# BIOAg Project Report

## Report Type: Final

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

## Principal Investigator(s) and Cooperator(s): Tobin Northfield (PI), Tawnee Melton (Cooperator)

## Abstract:

Codling moth is the key pest in Washington apples, a $2 billion industry. In organic blocks, however, codling moth shows signs of insecticide resistance to codling moth granulovirus, a key codling moth organic insecticide. Here, we present results from a student theoretical modeling and empirical project to provide paths forward for insecticide resistance management. First, we develop general theory to guide resistance management programs for reducing pest resistance in response to biopesticides. We consider two types of biopesticides: 1) one where the key agent is a parasite, like an entomopathogenic nematode, and 2) one where the key agent is a pathogen, like an entomopathogenic virus or fungus. These two systems have very different implications for resistance development and potential to use arms races to improve the efficacy of the entomopathogen in biopesticides. Next, we experimentally evaluated the potential of two codling moth pathogens, granulovirus and nematodes. Unfortunately, the combination of these entomopathogens on last instar and pupal codling moths was ineffective in our trials. Finally, we present models generally parameterized for codling moths to evaluate the impacts of predator conservation on the evolution of pest insecticide resistance. We find that predator conservation alone has little impact but aids in maintaining low pest densities while allowing insecticide-susceptible pest strains to outcompete resistant strains when insecticide use is reduced.

## Project Description:

*Introduction*

Codling moth is the key pest in Washington apple, as well as an important 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. A key codling moth insecticide for organic growers in particular is a granulovirus that is highly specialized to attack codling moth. However, resistance to the codling moth granulovirus relied on in organic apple orchards is already becoming commonplace in European codling moth populations and evidence suggests it is present in Washington as well. However, because the key ingredient, a virus, can itself evolve, it is not clear if it is possible to adapt the insecticide itself to resistant populations of codling moth. Therefore, a game plan is needed to reduce resistance to this key method of organic codling moth control. Here, we develop the first theoretical work we are aware of designed to specifically inform management of resistance to pathogens used as biopesticides. Although the theory was originally inspired by codling moth control, we evaluate general theory to apply to a wide range of agricultural systems. We focus on two types of entomopathogens often used as biopesticides: 1) a parasite that kills its host as part of transmission, such as a nematode, and 2) a pathogen that can spread between living hosts, such as a virus. In addition, we evaluate the potential for combining codling moth granulovirus with Steinernema feltiae nematodes that are commercially available for organic management. Finally, we evaluate a model using an empirically-derived codling moth life table to evaluate the effects of predators on insecticide resistance.

*Outputs*

**Overview of work completed**

In 2021 we conducted an experiment evaluating the effects of codling moth 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 larvae were placed in a virus-coated environment and nematodes 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 the biopesticide includes a parasite for which 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 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 type of entomopathogen (parasite or true pathogen), and the type of resistance provided (lower infection rates, or lower rates of pathogen-induced mortality). Finally, we evaluate models using empirically derived codling moth life tables to determine the relative impacts of reducing insecticide treatments and predator conservation on insecticide resistance evolution. We find that while predator conservation itself has little impact on slowing evolution of insecticide resistance, it does allow low pest densities to remain low while insecticides are not used. Thus, it is the reduction in insecticide use itself that slows resistance in our models, rather than predator conservation, per se.

**Objectives:**

**Objective 1: development of theory guiding management of resistance to biopesticides**

Recent research has found three forms of codling moth 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 parasite 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 pass through the 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, such as the case of an entomopathogenic virus. 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.

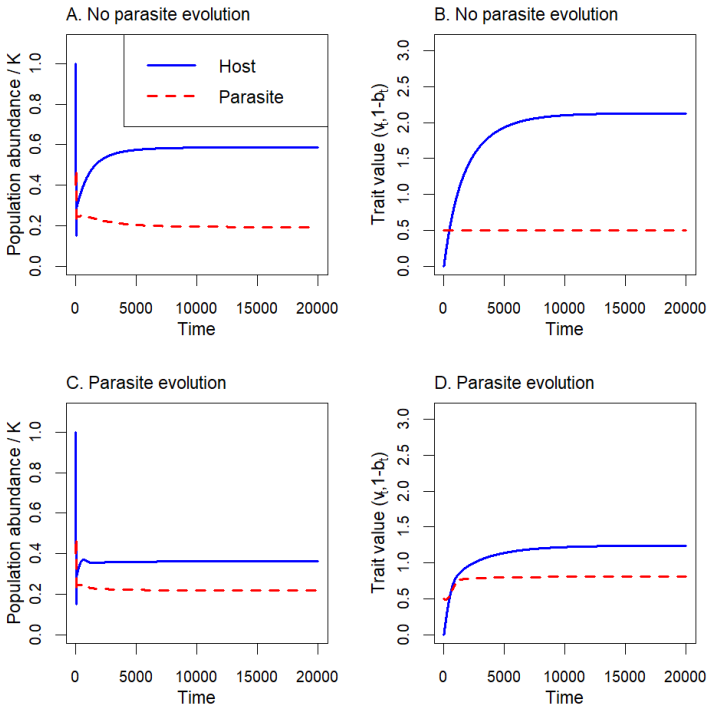


*Figure 1: Time series of population abundances or parameter values for the host (blue solid lines) or parasite (red dashed lines) populations. In (A),(B), there is no parasite evolution. In (C),(D), the parasite is allowed to evolve.*

*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 abundances (Figure 1 A,B). However, in the scenario where the parasite is allowed to evolve, it is able to keep the pest at lower densities (Figure 1 C,D). 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 is used in a biopesticide, arms races may be used to improve the biopesticide in response to resistant populations.

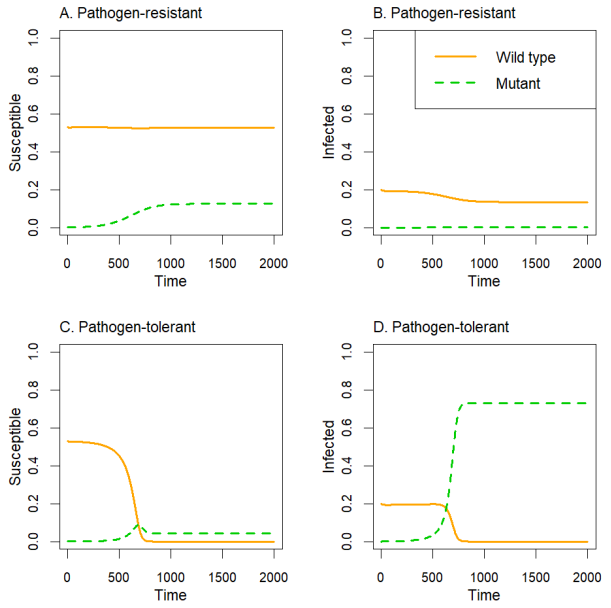


Figure 2. Time series (epidemiological model) of susceptible or infected population abundances for wild type (orange solid lines) and mutant (green dashed lines) populations. In (A),(B), mutant experiences pathogen-resistance. In (C),(D), mutant experiences pathogen-tolerance.

*Epidemiology models*. To evaluate the scenario where a true pathogen is used that can be transmitted between living pests, we repurposed models developed by Roy Anderson and Robert May (Anderson and May 1979), where they considered epidemiology of infectious disease. While the majority of epidemiological research has been motivated by human or vertebrate disease, 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. When these evaluations are conducted, it becomes clear that transmission resistance only benefits healthy (susceptible) individuals, whereas virulence resistance 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. 2). However, when the mutant can become infected but has a lower mortality rate, it 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.

**Objective 2: Evaluation of combining nematodes and granulovirus for codling moth control**

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 2021, when the codling moth larvae were placed in deli containers in groups of 10 and sprayed with either 1) an aqueous virus solution (concentration: $7.5 \times 10^{10}$ 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 larval 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.

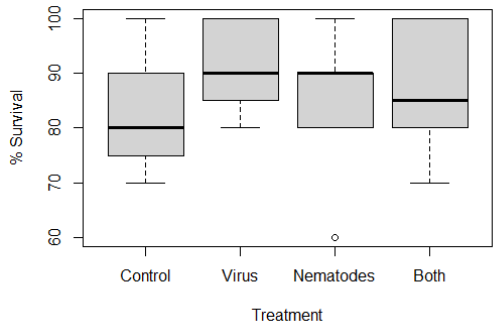


*Figure 3. Codling moth survival after 10 days when treated as larvae with granulovirus (Virus),* S. feltiae *nematodes (Nematodes), both granulovirus and S. feltiae nematodes (Both) or water (Control).*

Unfortunately, we found very high survivorship 10 days after application (Figure 3), and after 17 days we found very little codling moth pupa mortality relative to the controls from 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) (Figure 3). 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 shocking 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 larvae). 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.

**Objective 3: Evaluate the effects of reduced insecticide use and predator conservation on insecticide resistance**

A cornerstone of integrated pest management is the reduced use of insecticides to improve the conservation of natural enemies of pests. One benefit of this conservation of natural enemies has long been thought to include the slowing of insecticide resistance, by killing the pests that survive insecticide applications. However, there has been surprisingly little theoretical research to evaluate the likelihood of this occurrence, and whether it is the conservation of predators, or the reduction of insecticide applications that benefits insecticide resistance more. Here, we developed life table models parameterized specifically for codling moth and considered larval mortality caused by insecticide application, as well as pupal mortality caused by generalist predators. We used mortality rates measured for Washington codling moths by Ferro et al. (1975), and egg production data for Washington codling moths collected by Jones et al. (2008). We used the mortality and reproduction rates to develop a life table and following Leslie (1945) used the equation F(t+1) = A*F(t), where A is a life table, with the top row including fecundity at each life stage, and the other rows describing the probability of moving from one life stage to another. We assumed each day represents a different stage, such that A is a 53 row x 53 column matrix, representing a 53 day generation period, and the 53$^{rd}$ row and column represents adults that can stay in that life stage as long as they survive. The vector F(t) is a vector of densities at each life stage, and * represents matrix multiplication. A key larval period where codling moth caterpillars are exposed to insecticides, including granulovirus is the approximately three days

after egg hatch, but before they enter the apple. We imposed additional mortality at this stage to simulate insecticide treatment and allowed this mortality to vary between genotypes that differ in insecticide susceptibility. For simplicity, we assumed that the cost of resistance is applied to reproductive output. This could arise simply from a direct effect on egg production by resistant individuals, or an indirect effect that ages codling moths faster, effectively reducing their egg production in a similar fashion to delaying mating (Jones et al. 2008). We assume a single gene, two allele governing resistance, and assumed codominance, where the heterozygous moths exhibit half the resistance of the homozygous resistant moths. We assume that the homozygous resistant codling moth larvae are 90% resistant to the insecticide, and the heterozygous resistant moths are 45% resistant. We started with 4,000 eggs and no other life stages present and simulate population growth of each genotype for 20 generations (each generation is 53 days). For simplicity, we do not include winter-specific mortality in our simulations.

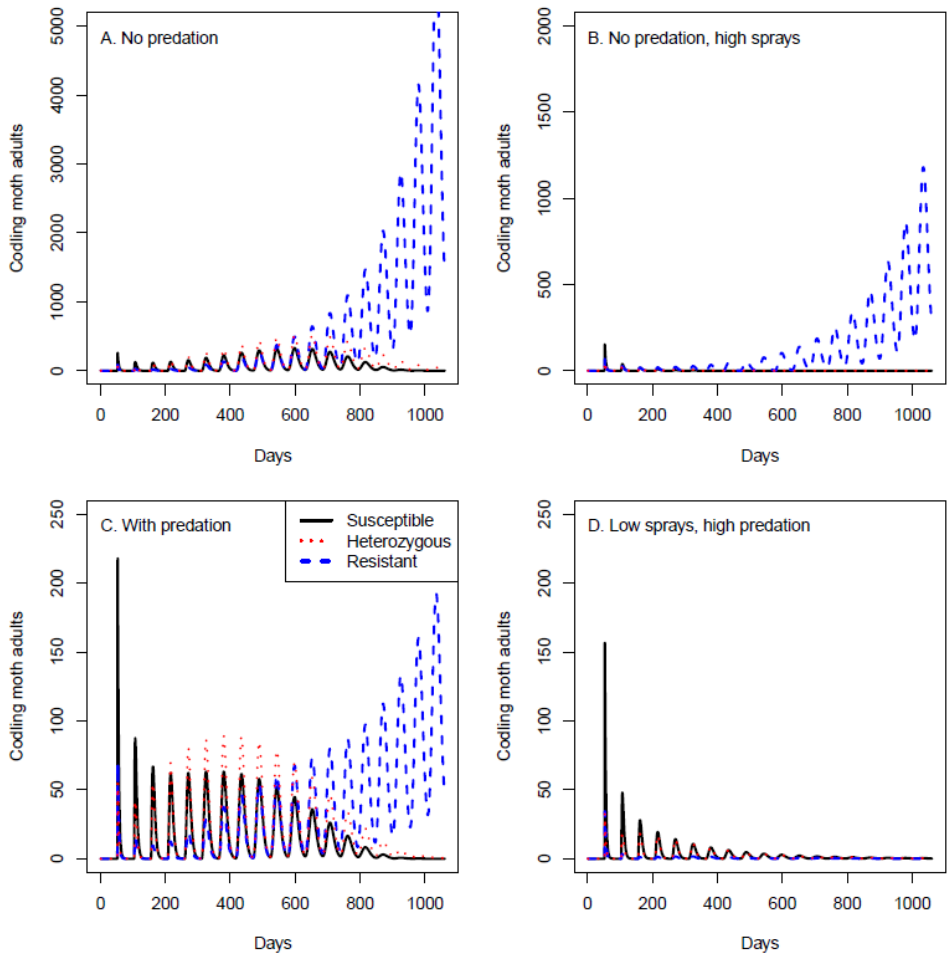


*Figure 4. Abundances of homozygous susceptible (black solid lines), homozygous resistant (dashed blue lines), or heterozygous (dashed red lines) codling moth adults with moderate insecticide mortality (A), higher insecticide mortality (B), with moderate insecticide mortality and predation at 20% the per diem rate of insecticides (C), or with low insecticide mortality and high predation (D). Note differences in scales across panels.*

In our simulations, we consider four scenarios: 1) moderate insecticide mortality with no predators, 2) higher insecticide mortality with no predators, 3) moderate insecticide mortality with low predation

(20% the per diem mortality of insecticides), and 4) low insecticide mortality with high predation (5 times the per diem mortality of insecticides). We find that increasing the mortality caused by the insecticides does reduce the overall numbers of codling moth adults, but the resistant allele quickly becomes fixed in the population, allowing high numbers of codling moths (Figure 4A,B). Similarly, the addition of predators does have a small effect on slowing resistance development by increasing overall mortality, but ultimately the resistant allele becomes fixed in the population (Figure 4A,C). In contrast, a combination of reducing insecticide sprays and increasing predation rates allows for low codling moth numbers, while simultaneously limiting resistance in the population (Figure 4A,D). These results highlight that it is the reduction in insecticides, rather than predation per se that reduces insecticide resistance in pest populations such as codling moths. However, the conservation of predators allows for low pest populations while resistance is being managed, allowing for improved yields and fruit quality.

*References:*

Anderson, R.M., May, R.M., 1979. Population biology of infectious diseases: Part I, Nature. 280, 361–367.
Ferro, D.N., R.R. Sluss, T.P. Bogyo. 1975. Factors contributing to the biotic potential of the codling moth, Laspeyresia pomonella (L.) in Washington. Environmental Entomology, 4: 385-391.
Jones, V.P., N.G. Wiman, J.F. Brunner. 2008. Comparison of delayed female mating on reproductive biology of codling moth and obliquebanded leafroller. Environmental Entomology, 37: 679-685.
Leslie, P.H. 1945. On the use of matrices in certain population mathematics. Biometrika, 33: 183-212.

*Resulting publication:*

Roberts, J.P., and T.D. Northfield. In revision. Entomopathogen-host evolution and implications for biopesticide resistance management. Preprint: **doi:** https://doi.org/10.1101/2022.10.28.514078

**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. Similarly, he presented the findings at the Pacific Branch of the Entomological Society of America as part of a symposium focused on mathematical model in applied systems. Although it will take place after the end date, Roberts will also present his results at the Orchard Pest and Disease Management Conference in Portland, Oregon, which includes a combination of researchers, insecticide industry representatives and crop consultants. 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 a member. This is an important meeting that includes many stakeholders, such as growers and crop consultants.

## Impacts

Short term: Based on our research, we find that further methods need to be developed before nematodes are used to effectively control codling moth pupae when they are not in the soil (e.g., when pupating on tree bark).

Mid term: Informing biopesticide resistance management programs, and identifying potential avenues for biopesticide improvement by selecting for better entomopathogens.
Long term: Improved long-term sustainability of biologically-based insecticides through the integration of resistant pests in pathogen rearing.

## Additional funding applied for/secured:

Applied for:
Northfield, TD, R Gomulkiewicz, WR Cooper, USDA AFRI. "Disrupting disease epidemiology with vector entomopathogens." $749,563. Not funded.

Secured:
$2 million/year congressional appropriations for collaborative research between WSU and USDA ARS Wapato on X-disease and Little cherry virus pathogen systems. This research will include evaluation of entomopathogens used, informed by the theory developed here.

## Graduate students funded:

Jeremy Roberts, Entomology, Wenatchee TFREC, MS in Entomology, 2022

## Recommendations for future research:

PI Northfield is currently funded by a USDA Specialty Crop Research Initiative project focused on integrated management of spotted wing drosophila. There is concern for insecticide resistance evolution in spotted wing drosophila, and Northfield will build on the research developed here to inform sustainable management of this important fruit pest.