FINAL PROJECT REPORT

Project Title: Assay development to monitor insecticide resistance in codling moth

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Total Project Request: **Year 1:** $24,150 **Year 2:** $24,960
Cooperators:

Other funding sources: None

Total Project Funding: 49,110

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	15,000	15,750	
Benefits	1150	1210	
Wages			
Benefits			
Equipment			
Supplies	8000	8000	
Travel			
Miscellaneous			
Total	24,150	24,960	

ORIGINAL OBJECTIVES

1) Develop assays that determine the levels of enzymes that degrade pesticides.
2) Clone transcripts that encode known enzymes that confer insecticide resistance.
3) Clone transcripts that encode known targets of insecticides currently used in the orchard.
4) Develop assays to determine target mediated resistance.

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

- Optimized conditions for assays used to measure enzymes involved in insecticide resistance.
- Used enzyme assays to determine differences between lab and field populations of codling moth.
- Identified gene transcripts that encode enzymes that confer insecticide resistance.
- Used enzyme assays to determine differences in field populations of codling moth either susceptible or resistant to organophosphates or neonicotinoids.

RESULTS AND DISCUSSION

There are two broad mechanisms by which insect pests develop resistance to insecticides. They may produce large amounts of detoxifying enzymes which either break down the insecticide molecule or bind to it so tightly that it cannot function (a process known as sequestration). The second mechanism, and much less frequent, involves mutation of the insecticide target site, such as the acetylcholinesterase enzyme in the nervous system. This effectively blocks the action of the insecticide. Both types of mechanism have been studied in various species of insect.

Detoxification enzymes are a natural part of the insect defense system against foreign agents, such as toxic plant compounds. These enzymes also function to inactivate insecticides. There are three main classes of detoxification enzymes; cytochrome P450 monooxygenases (P450s), esterases, and glutathione-*S*-transferases (GST). P450s have broad substrate specificities so this class of enzyme can mediate resistance to all classes of insecticides. This broad substrate specificity and the fact that 600 genes encoding P450s have been identified in insects makes this family of enzymes a major contributor to insecticide resistance. Glutathion-*S*-transferases play a role in the defense by attaching a glutathione molecule to a foreign molecule, an insecticide for example. Once the glutathione is attached, the foreign molecule with glutathione is sequestered by the insect, making it unable for the insecticide to reach its target site. Esterases are the third important group of detoxification enzymes. An esterase is an enzyme that splits ester bonds into an acid and an alcohol in a chemical reaction with water. Esterases have been well documented for their role in insecticide resistance either by a mutation in the enzyme that causes it to bind tightly to organophosphates or by over expression of the gene which is responsible for detoxification of carbamates and pyrethroids.

Enzyme assays to determine esterase, GST, and P450 levels in codling moth males and females were developed using the insects from lab colony at YARL. Once the assays were optimized, enzyme levels were determined for 30 – 50 individuals. Enzyme activity levels for males and females are listed in Table 1. Enzyme levels were different in males and females. This sex specific difference indicates the importance in treating males and females separately when determining a baseline level of enzyme activity. Determination of the baseline enzyme levels for the moths from lab colony gave us the ability to compare those to field collected insects. Dr. Alan Knight provided me with field collected codling moth, and presumably organophosphate and neonicotinyl resistant, from a highly sprayed orchard (LatA). Significant increases in esterase and GST enzyme activity was observed in the field collected insects (Table 1).

Table 1. Enzyme Activities for Laboratory Reared and Field Collected Adult Codling Moth

	Lab male	***Lab female***	***Lab M+F***	***Field male***	***Field female***	***Field M+F***
Esterase	612 ± 111	435 ± 137	525 ± 152	826 ± 424	852 ± 253	835 ± 369
P450s	12.2 ± 3.9	15.2 ± 3.1	13.7 ± 3.8	15.4 ± 6.3	6.6 ± 3.4	11.9 ± 7.2
GST	12.2 ± 5.1	8.7 ± 3.7	10.3 ± 4.8	31.9 ± 14.3	31.5 ± 8.0	31.8 ± 12.2

This initial study showed the potential utility of the enzyme assays, and this year a more thorough group of insects was tested with the hopes that the assays will allow us to define levels where field resistant populations could be predicted using this procedure.

A much more exhaustive study was done this year, using insects collected from 18 field sites, with codling moth populations displaying various resistance levels to organophosphates and neonicotinoids. Acetylcholinesterase activity (Figure 1), glutathione *S*-transferase activity (Figure 2), mixed function oxidase (cytochrome P450s) activity (Figure 3), and non-specific carboxylesterase activity (Figure 4) were determined for about 30 males and 30 females from each collection site. Differences in male and female enzyme levels can be seen among many of the field populations. Our results indicate that esterase levels (Figure 4) appear to correlate with resistance (when compared to the lab colony), but this does not hold true for males from each of the populations. Oxidase levels do not give clear results (Figure 3) nor does glutathione *S*-transferase activity (Figure 2). Acetylcholinesterase levels seem to correlate with resistance, but only in females (Figure 1). Our results seem to indicate the complexities of using enzyme levels to predict insecticide resistance.

Our conclusions have recently been supported by the same research group that called for standardization of enzyme assays to monitor codling moth for insecticide resistance (Reyes et al., In Press, Pesticide Biochemistry and Physiology available online). The authors conclude that 1) "The contrasting responses of the sensitive and resistant strains to azinphos-methyl and to various esterase substrates indicates that partial investigations can lead to erroneous conclusions about the involvement of different mechanisms in the resistance of codling moths to OPs. It is likely that our knowledge in this area remains incomplete." 2) They further conclude that "The resistance ratios for azinpho-methyl were quite related to the enhanced MFO activity observed for these resistant strains. Thus, the simple measurement of one detoxification system associated with bioassays would therefore conclude that this detoxification system is exclusively involved in the resistance to the insecticide in question. However this may not tell the whole story and be misleading".

Based on the results shown in their paper and the results from tests we have run there is an abundance of information to be obtained by using different substrates and assay protocols. Different substrates measuring different enzyme activities and resistant levels show that using one assay or substrate gives a very small view of the greater picture involved in insecticide resistance. "The resistance of the codling moth to the OPs appears complex. Depending on the resistant strain considered, it may be the result of the combination of several mechanisms". Much more research is needed to clarify this situation before enzyme assays alone can be used to predict insecticide resistance in the orchard.

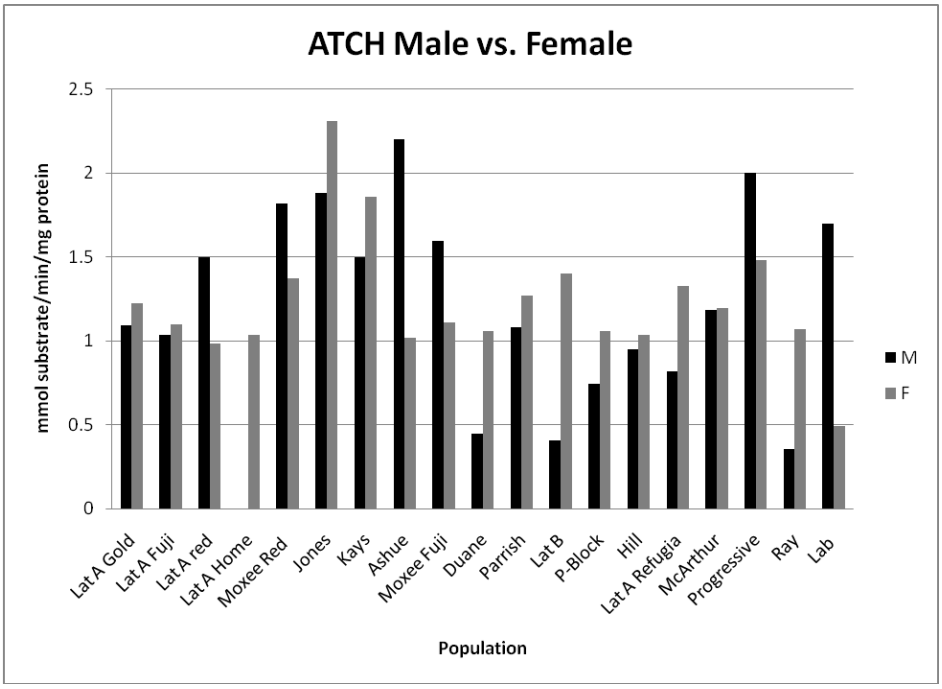

Figure 1. Acetylcholinesterase activity in male and female Codling Moth collected in the Yakima Valley.

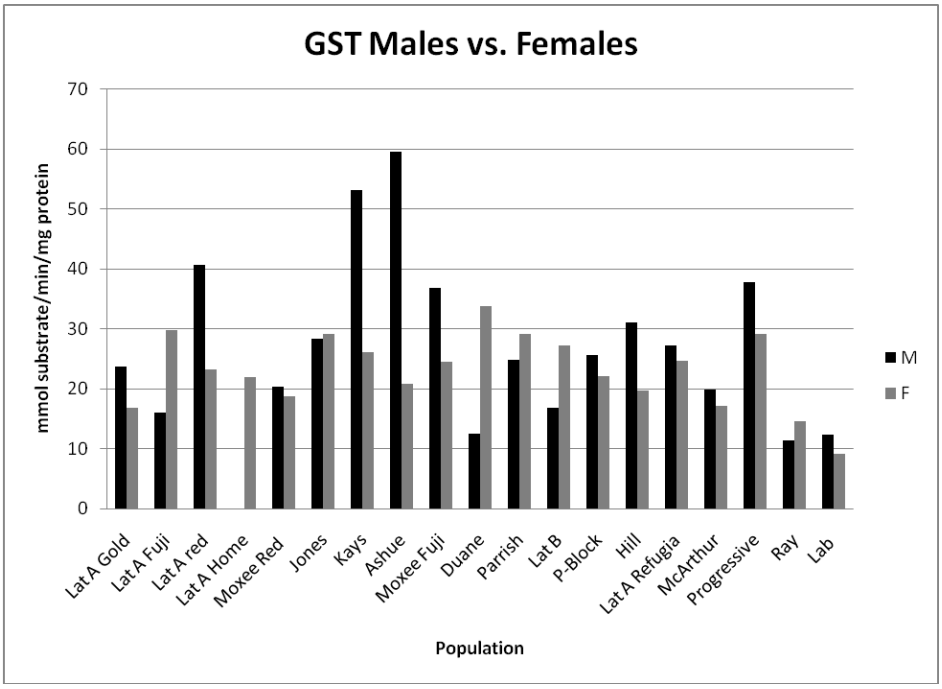

Figure 2. Glutathione S-Tranferase activity in male and female Codling Moth collected in the Yakima Valley.

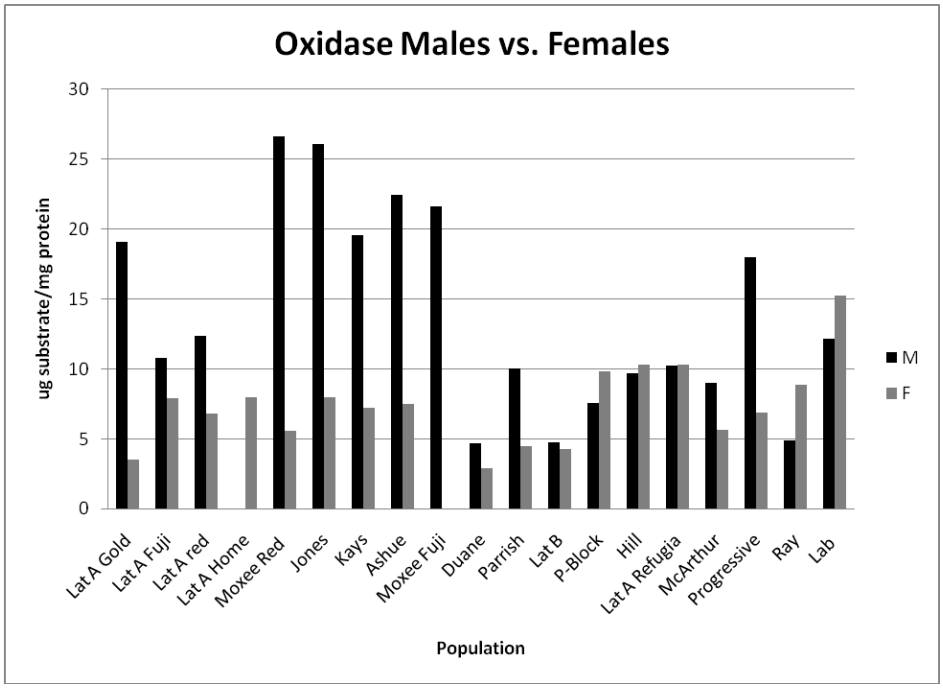

Figure 3. Mixed-Function Oxidase activity in male and female Codling Moth collected in the Yakima Valley.

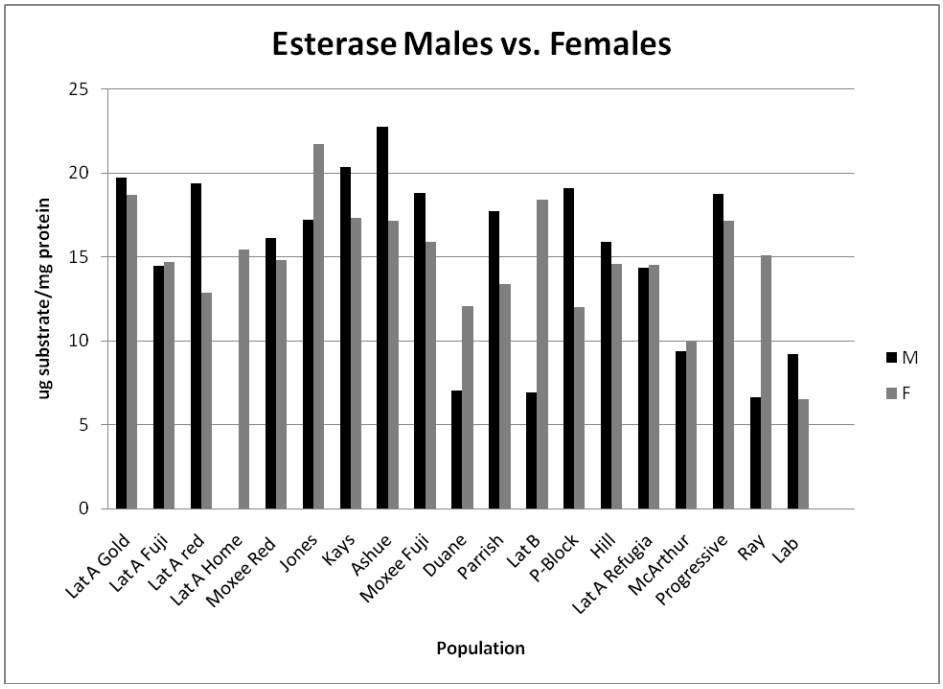

Figure 4. Non-specific Esterase activity of male and female Codling Moth Collected in the Yakima Valley.

Another goal of this project was to determine the nucleotide sequences of these enzymes as expressed in the codling moth. Analysis of the codling moth transcriptome allowed us to identify transcripts encoding 10 glutathione *S*-transferases, 7 carboxylesterases, and 20 mixed function oxidases (Table 1). Future work would have to include expression of each of these enzymes and then to determine if they detoxify individual chemical insecticides. Only through this type of analysis would we be able to pinpoint the specific enzyme(s) that are able to degrade the chemical compounds to a non-toxic form.

Table 2. Insecticide resistance enzymes annotated from codling moth transcriptome.

Tissue Source	Annotated Hits by Homology	GST	Esterase	Cyt P450
Male Antennae	**542**	**4**	**1**	**5**
Female Antennae	**475**	**4**	**0**	**3**
Male Legs and Mouthparts	**431**	**3**	**0**	**5**
Female Legs and Mouthparts	**350**	**1**	**4**	**6**
Eggs (Embryos)	**660**	**2**	**0**	**3**
Neonate Larvae	**617**	**4**	**1**	**4**
All Tissues	**2267**	**10**	**7**	**20**

Glutathione S-transferase (GST), Carboxylesterase (Esterase), mixed function oxidase (Cyt P450).

EXECUTIVE SUMMARY

Resistance to chemical insecticides used to control codling moth in the orchard is a major concern and is potentially costly to orchardists. The goal of this project was to develop assays that can be used to determine the mechanism of insect resistance in orchard populations of codling moth. Insecticide resistance can either manifest itself as an increase in detoxification enzymes or as a modification to the specific target of a given pesticide. Determination of the mechanism of insecticide resistance in an orchard population, either target site or detoxifying enzyme, would allow the orchardist to select appropriate control measures. For example, if the resistance is due to a detoxification enzyme, the population could be cross resistant to other chemicals and an alternate class of pesticide could then be selected to provide adequate control. If rapid assays were available to determine the resistance mechanism, this would provide useful information in the orchardist's choice of control measures.

Progress was made in the development of assay procedures to monitor detoxification enzyme activities. Our results seem to indicate the complexities of using enzyme levels alone to predict insecticide resistance. These results were confirmed by the original research group that called for standardization of methods to determine detoxification enzyme levels. We detected variation in enzyme levels among different field populations, as well as differences based on the sex of the insect. Our conclusions, as well as those of the French researchers, are that insecticide resistance is complex and that more specific assays are needed before we can even think about using them to predict resistance in the orchard.

Future directions for this project include using the sequence information derived from the codling moth transcriptome to examine the role of each of the detoxification enzymes in degrading specific chemical insecticides. While a project of this sort is possible, it would take much effort and resources to complete. If needed, we have laid the groundwork for such a project by determining the nucleotide sequences for many of the codling moth enzymes. Expression of these enzymes and chemical degradation assays would need to be done in order to identify the particular detoxification method used by resistant codling moth populations.