cardboard bands around trunks of trees in a small untreated apple block at Wenatchee TFREC, which has very high codling moth densities, and collecting them after larvae entered the cardboard to pupate. The bands with pupae will be brought back to the lab and treated with one of four treatments: 1) water, 2) virus, 3) nematodes, or 4) granulovirus and nematodes. Cardboard bands were set up on 9 July 2021, and collected 22 July, when the codling moth larvae were placed in deli containers in groups of 10 and sprayed with either 1) an aqueous virus solution (concentration: 7.5×10^{10} occlusion bodies/L), or 2) water and left in the containers for two hours to allow virus to contact larvae. The larvae were then transferred to larger deli containers with 2 cardboard strips to allow pupation. We used this technique, rather than allowing larvae to pupate in soil, 1) because our preliminary study observed high larvae mortality in the soil across all treatments, and 2) to replicate codling moth pupae pupating on tree bark. Commercial nematode labels suggest spraying nematodes directly onto trees, but we are not aware of research evaluating this mode of application, rather than treating pupae within soil. Codling moth larvae were then sprayed directly with *Steinernema feltiae* nematodes at field rates (approximately 3 nematodes/cm^2), or water. We used *S. feltiae* instead of *S. carpocapsae*, due to findings by Lacey et al. that it was the more effective species for codling moth. We then evaluated mortality after 7 and 10

days, as well as after 17 days. To analyze the data, we used generalized linear models that assumed a binomial error distribution and logit link function.

Resistant modeling. Recent research has found three forms of granulovirus resistance in European codling moths, as well as some preliminary evidence of resistance within Washington. However, while there has been extensive research developing theory for how resistance to chemicals evolve, it is not clear how insect pests evolve in response to biopesticides. A key difference between biopesticides and chemical insecticides is that biopesticides can evolve in response to evolving pest populations in the field and laboratory colonies. These evolutionary processes are thought to allow for the development of more impactful biopesticides. For example, a granulovirus strain (Cyd-x HP) was collected from a region of France and has shown stronger efficacy than the original strain for resistant populations. However, it has been unclear how arms races between pests and pathogens can be used to develop more effective biopesticides. Because codling moths spend the majority of the larval stage within fruit, it is thought that transmission in the field is limited. For this reason, transmission has not been studied extensively. However, transmission rates are likely high in colonies where larvae are reared on diet, and exposed to each other, as opposed to individual larvae being contained within different fruit. Thus, we must consider transmission of the virus within colonies, to see if introducing resistant moths to virus rearing methods will allow evolution of better virus strains. The virus typically kills the host within 3 days, and such high virulence may suggest that transmission may be highest from codling moth larval cadavers than through transmission from living larvae. However, due to the lack of clarity for how transmission is most likely to occur, we used two model frameworks, with different assumptions to gain new insights into biopesticide resistance management. The first model is inspired by host-parasite systems where the pathogen must kill the host to disperse. For example, in order to disperse from a host, nematodes build up in a host, feeding on all the internal components of the insect before killing it and dispersing. In this case, we assume that the host has a defense against the pathogen (e.g., strong exoskeleton to reduce nematode penetration), and pathogens have traits that allow them to overcome this defense (e.g., particularly sharp mouthparts that allow the nematode to bypass exoskeleton). The second model uses a more epidemiological approach where hosts are modeled and considered either susceptible to infection (healthy), or infected. In this case, transmission can occur between infected individuals without killing the host, such that a single infected host can infect many susceptible hosts. Here, evolution occurs through the introduction of a mutant pathogen strain or host, and we model the competition between the new mutant and the wild type. Because the transmission is not dependent on host mortality, we can evaluate the likelihood of evolution between host and pathogen leading to two scenarios: 1) the host evolves resistance to infection, limiting infection across the population, or 2) the host evolves tolerance to infection, such that pathogen-induced mortality is low, but transmission rates remain high. These two scenarios have different needed responses, so understanding which is more likely is key to managing biopesticide resistance.

Results (year 1)

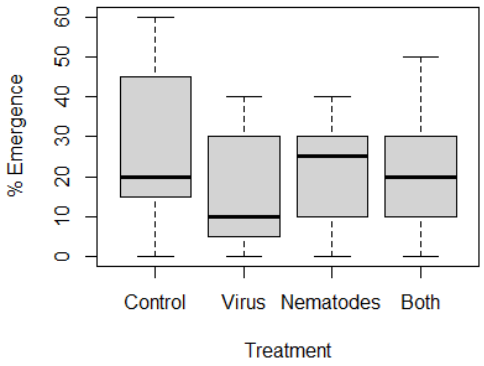

Figure 1. Codling moth emergence after 17 days when treated as larvae with granulovirus (Virus), *S. feltiae* nematodes (Nematodes), both granulovirus and *S. feltiae* nematodes (Both) or water (Control).

Nematode, granulovirus experiment. Unfortunately, after codling moth emergence 17 days we found very little codling moth pupa mortality in response to the granulovirus (generalized linear model: z = -1.629, *P* = 0.103), nematodes (generalized linear model: z = -0.626, *P* = 0.531), or the combination of the pathogens (generalized linear model: z = 0.808, *P* = 0.419). Similar results were observed at day 7 or 10 mortality, which had median survivorships of 90% and 85% when averaged across the treatments. These findings suggest that while nematodes may be effective for codling moths pupating in soil, as has been shown previously by Lerry Lacey and colleagues, it may not be effective when sprayed directly on trunks, where nematodes are likely to either desiccate, or need to be sprayed directly onto nematodes. It is not surprising that granulovirus applied to last instar larvae was not enough to kill codling moth larvae on their own, as this product is typically used to treat newly emerged larvae that feed on virus-coated leaves just after emerging from eggs (we are not aware of previous studies evaluating virus efficacy on last instar laravae). However, we also show that the presence of virus just before nematode exposure is not enough to weaken the larvae's immune system to allow nematode infection.

Models to inform biopesticide resistance management: *Host-parasite models*. We first model a scenario where pathogens are used as a biopesticide, such that the pathogen is not allowed to evolve, and track host and parasite abundance, as well as their trait values. For the pest (host) this trait represents resistance, and for the parasite this represents the effectiveness of this resistance. First, we consider a scenario where the pest can evolve resistance to the parasite, but the parasite does not evolve. This scenario is useful for two reasons. First, it provides a baseline to allow us to determine the ultimate effects of parasite evolution on pest density, and second, it reflects the scenario where parasites are mass produced in the absence of the host and not allowed to co-evolve with the pest. In this case, the pest is

initially controlled, but then evolves resistance to the parasite, allowing it to reach much higher

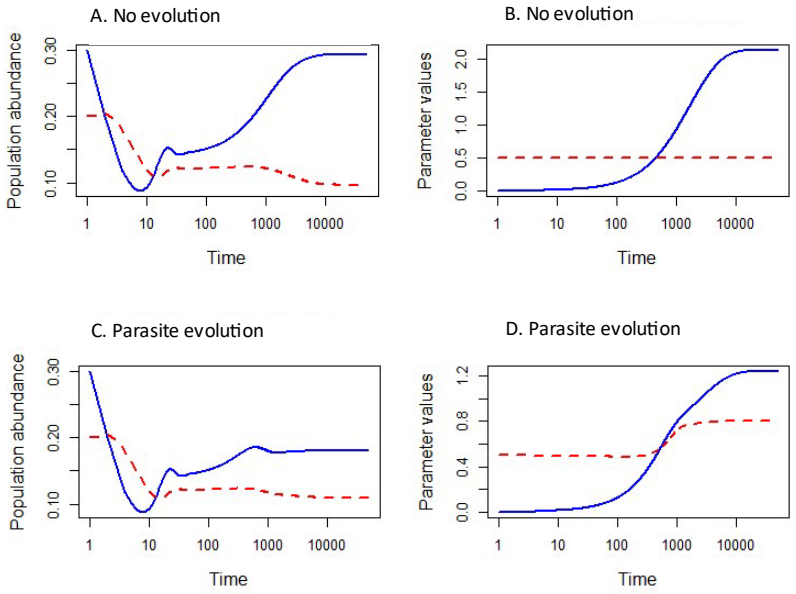

Figure 2. Densities of the pest (blue) and pathogen (red) for densities (A,C), or traits (B,D), where the pathogen does not evolve (A.B), or does evolve (C,D). The pest trait represents resistance to the pathogen, and the pathogen trait (effectiveness of pest resistance) is constant. Time is shown on a log scale and is parameterized such that time steps are very short, for example, in hours, rather than days or years.

abundances (Figure 2 A,B). However, in the scenario where the parasite is allowed to evolve, it is able to keep the pest at lower densities. For the typical biopesticide scenario where resistance is allowed to develop in the pest, but the parasite does not evolve, we considered the effects of reintroducing the resistant pest to the parasite and allowing them to coevolve. This scenario represents an example of including resistant populations into the biopesticide rearing process. In this case, the parasite coevolved quickly with the resistant pest and provided better control (data not shown). Thus, when the parasite must kill the host to transmit, arms races may be used to improve the biopesticide.

Epidemiology models. When we considered the scenario where transmission can occur from living pests, we repurposed old models developed by Roy Anderson and Robert May, where they considered epidemiology of infectious disease. While most epidemiological research has been motivated by human disease (with some exceptions), where host evolution is not considered, we focus on the development of resistance within host (pest) population. The models track the abundance of susceptible (healthy) individuals and infected individuals, where susceptible individuals become infected at a rate proportional to the number of infected. We assume that infected individuals die at a rate that is higher than susceptible individuals, and we refer to the magnitude of this increased mortality rate as “virulence”. Thus, we begin with a system of susceptible and infected individuals, and we introduce a mutant strain of the hosts. After introduction, we have four classes of hosts: susceptible and infected wild types, and susceptible and infected mutants. We assume that mutants have an advantage in either lower likelihood of getting sick

(transmission resistance), or lower risk of mortality when infected (virulence resistance). For either case, we assume that resistance comes at a cost of lower reproduction rate. One advantage of these models is that they have been extensively evaluated algebraically. Evaluating the equations algebraically, it becomes clear that transmission resistance only benefits healthy (susceptible) individuals, whereas virulence resistance

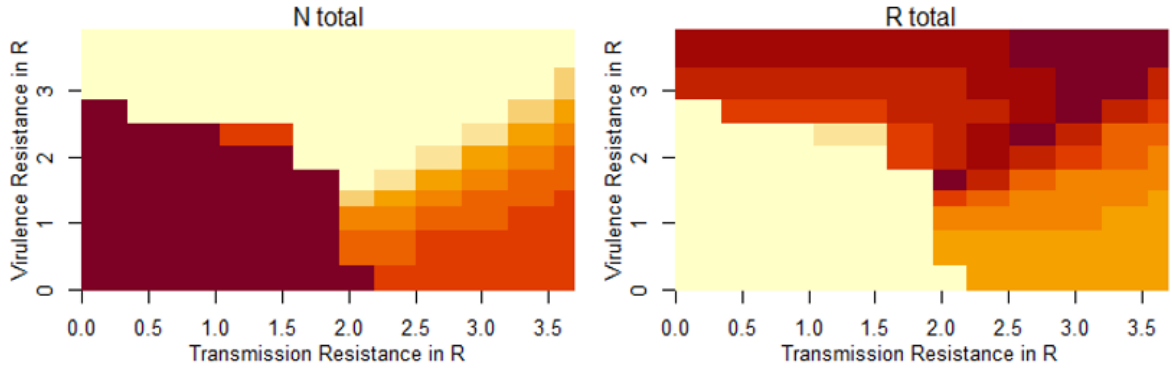

Figure 3. Final (equilibrium) abundance of wild type (left panel), or resistant individuals (right panel), when the mutant hosts have either resistance to transmission risk (x axis), or resistance in the form of lower virulence (y -axis). Darker colors represent higher densities. When the mutant population has low resistance for either transmission or virulence (bottom left corner of each panel), the wild type outcompetes the mutant, due to lower reproductive rates of the mutant. Resistance associated with lower transmission only (lower right corner of each panel) allows invasion of the mutant, but the wild type is able to persist. Whenever the mutant has low virulence (top of each panel), the mutant drives the wild type to extinction, such that resistance fixes in the

only benefits infected individuals. Thus, the selection pressure for each type of resistance depends on the prevalence of the infection within the mutant population. This is also intuitive: lower transmission rate does not benefit an individual that is already sick, and lower pathogen-induced mortality doesn't affect healthy individuals. The type of "resistance" in the host population also influences the persistence of the original population. When a new mutant experiences lower transmission risk, it lowers the total number of infected individuals, and allows the original, wild type to persist (Fig. 3). However, when the mutant can become infected but has a lower mortality rate, it actually increases the number of infected individuals in the population (by allowing infected host to survive longer), and exaggerates the mortality rate of the original, wild type population. Because insecticide resistance management typically focuses on maintaining populations of wild type individuals that can mate with mutants and dilute the prevalence of resistant genes, this is an important finding. We also considered the scenario where lower virulence is tied to rapid clearance of the pathogen, such that the infected individuals revert to healthy individuals. In this scenario, the system becomes more like the transmission-resistance, because it ultimately reduces the amount of infection in the population, allowing susceptible individuals to persist. Thus, the type of resistance that emerges is important to understand the spread of resistance through the pest population. If mutation simply allows pests to survive infection for longer, then introducing the resistant pests into the rearing process of the biopesticide will not lead to a more effective biopesticide. This is because low host mortality does not inhibit the pathogen as long as it is able to transmit. So, there won't be selection pressure for pathogen improvement like there is in the host-parasite scenario where host mortality is needed for transmission.

Outreach.

Jeremy Roberts, the MS student in this project presented his research on the nematode and granulovirus experiment at the national meeting of the Entomological Society of America. This meeting is

attended by both entomological researchers and crop consultants. These findings are important for future research and management decisions, and these findings will help inform those decisions. PI Northfield also shared the findings with key members of the codling moth taskforce (stakeholders and researchers) that were considering using nematodes to control codling moths. In addition, PI Northfield organized a session at the annual meeting of the Washington State Tree Fruit Association that included an update from the codling moth taskforce, of which PI Northfield is an active member. This is an important meeting that includes many stakeholders, such as growers and crop consultants.

Next steps. The next step for the project, which will be conducted in 2022 is to a) publish the work described above, and b) to use models to describe the effect of lower pesticide rates on the evolution of pesticide resistance when natural enemies are considered.

Impacts

None so far

Additional funding applied for/secured:

None so far

Graduate students funded:

Jeremy Roberts, Entomology, Wenatchee TFREC

Recommendations for future research:

We are continuing the modeling work to better understand (bio)pesticide resistance, including when natural enemies are better conserved.