2009 Apple Research Review
January 21-23, 2009
Hoilday Inn at TRAC
Pasco, WA
22 January

Time	Page	PI	Project Title	Funding period
8:00		T. Schmidt	Introduction	

Apple Crop Protection

			Final Reports	
1:00		Hanrahan	Introduction	
1:15		McFerson	Technology Roadmap update/SCRI report	
1:30	1	Fazio	Replant disease tolerance of Geneva rootstocks	06-08
1:45	12	Xiao	Decay control and management of fungicide resistance (extension)	07
2:00	22	Landolt	Sprayable foam for trap and kill of cocooning codling moth larvae	06-08
2:15	27	Garczynski	Molecular characterization of taste, smell and feeding in codling moth	07-08
2:30	34	Garczynski	Identification of Bt toxin targets in codling moth larvae	07-08
2:45	36	Yee	Apple maggot host attractants	08
3:00	48	Yee	DNA and morphometric diagnostics for apple and snowberry maggot flies	08
3:15	59	Neven	Fate of codling moth in apples after harvest	07-08
Group			**Poster Session Continuing Reports 5:00- 6:30pm**	
1	70	Xiao	Control of postharvest fruit rots in apple	08-09
1	77	Campbell	Augmenting fungal control in apples with natural compounds	07-09
1	85	Beers	Integrated biological control of wooly apple aphid	08-10
2	91	Jones	Interaction of dispersal and management of CM and OBLR	07-09
2	97	Jones	Defining natural enemy biology and phenology to improve IPM: Poster 1	08-10
2	105	Brunner	Management of codling moth and leafrollers in apple orchards	08-09

FINAL PROJECT REPORT

Project Title: Replant disease tolerance of Geneva rootstocks

PI:	Dr. Gennaro Fazio	**Co-PI(2):**	Dr. Mark Mazzola
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State/Province/Zip	NY	**State/Province/Zip:**	WA

Cooperators: Tom Auvil, Tim Smith and others at WTFRC

Other funding sources: None.

Total Project funding: $95,230

Budget History

Item	2006	2007	2008
Salaries[1]	17,000	17,000	21,000
Benefits	5,000	5,000	6,000
Wages			
Benefits			
Equipment			
Supplies[2]	6,000	6,000	6,000
Travel[3]	1,530	1,530	1,530
Miscellaneous[4]			
Total	30,000	30,000	35,320

Footnotes: [1]Technician salary for part-time assistance in propagation budding and maintenance of stoolbeds.
[2]Includes cost for rootstock liners, trees, support system, laboratory supplies etc.
[3]Travel to and from trials.
[4]Includes shipping expenses, communication costs etc.

Progress Report
Objectives

1. To study the relative performance of Geneva dwarfing apple rootstocks compared to commercial controls in replant soils and the study of genetic mechanisms related to tolerance to ARD.
2. In the most recent visits we have come to appreciate the need by a certain segment of the industry to plant liners in place in the orchard either as sleeping eyes or as bench grafts. We would like to modify our existing protocol to discover "nursery in place" properties of rootstocks and how they interact with replant disease when the plants are so young. The question we are trying to answer is: how well do ARD tolerant sleeping eyes and bench grafts do in a replant situation?
3. To set up an early evaluation protocol for newly developed genotypes that screens for components of apple replant disease resistance in the early stages of breeding.

SIGNIFICANT FINDINGS AND DEVELOPMENTS

- **General replant tolerance has been confirmed in certain Geneva rootstocks (CG4214, G.41, G.935, and CG5890). Even though we did not intend to make these trials about fire blight resistance, this disease has killed several known susceptible rootstocks (M.9 Pajam 2 came in first with 35% dead and Supporter 1 and 2 with 20% trees each). Fire blight resistant B.9 continues to be one of the weakest and least productive rootstocks in all the replant experiments that have been planted so far. Malling 9 survival has been compromised by several fire blight events.**
- **Fumigation's positive effect on season's tree growth treatment disappeared in the fourth season, however, the initial boost in growth increased the cumulative effect on TCSA and Fruit Yield – it seems as if the replant susceptible rootstocks are behind one or two seasons.**
- **It is critical to plant replant and fire blight resistant rootstocks in orchards destined for organic management since losses due to rootstock susceptibility alone can amount to over 50% of the potential yield.**
- **First yield data from a graft in place replant experiment containing the widest selection of Geneva rootstocks ever tested in Vantage WA indicates that some new Geneva rootstocks match or surpass control rootstocks.**

OBJECTIVE 1. During this granting period we field tested a number of rootstocks (Table 1) at several documented diverse replant sites, including Wapato, Chelan, Brewster, Vantage, and Naches. These sites represent different soil, management and agro-ecological conditions and are a good representative test of the reliability of new rootstock genotypes in WA. Thanks to the efforts of the Washington Tree Fruit Research Commission, these trials also cover other traits that may be impacted by rootstocks such as fruit size, maturity, and other fruit disorders. Plans to establish larger plantings with selections from these experiments are well on their way. These trials will impact the apple production machinery in WA in a very significant way as federal and state regulation of fumigants increases and the amount of virgin land optimal for apple production decreases.

The study of the genetic factors that are impacting replant tolerance found in the Geneva breeding program is still in its early life. We have accomplished a very important step in this process: the confirmation that there are genetic differences in the interaction between components of ARD and a diverse pool of apple rootstocks. For example, Geneva rootstocks that shared common ancestry supported lower populations of lesion nematodes (*Pratylenchus penetrans*) and had lower incidence of *Phythium* infection in replant soils (Mazzola et. al, 2009 Plant Disease 93:51-57). Another example is a root morphological trait which produces a preponderance of fine roots and is shared by several replant tolerant Geneva apple rootstocks. We are in the process of characterizing this trait and its impact on nutrition, replant tolerance and productivity.

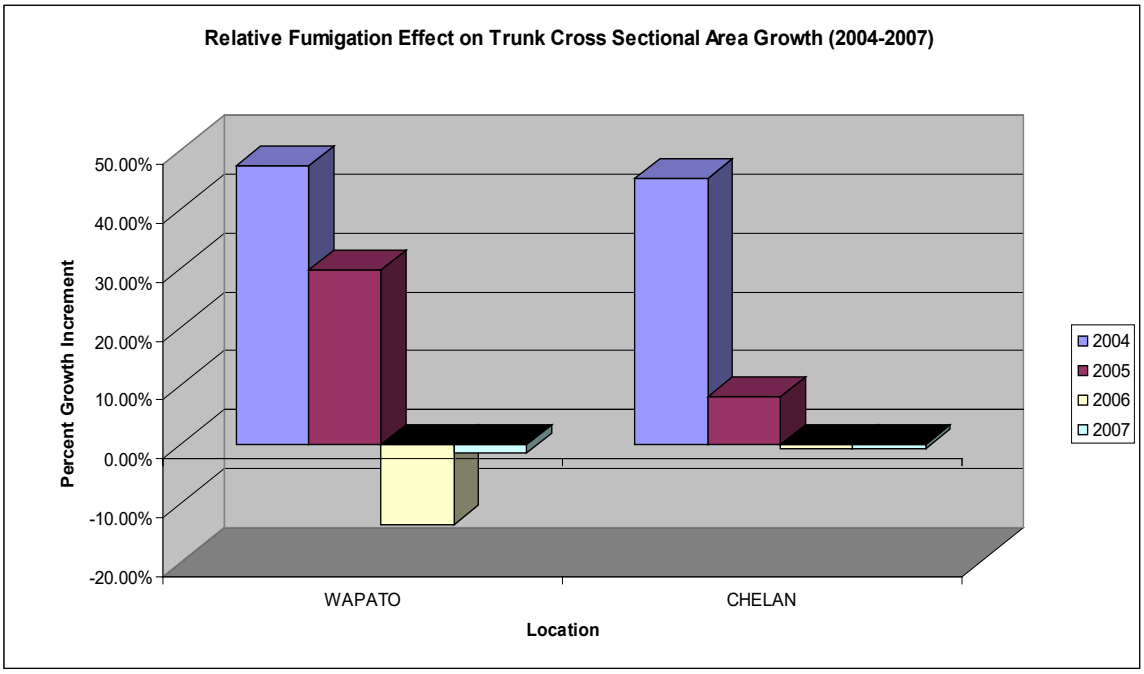


Figure 1. This figure depicts the effect of fumigation on growth relative to unfumigated samples over three years. It is evident that the effect on tree growth is significant in the first two years and then dissipates in following years. To have a major impact on production planting must follow soon after fumigation (within safety limits) otherwise the benefits disappear.

Table 1. Locations and rootstocks planted in ARD trials 2003-2008.

Rootstock	Location*	Scion Varieties
G.16	WA, CH, NA	Brookfied Gala, Honeycrisp
G.11	WA, CH, BR, VA	Brookfied Gala, Torres Fuji, Aztec Fuji
G 3041	WA, CH, VA	Brookfied Gala, Aztec Fuji
G 5935	WA, CH, NA, VA	Brookfied Gala, Honeycrisp, Aztec Fuji
PiAU-56-83	WA, CH	Brookfied Gala
Pajam 2	WA, CH	Brookfied Gala
M.26 EMLA	WA, CH, NA	Brookfied Gala
Bud 9	WA, CH, NA	Brookfied Gala
Supporter 1	WA, CH	Brookfied Gala
Supporter 2	WA, CH	Brookfied Gala
Supporter 3	WA, CH	Brookfied Gala
4214	WA, NA, BR, VA	Brookfied Gala, Torres Fuji
4003	NA	Honeycrisp
4814	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
4210	NA, BR, VA	Honeycrisp, Torres Fuji, Aztec Fuji
G.30	NA, VA	Honeycrisp, Aztec Fuji
5087	NA, VA	Honeycrisp, Aztec Fuji
G 4202	NA	Honeycrisp
4013	NA	Honeycrisp

Rootstock	Location*	Scion Varieties
4213	NA	Honeycrisp
M.9 EMLA	NA, BR	Honeycrisp, Torres Fuji
5757	BR	Torres Fuji
G.202	BR, VA	Torres Fuji, Aztec Fuji
6879	BR	Torres Fuji
MM.106	BR	Torres Fuji
6006	BR	Torres Fuji
7707	BR	Torres Fuji
5257	BR, VA	Torres Fuji, Aztec Fuji
3007	BR, VA	Torres Fuji, Aztec Fuji
4011	BR, VA	Torres Fuji, Aztec Fuji
5935	BR	Torres Fuji
5463	BR, VA	Torres Fuji, Aztec Fuji
4003	BR	Torres Fuji
6001	BR	Torres Fuji
6210	WA	
M.7	BR, WA	Torres Fuji
JTE-B	BR	Torres Fuji
Ottawa 3	BR	Torres Fuji
JTE-C	BR	Torres Fuji
5890	BR	Torres Fuji
2034	VA	Aztec Fuji
2406	VA	Aztec Fuji
3001	VA	Aztec Fuji
4002	VA	Aztec Fuji
4004	VA	Aztec Fuji
4013	VA	Aztec Fuji
4172	VA	Aztec Fuji
4288	VA	Aztec Fuji
5046	VA	Aztec Fuji
5179	VA	Aztec Fuji
5202	VA	Aztec Fuji
4019	VA	Aztec Fuji
Mark	VA	Aztec Fuji
Supporter 4	VA	Aztec Fuji

*** WA=Wapato, CH=Chelan, NA=Naches, VA=Vantage, BR=Brewster**

FINDINGS BY LOCATION:

2004 CHELAN REPLANT TRIAL – HOW IMPORTANT ARE ROOTSTOCKS UNDER ORGANIC MANAGEMENT?

This was the fifth growing season for this trial. We have learned that rootstocks play a very big role in the success of an organic orchard. Pervasive tree death due to fire blight or vole damage was predominant in M.9 trees. B.9 survived fire blight but several trees were lost and the surviving ones failed to fill their space and looked extremely stunted in both fumigated and non-fumigated treatments. The fumigation effect on tree productivity is still significant in the overall planting, especially for susceptible rootstocks; the difference in fruit per tree between treatments (Figure 2) is significant. That difference may be due to the increased scaffold build in the first two seasons of growth due to the fumigation effect. Overall, the initial growth spurt due to fumigation is still

noticeable throughout the orchard but as shown in Figure 3 there were no differences in growth due to the fumigation treatment this year. When we look at the performance of the individual rootstocks (figures 2 and 3 we notice that some are relatively unaffected by the replant problem and seem to do relatively well in fumigated and non-fumigated soils. G.41 and G.935 continue to perform well in this trial and anecdotally tree deaths due to vole damage seem to be less in Geneva rootstocks than other rootstocks. In the extra non fumigated rows of this trial are several plants of CG4214 (data not shown) that performed relatively well compared to G.41 and G.935.

2004 WAPATO REPLANT TRIAL – This trial has come into full production and most rootstocks (B.9 being the exception) have filled the canopy space. In this planting in the overall effect of the fumigation is still detectable. CG4214, G.41, G.935 have performed well and have shown that having fire blight resistance along with apple replant tolerance is a very good thing.

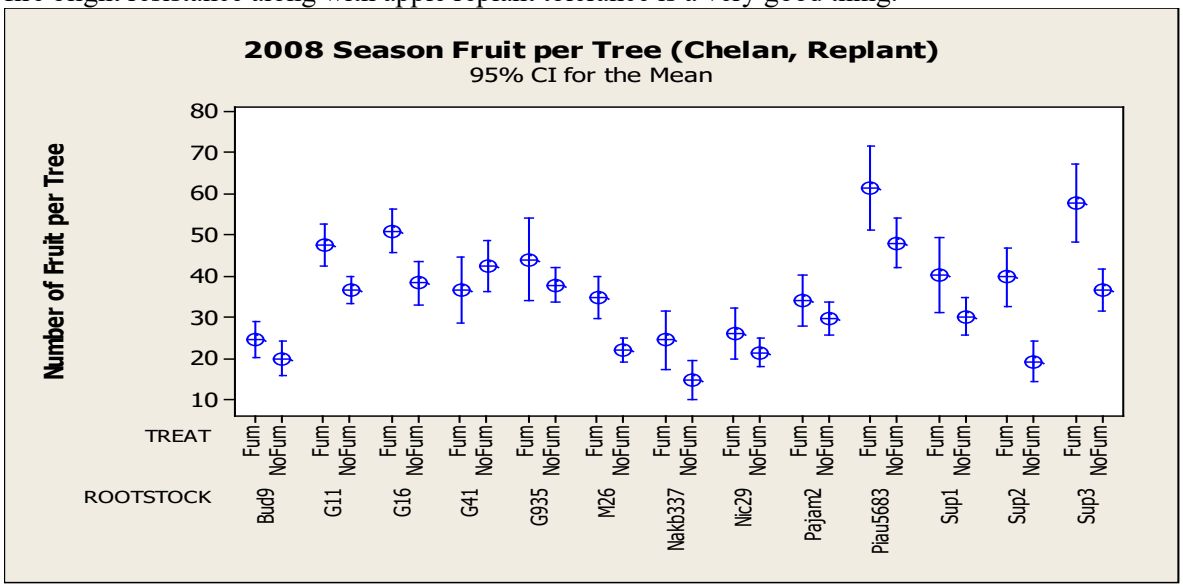


Figure 2. Yield per tree 2007. In this organic planting in Chelan the overall effect of the fumigation is still detectable. Some rootstocks however do not seem to be affected as much (3041 aka G.41, 5935 aka G.935). A mixture was identified in G.41 rootstock. Every G.41 tree in the trial was DNA fingerprinted resulting in the identification of all misidentified trees (roughly 20% of the total). This rootstock was labeled 27R5-1.

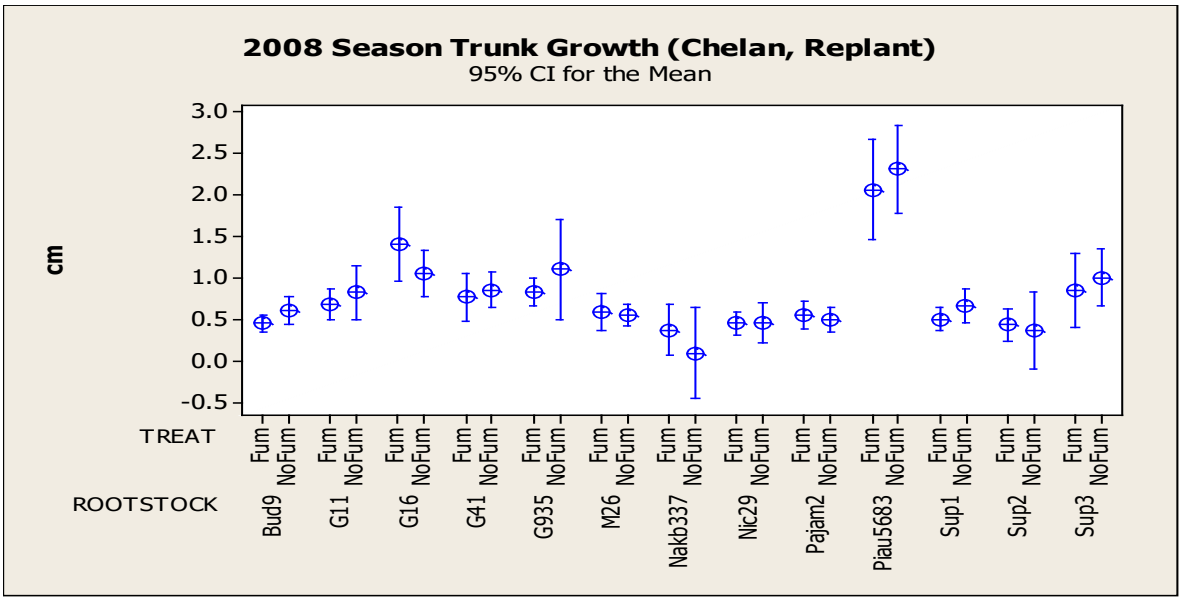


Figure 3. 2008 Season trunk growth shows virtually no difference between the fumigated and non fumigated treatment: a sign that the effect is gone and that the productivity now and in the future is in the hands of genetic resistance of individual rootstocks.

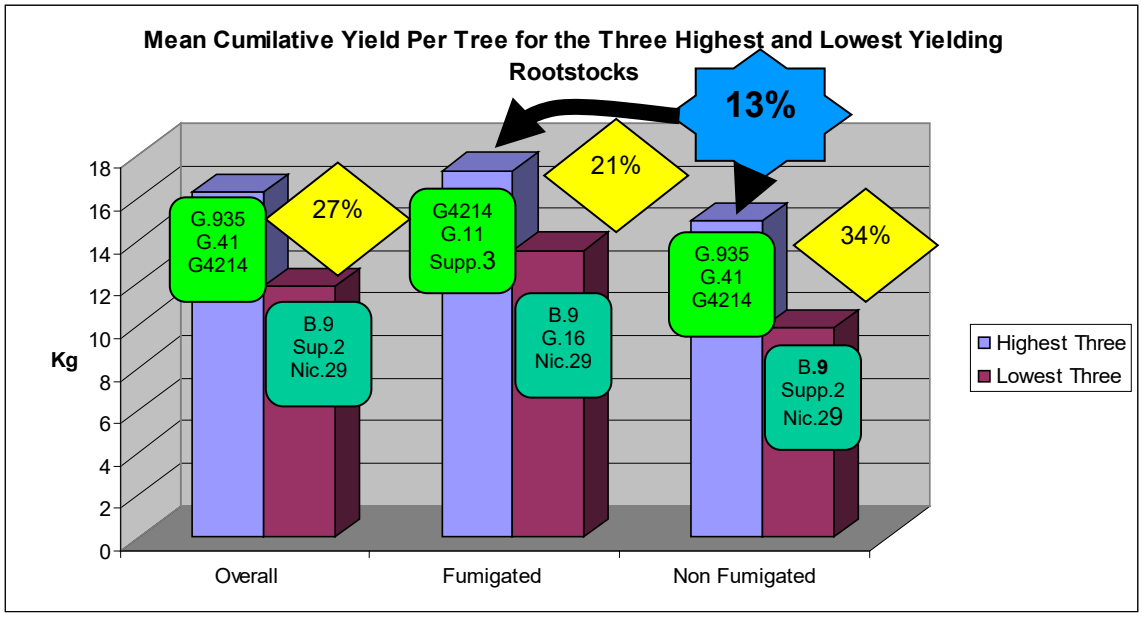


Figure 4. To generate the above graph we took the best and worst three performers in the different categories and calculated the means of cumulative yield. In Wapato G.935, G.41 and G.4214 are consistently the best rootstocks producing up to 34% more apples per tree that the three low producing rootstocks. In the fumigated block Supporter 3 and G.11 ranked in the top three indicating yield potential in fumigated or virgin soil. While fumigation increases production by 13% in the best genetic scenario (resistant rootstocks), using resistant rootstocks increases production by 21% in fumigated ground and by 34% in non fumigated ground (data up to 2007).

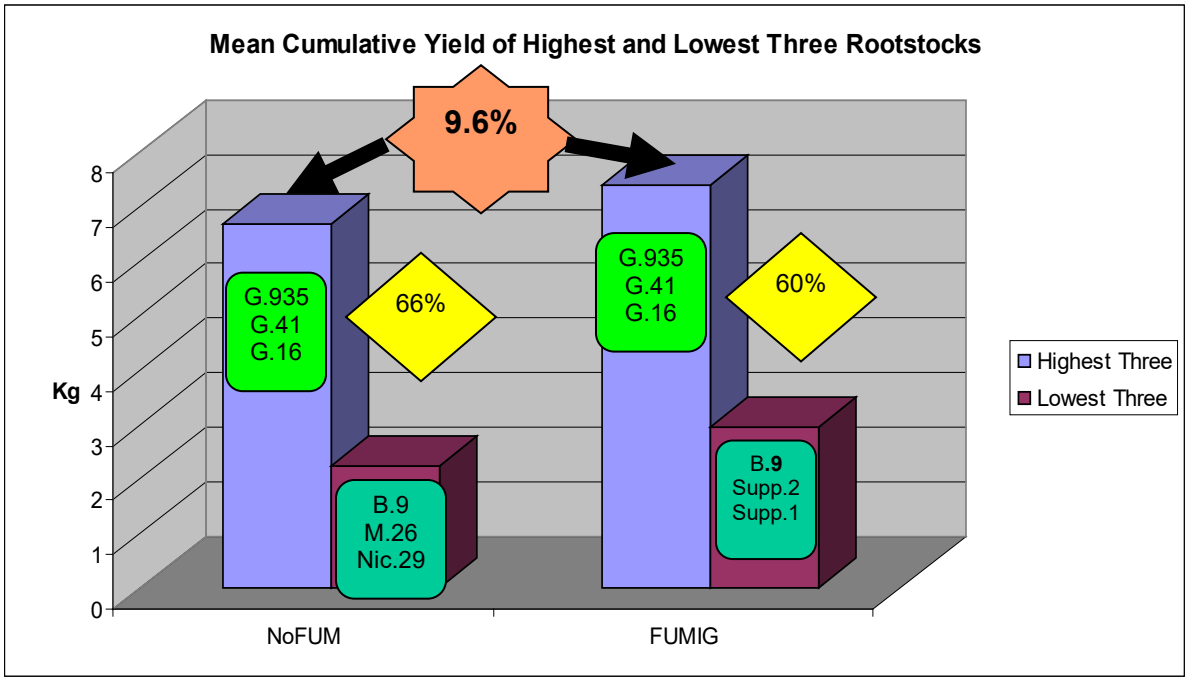


Figure 5. Same analysis as in Figure 4 but for the Chelan Replant Trial. The Advantage of planting resistant rootstocks is very clear.

2006 BREWSTER REPLANT TRIAL

This trial is set in the quintessential replant location having been cultivated in apple for over 100 years. This trial is a good indication that trying to escape replant by fumigating and planting vigorous rootstocks such as MM106, M.7 or 5463 is futile – they produced too much unproductive wood and were hard to manage. Despite the harsh replant environment a few Geneva rootstocks performed well and had a good crop load in both fumigated and non fumigated treatments. CG5980 is one of those rootstocks in the semi-dwarf category that performed well in this trial and other trials in NY state with Honeycrisp as the scion. Along with 4214 it is slated for release in the near future.

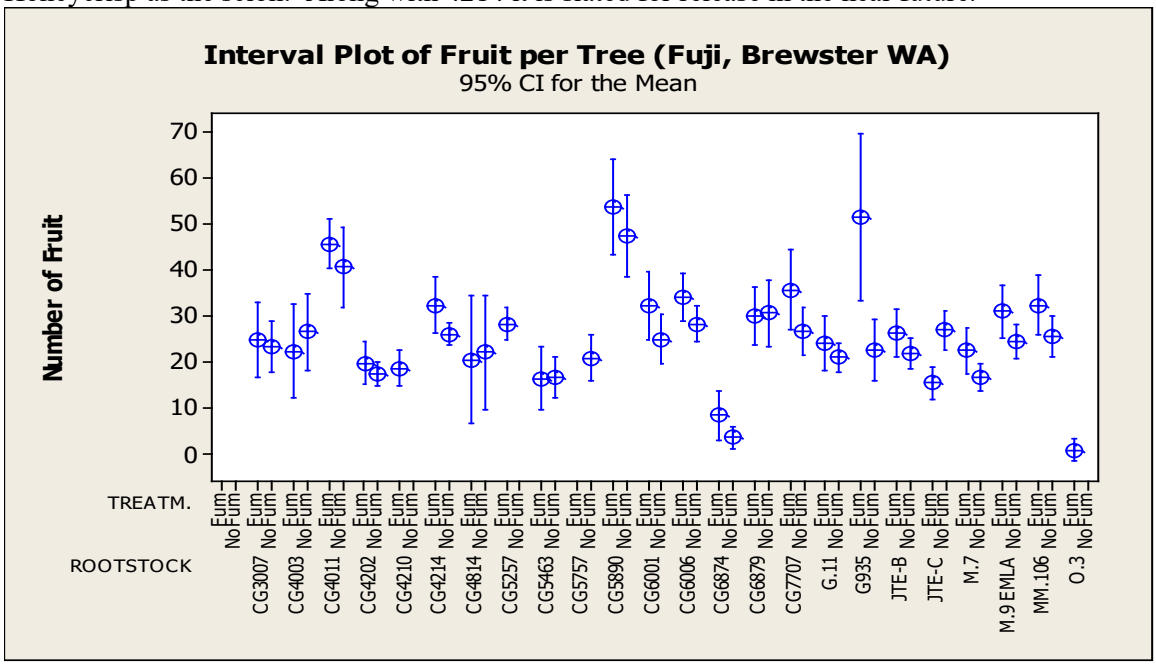


Figure 6. Brewster Trial planted in 2006. 5890 and 4011 are the top performers. Vigorous rootstocks in this trial were inefficient.

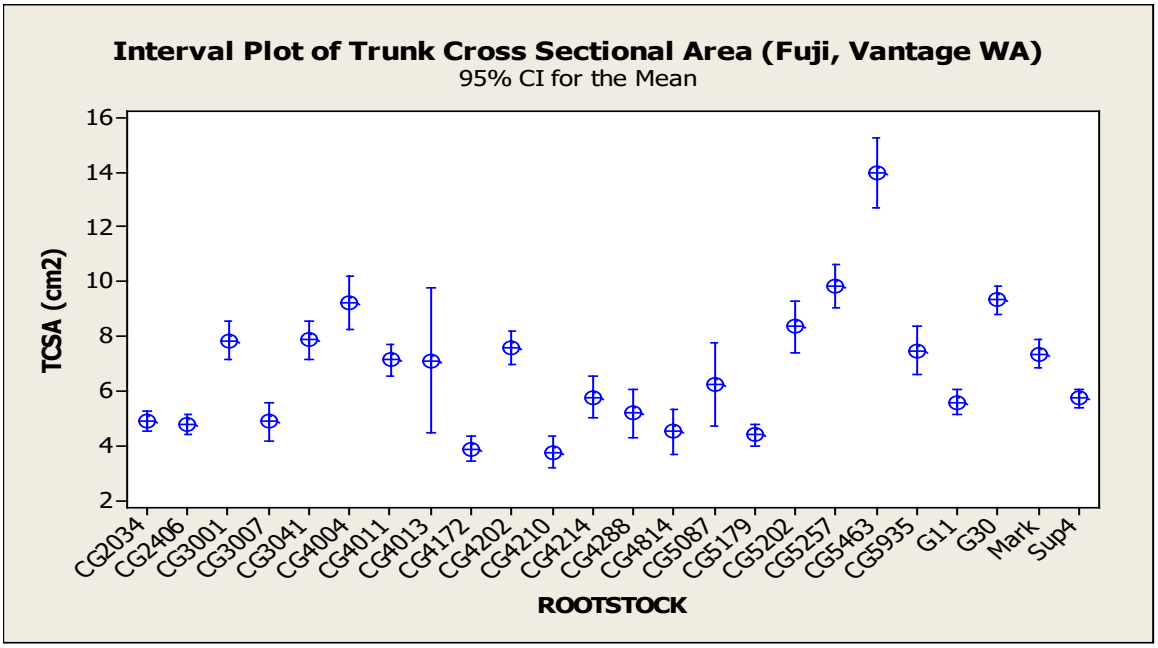


Figure 7. Tree size after two seasons. CG5202, CG5463, CG4004 and G.30 may be too vigorous for this type of management.

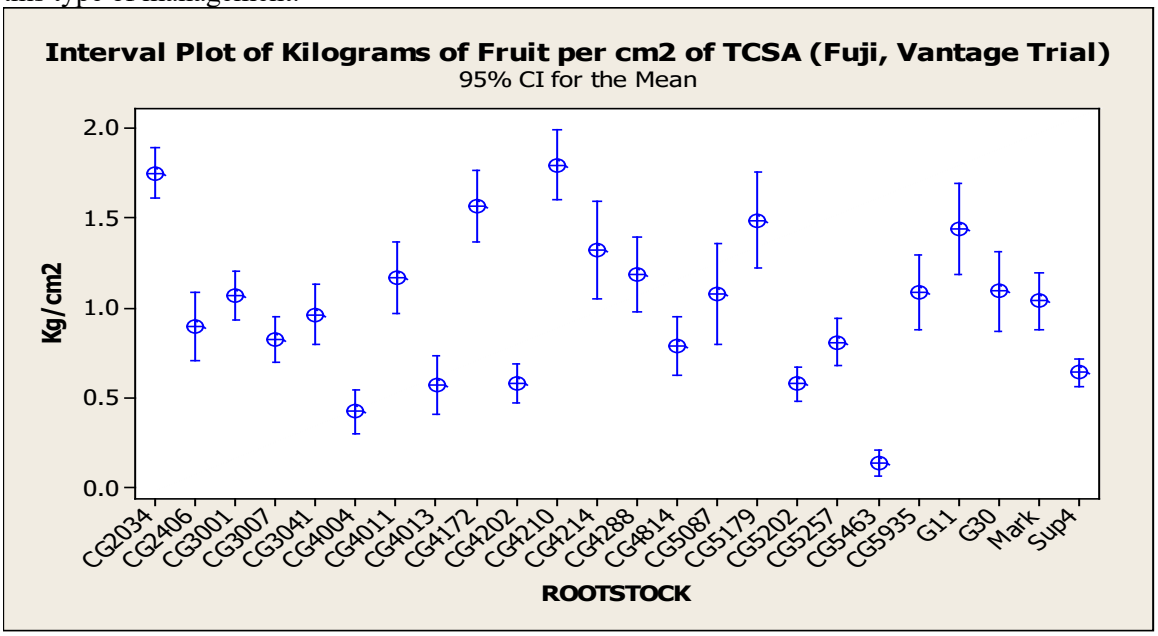


Figure 8. Several Geneva rootstocks were as yield efficient as Mark. CG.5463 will have to be removed from this trial.

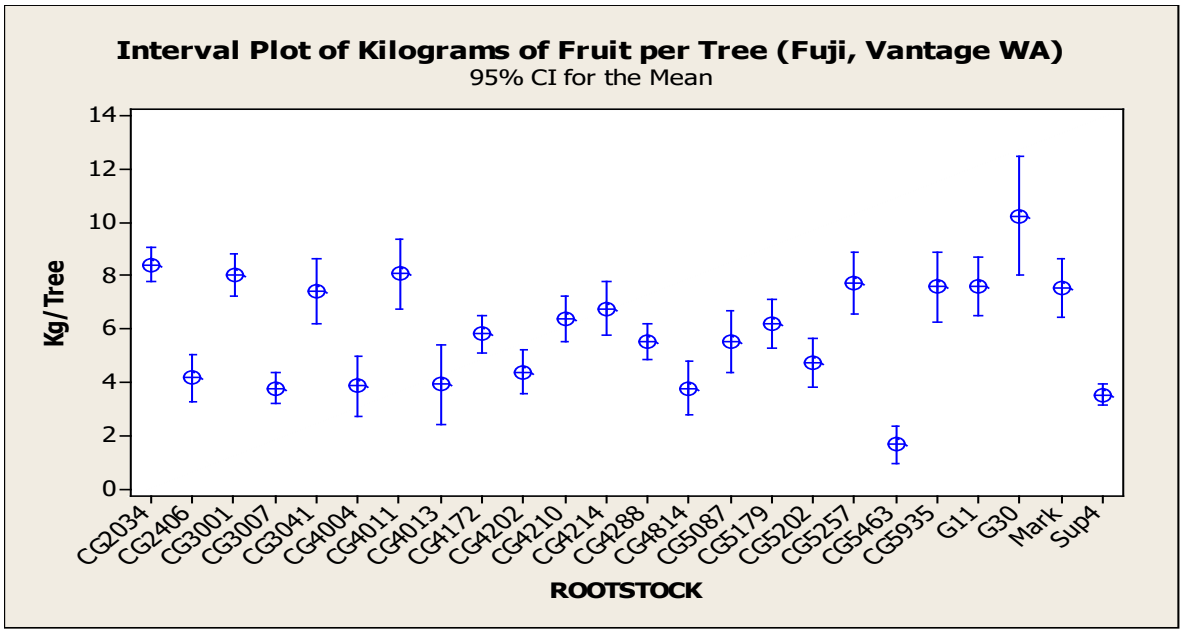


Figure 9. Several Geneva rootstocks were able to produce a "target" number of fruit per tree similar to the check Mark. S

OBJECTIVE 2

This trial represents one of the most diverse trials in WA in terms of new rootstocks from the Geneva breeding program (Figures 7-10). This year represented the first crop on the BenchGrafts (BG) replant trial at the Auvil Fruit Tree Farm (Vantage, WA). This is a very discriminatory trial because of the intensive precision management intended to push the rootstocks to the maximum of their ability. As a result we have eliminated several rootstocks that seem to be overwhelmingly susceptible to latent viruses since the benchgrafting material was not clean. A few new rootstocks such as CG2034 and CG3001 have shown promise under this type of management. Other rootstocks such as G.41 (CG3041), G.11, G.935 all performed as well as the check rootstock Mark. It will be interesting to watch CG2034 to see if it comes back with a similar crop in the next season.

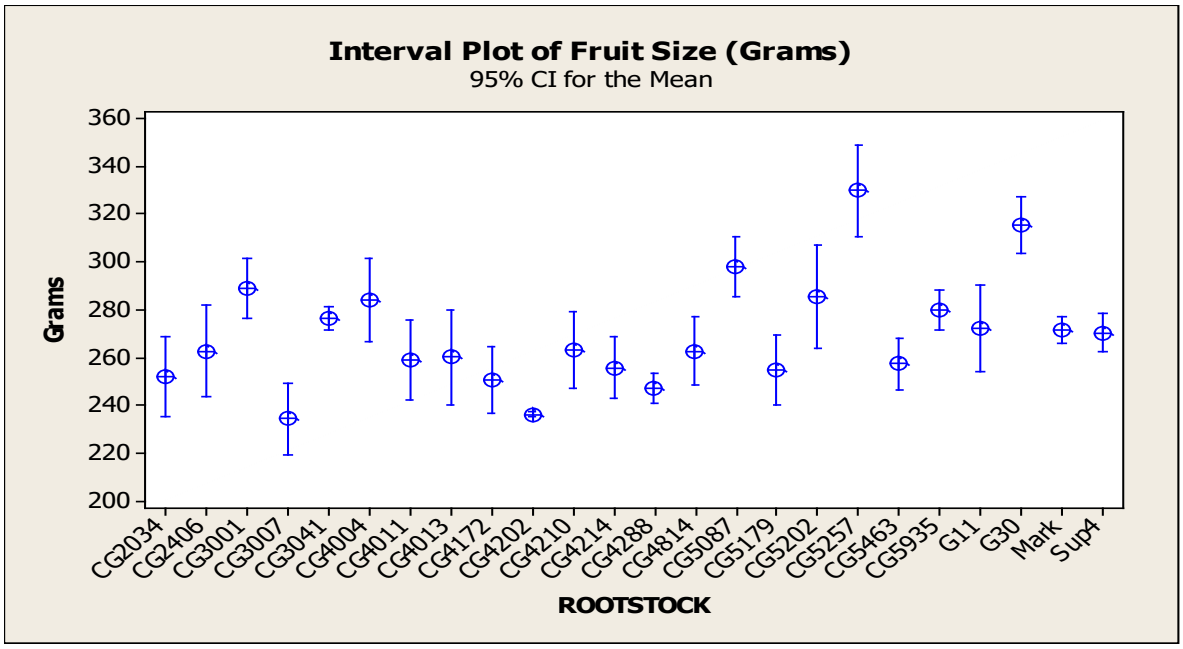


Figure 10. Fruit size varied somewhat in the first production year of this trial. CG3001, G.41 stands out as having a high crop load and yet maintaining fruit size.

OBJECTIVE 3

We planted a large replant experiment in Geneva this year that included some of our more advanced selections as well as commercial checks – all rootstocks were made into bench grafts with the Brookfield Gala as the scion variety. This experiment was planted in pots using two different soils (Clay Loam and Sandy Loam) where half of each soil was steam pasteurized. Sensitivity or resistance to ARD was evaluated by measuring tree height, stem diameter, fresh total plant weight, fresh scion and rootstock weight, increase of total and rootstock fresh weight, number of feathered trees, number of branches and total branch length. This was a destructive experiment since we also measured root mass and took data on root architecture differences. We have collected leaves for mineral analysis. This experiment will help us develop better screening techniques for future releases. Preliminary results show that there were significant differences in the sensitivity of certain rootstocks depending on the type of soil. There was good correlation between WA field experiments and the resistance or sensitivity to ARD in this experiment.

AKNOWLEDGEMENTS

We would like to express heartfelt gratitude to the many growers that are cooperating in this effort by hosting trials as well as the staff members at the WTFRC that have worked very, very, hard to obtain this data.

EXECUTIVE SUMMARY

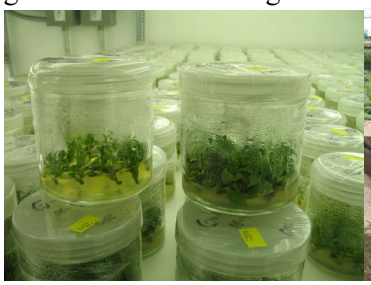
We have learned that the future survival of the apple industry will be highly dependent on the implementation of new scion and rootstock varieties obtained through advances of breeding, genetics and genomics. In relation to apple rootstocks these sets of experiments showed that breeding for disease resistance and increased yield was successful and that even though these rootstocks were selected under New York conditions there was enough genetic diversity in the group to show adaptability to Washington conditions. These trials will impact the apple production machinery in Washington in a very significant way as federal and state regulation of fumigants increases and the amount of virgin land optimal for apple production decreases. **With regards to fumigation – the positive effect on tree growth disappeared after the second season. The initial boost in growth increased the cumulative effect on TCSA and Fruit Yield – BUT – this increase was less than half of what planting genetically tolerant rootstocks did in the same seasons.** This productivity due to genetic resistance is maintained throughout the life of the orchard.

It is critical to plant replant and fire blight resistant rootstocks in orchards destined for organic management since losses due to rootstock susceptibility alone can amount to over 50% of the potential yield. General replant tolerance has been confirmed in certain Geneva rootstocks (CG4214, G.41, G.935, and CG5890). Even though we did not intend to make these trials about fire blight resistance, this disease has killed several known susceptible rootstocks (M.9 Pajam 2 came in first with 35% dead and Supporter 1 and 2 with 20% trees each). Although fire blight resistant B.9 continues to be one of the weakest and least productive rootstocks in all the replant experiments that have been planted so far. Malling 9 survival has been compromised by several fire blight events.

First yield data from a bench-graft plant in place replant experiment under intensive precision management intended to push the rootstocks to the maximum of their ability in Vantage WA indicates that some Geneva rootstocks match or surpass control rootstocks. This trial has the widest selection of Geneva rootstocks ever tested in Washington state and promises to uncover other useful qualities about Geneva rootstocks.

We have discovered that there may be several components to the genetic resistance of Geneva rootstocks to apple replant disease. These genetic components may act as traditional disease resistance genes, as genes that control nutrient uptake and genes that modify morphological characters of the root system that increase soil profile exploration. Thanks to the support of these grants we are closer to understanding which of these components stand out and are selectable in our large breeding pool – so that future rootstocks releases from this program will have improved performance with regard to apple replant disease.

With regards to the availability and propagation of G.41 and G.935, we have spearheaded a massive effort to micropropagate the material. We realize that the conversion to these new rootstocks by the nursery industry is somewhat viscous because of the nature of propagation of apple rootstocks, some lack of capital and in some cases because of the mediocre propagation ability of these new genotypes. We are trying to provide as much support as possible to the nurseries to foster such conversion by helping in the Tissue Culture process, certifying the material through DNA fingerprinting and researching better ways to propagate this new material. We have had some success in tissue culture and this spring our collaborators may be able to produce up to 300,000 plantlets of G.41 which will get us closer to our target of 1.5 million liners of G.41 in two or three years.



FINAL PROJECT REPORT

Project Title: Decay control and management of fungicide resistance

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State/Zip:	WA/98801	**State/Zip:**	CA/94710

Cooperators: Selected packinghouses across the state

Other Funding Sources: None

Total Project Funding: $90,000

Budget History:

Item	Year 1: 2007
Salaries	50,000
Benefits	15,850
Wages	10,000
Benefits	1,150
Equipment	0
Supplies	10,000
Travel	3,000
Total	**90,000**

Objectives:

1. Develop preharvest fungicide and postharvest fungicide integrated programs for decay control.
2. Develop preharvest fungicide and postharvest biocontrol agent integrated programs for decay control.
3. Develop pre- and post-storage integrated programs for decay control.
4. Develop pre- and postharvest fungicide programs for control of Sphaeropsis rot.
5. Evaluate various programs that not only control decay but also minimize or control the development of resistance in *P. expansum* to pyrimethanil and fludioxonil.
6. Evaluate thermofogging-based programs for decay control.
7. Collaborate with Bruce Campbell in evaluating natural compounds for decay control.

Significant findings:

- Residual activity of Pristine in apple fruit was still evident 5 months after harvest. Preharvest Pristine in combination with postharvest Bio-Save was more effective than Pristine alone in reducing blue mold at storage temperature. More research to explore this strategy for blue mold control is needed.
- Residues of Penbotec in apple fruit can provide a long time protection against blue mold. Scholar drench applied prior to storage in combination with Bio-Save applied at packing could be a viable option for blue mold control.
- Similar to what we previously observed on Red Delicious, when Penbotec and Scholar were applied as drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Fuji fruit in CA storage conditions. Even after 7 months in cold storage, the residues of Penbotec and Scholar in the drenched fruit still protected wounds from infection by *Penicillium expansum*. It appeared that Penbotec had a better residue protection than Scholar.
- Although infections of apple fruit by *Sphaeropsis pyriputrescens* occurs during the fruit-growing season in the orchard, a preharvest fungicide spray or a postharvest fungicide drench reduced Sphaeropsis rot incidence in storage and that a postharvest drench with one of the three apple-postharvest fungicides was highly effective in controlling this disease.
- Although pyrimethanil reduced blue mold incited by pyrimethanil-resistant strains of *P. expansum*, use of DPA in combination with pyrimethanil can compromise the efficacy of pyrimethanil for control of pyrimethanil-resistant strains. In order to avoid the development of resistance to pyrimethanil in *P. expansum* populations, strategies for fungicide resistance management, such as rotation among postharvest fungicides or use of preharvest fungicides instead of postharvest drench with fungicides, should be implemented.
- Thermfogging pyrimethanil or fludioxonil was able to reduce blue mold and gray mold. However, in commercial operations, a fungicide treatment applied by thermofogging to the fruit in a storage room may be delayed for 1-3 days after harvest until the room is filled with bins of fruit. A 1- to 3-day delay of the thermofogging treatment significantly compromised the effectiveness of the treatment, particularly for blue mold control.
- 2,5-DHBA or 2,3-DBAld as a chemosensitizing agent was not able to overcome fludioxonil resistance of *P. expansum* on apple fruit, though these two compounds in combination with fludioxonil controlled a fludioxonil-resistant strain in an in-vitro test. More research is being conducted in Campbell and Xiao labs to evaluate natural compounds as chemosensitizing agents to overcome fungicide resistance.

Methods:

In 2007, we set up a few experiments to evaluate various pre- and postharvest integrated programs for control of storage diseases in Red Delicious and Fuji apples before packing as well as decay after packing. Selected fungicides were applied within two weeks before harvest. Pre- and postharvest drench treatments had also been applied to the fruit. Various fungicides and biocontrol treatments were applied to the fruit 5 and 7 months after harvest. Fruit were evaluated for decay development.

Experiments were set up to evaluate various postharvest fungicide treatments applied in various combinations of pre-storage treatments and online treatments for control of decay. This experiment was to simulate commercial operations in which fruit are drenched with fungicides prior to storage and then treated again with fungicides or biocontrol agents on the packing line at packing. Various on-line fungicides and biocontrol treatments were applied to the fruit 5 and 7 months after harvest. Fruit were evaluated for decay development.

An experiment was conducted on Golden Delicious to evaluate effects of timing of infection of apple fruit by *Sphaeropsis pyriputrescens* on effectiveness of pre- and postharvest fungicide applications for control of Sphaeropsis rot. Apple fruit were inoculated with the pathogen at 5 and 2 weeks before harvest. Fruit were either treated with preharvest Pristine and Topsin M or drenched with postharvest fungicides. Decay development was assessed monthly for up to 7 months after harvest, starting 3 months after harvest.

Effects of DPA in combination with either Scholar or Penbotec in the drench solution on the control of fungicide-resistant mutants of *Penicillium expansum* on apple fruit were evaluated.

An experiment was conducted to evaluate thermofogging fungicides for control of postharvest diseases. Commercially harvested fruit were used for this experiment. Both fludioxonil and pyrimethanil as themofogging treatments were tested.

In collaboration with Bruce Campbell, an experiment was conducted to evaluate 2,5-dihydroxybenzoic acid and 2,3-dihydroxybenzaldehyde for management of fludioxonil-resistant strains of *Penicillium expansum* and for decay control.

Results & Discussions:

Preharvest Pristine in combination with postharvest biocontrol agent or fungicide for blue mold control.

Pristine was applied to Fuji apples 7 days before harvest. Fruit were stored in CA for 5 months, washed and brushed through a research packing line, wounded and inoculated with *P. expansum*. Inoculated fruit were stored at 32°F in air for 8 weeks and one additional week at room temperature.

Preharvest Pristine without any postharvest biocontrol or fungicide reduced blue mold incidence to 20% after 8 weeks at 32°F. Preharvest Pristine in combination with BioSave further reduced blue mold to only 2.5%. The effectiveness of Pristine and BioSave was diminished after the fruit were stored at room temperature for one additional week (Table 1). However, the size of decay on the fruit treated with preharvest Pristine and postharvest Bio-Save was significantly smaller than that on the fruit treated with preharvest Pristine without postharvest Bio-Save. Virtually no decay developed on the fruit that were treated with Scholar or Penbotec. Our results indicate that residual activity of Pristine in apple fruit was still evident 5 months after harvest. Preharvest Pristine in combination with

postharvest Bio-save appears to be more effective than Pristine alone in reducing blue mold at storage temperature. More research to explore this strategy for blue mold control is needed.

Table 1. Preharvest Pristine in combination with postharvest fungicide and biocontrol agent for control of blue mold

Preharvest Treatment	Fungicide applied 5 months post drenching	8 weeks at 32F post inoculation		1 week at room temp after cold storage	
		% decay	Lesion (mm)	% decay	Lesion (mm)
Nontreated	No Fungicide	100.0a	30.0a	100.0a	64.1a
	Scholar	0.0e	0.0d	0.0d	0.0d
	Penbotec	0.0e	0.0d	0.0d	0.0d
	TBZ	100.0a	30.3a	100.0a	63.7a
	BioSave	31.7c	6.9c	92.4ab	23.1b
Pristine	No Fungicide	20.0d	14.1b	78.8c	23.2b
	Scholar	0.0e	0.0d	0.0d	0.0d
	Penbotec	0.0e	0.0d	1.3d	8.8c
	TBZ	47.5b	12.7b	95.0ab	31.1b
	Biosave	2.5e	0.9d	97.5ab	8.8c

Postharvest drench treatments in combination with postharvest biocontrol agent or fungicide for blue mold control.

Experiments were conducted on both Red Delicious and Fuji apples. Penbotec applied as a pre-storage drench treatment had an excellent residual activity in apple fruit against blue mold. No decay developed on Penbotec-drenched fruit that were inoculated with the pathogen 5 or 7 months after the drench treatment (Tables 2 and 3). Scholar also exhibited a very good residual activity in apple fruit against *P. expansum*, but the residual activity of Scholar was reduced after the fruit had been moved to room temperature. However, Bio-Save applied at packing provided additional benefits than Scholar drench without Bio-Save. Our results suggest that residues of Penbotec in apple fruit can provide a long time protection against blue mold and that Scholar drench applied prior to storage in combination with Bio-Save applied at packing could be a viable option for blue mold control.

Table 2. Postharvest drench with Penbotec or Scholar in combination with Bio-Save for control of blue mold in Red Delicious apples

Drench treatment applied prior to storage	Fungicides applied at packing 5 or 7 months post drenching	5 months post drench treatments		7 months post drench treatments	
		% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage
		TBZ-R	TBZ-R	TBZ-R	TBZ-R
Nontreated	No fungicide	100.0a	100.0a	98.8a	98.8a
	Scholar	0.0c	10.0d	0.0c	0.0d
	Penbotec	0.0c	0.0e	0.0c	0.0d
	Mertect	100.0a	100.0a	100.0a	100.0a
	Bio-Save	25.0b	92.5b	35.0b	100.0a
Scholar	No fungicide	0.0c	20.0c	0.0c	36.3b
	Bio-Save	0.0c	10.0d	0.0c	25.0c
Penbotec	No fungicide	0.0c	0.0e	0.0c	0.0d
	Bio-Save	0.0c	0.0e	0.0c	0.0d

Table 3. Postharvest drench with Penbotec or Scholar in combination with Bio-Save for control of blue mold in Fuji apples

		5 months post drench treatments		7 months post drench treatments	
Drench treatment applied prior to storage	Fungicides applied at packing 5 or 7 months post drenching	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage
		TBZ-R	TBZ-R	TBZ-R	TBZ-R
Nontreated	No fungicide	98.8a	98.8ab	98.8a	100.0a
	Scholar	0.0c	0.0e	0.0c	1.3c
	Penbotec	0.0c	0.0e	0.0c	0.0c
	Mertect	98.8a	100.0a	98.8a	100.0a
	Bio-Save	61.3b	96.3b	61.3b	97.5a
Scholar	No fungicide	0.0c	16.3d	1.3c	6.3b
	Bio-Save	1.3c	10.0d	0.0c	1.3c
Penbotec	No fungicide	0.0c	0.0e	0.0c	0.0c
	Bio-Save	0.0c	40.0c	0.0c	0.0c

Residual activity of Penbotec and Scholar in Fuji apple fruit against Penicillium expansum.

In a previous study, we reported that when applied as a pre-storage drench treatment, residues of Penbotec and Scholar in Red Delicious apple fruit were stable and can protect fruit from infection by *P.expansum*. In the current study, we evaluated whether or not this residual activity also occurs on Fuji apple fruit.

Table 4. Residual activity of fludioxonil and pyrimethanil in Fuji apple fruit against *Penicillium expansum*

		5 months post drench treatments		7 months post drench treatments	
Drench treatment applied prior to storage	Fungicides applied at packing 5 or 7 months post drenching	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage	% infected fruit at 8 weeks at 0ºC post packing	% infected fruit at one additional week at room temperature after storage
		TBZ-R	TBZ-R	TBZ-R	TBZ-R
Nontreated	No fungicide	98.8	98.8	100.0	100.0
	Scholar	0.0	0.0	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
	Mertect	100.0	100.0	100.0	100.0
Mertect	No fungicide	98.8	100.0	97.5	97.5
	Scholar	0.0	1.3	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
Scholar	No fungicide	1.3	5.0	0.0	10.0
	Mertect	8.8	55.0	6.3	35.0
	Penbotec	0.0	0.0	0.0	0.0
Penbotec	No fungicide	0.0	0.0	0.0	0.0
	Mertect	2.5	15.0	0.0	2.5
	Scholar	0.0	0.0	0.0	0.0

Similar to what we previously observed on Red Delicious, when Penbotec and Scholar were applied as drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Fuji fruit in CA storage conditions (Table 4). Even after 7 months in cold storage, the residues of Penbotec and Scholar in the drenched fruit still protected wounds from infection by *Penicillium expansum*. It appeared that Penbotec had a better residue protection than Scholar. TBZ residue in drenched fruit did not provide a satisfactory protection after 7 months of CA storage, even against TBZ sensitive strain of *Penicillium expansum* (data not shown). An additional online treatment with either Penbotec or Scholar provided an excellent protection of the fruit from infection by either TBZ-R or TBZ-S strains of *P. expansum*.

Control of Sphaeropsis rot with pre- and postharvest fungicides.

Preharvest applications of Pristine and Topsin M and postharvest drench treatments with three postharvest fungicides were evaluated for control of Sphaeropsis rot. Two different timings of infection were included in the test to evaluate whether timing of infection affects the effectiveness of fungicide treatments (Table 5). Both Pristine and Topsin M significantly reduced Sphaeropsis rot compared to the nontreated control. The three postharvest fungicides statistically were equally effective, but Penbotec drench completely eradicated infections regardless of timing of infection before harvest. Ziram was effective, but Serenade was not effective in controlling Sphaeropsis rot.

Our results indicate that although infections of apple fruit by *Sphaeropsis pyriputrescens* occurs during the fruit-growing season in the orchard, a preharvest fungicide spray or a postharvest drench can still reduce Sphaeropsis rot incidence in storage and that a postharvest drench with one of the three apple-postharvest fungicides is highly effective in controlling this disease.

Table 5. Control of Sphaeropsis rot in Golden Delicious apples with pre- and postharvest fungicides

	Incidence of Sphaeropsis rot (%) 9 months after harvest	
Treatment	Fruit inoculated 5 weeks before harvest	Fruit inoculated 2 weeks before harvest
Nontreated control	75a	72.5a
Pristine 7d before harvest	46.3b	31.3b
Topsin 7d before harvest	66.3ab	47.5b
Ziram 14 d before harvest	43.8b	
Serenade 7 d before harvest		80.3a
Scholar drench after harvest	3.8c	1.3c
Penbotec drench after harvest	0c	0c
Mertect drench after harvest	6.3c	3.8c

Effects of Diphenylamine (DPA) on the control of blue mold on apple fruit incited by fludioxonil-resistant or pyrimethanil-resistant strains of Penicillium expansum.

DPA in combination with one of the three apple-postharvest fungicides was evaluated for control of blue mold on Red Delicious apple fruit incited by fludioxonil-resistant or pyrimethanil-resistant strain of *P. expansum*. The strain FR2 of *P. expansum* is resistant to fludioxonil but sensitive to pyrimethanil and TBZ. Fludioxonil alone or in combination with DPA was not effective in controlling blue mold incited by FR2. Pyrimethanil and TBZ almost completely controlled blue mold incited by FR2 (Table 6).

The strain PR2 is resistant to both pyrimethanil and fludioxonil but sensitive to thiabendazole (TBZ). DPA alone did not control this strain. Fludioxonil in combination with DPA provided a better control

of blue mold caused by PR2 than fludioxonil alone, but the difference in the effectiveness between these two treatments was diminished after the fruit were stored at room temperature for an additional week after cold storage. TBZ alone and TBZ in combination with DPA were equally effective against blue mold incited by PR2. However, pyrimethanil in combination with DPA was less effective than pyrimethanil alone in controlling blue mold incited by pyrimethanil-resistant strain of *P. expansum* (Table 6), while pyrimethanil alone or in combination with DPA almost completely controlled blue mold incited by pyrimethanil-sensitive strains of *P. expansum* (data not shown).

Table 6. Effects of Diphenylamine (DPA) alone or in combination with postharvest fungicides on the control of blue mold on apple fruit incited by fludioxonil-resistant or pyrimethanil-resistant strains of *Penicillium expansum*

Strain	Treatment	12 wk at 32°F		12 wk at 32°F+7d at room temp
		Incidence (%)	Lesion (mm)	Incidence (%)
FR2 (fludioxonil-resistant strain)	CK	85.8 ab	5.0 b	100 a
	Diphenylamine	54.2 c	3.6 c	98.3 a
	Thiabendazole	0 d	0 d	0 b
	Diphenylamine +Thiabendazole	0 d	0 d	0 b
	Fludioxonil	70.8 bc	6.2 a	91.7 a
	Diphenylamine +Fludioxonil	89.2 a	5.7 ab	100 a
	Pyrimethanil	0 d	0 d	0 b
	Diphenylamine +pyrimethanil	0 d	0 d	3.3 b
PR2 (pyrimethanil-resistant strain)	CK	100 a	21.1 a	100 a
	Diphenylamine	100 a	16.0 b	100 a
	Thiabendazole	1.7 d	1.2 ef	5.0 c
	Diphenylamine +Thiabendazole	0.8 d	1.2 ef	8.3 c
	Fludioxonil	29.2 c	3.9 ed	88.3 b
	Diphenylamine +Fludioxonil	1.7 d	0.8 f	86.7 b
	Pyrimethanil	31.7 c	4.8 d	88.3 b
	Diphenylamine +pyrimethanil	86.7 b	8.0 c	100 a

In a previous study, we found that if *P. expansum* develops resistance to pyrimethanil, the resistance can extend to fludioxonil and TBZ and thus the strains become multi-drug resistance. The results we reported here indicate that although pyrimethanil can still reduce blue mold incited by pyrimethanil-resistant strains, use of DPA in combination with pyrimethanil can compromise the efficacy of

pyrimethanil. In order to avoid the development of resistance to pyrimethanil in *P. expansum* populations, strategies for fungicide resistance management, such as rotation among postharvest fungicides or use of preharvest fungicides instead of postharvest drench with fungicides, should be implemented.

Thermofogging postharvest fungicides for decay control.

In 2007, 24 bins of Red Delicious harvested from a commercial orchard were used for the evaluation. Eight replicate bins were either not treated as a control or thermofogged with pyrimethanil or fludioxonil. Decay was assessed 8 months after harvest. The decay from natural infections was relatively low. A thermofog treatment with pyrimethanil or fludioxonil was equally effective and reduced both total decay and gray mold as compared to the nontreated control (Table 7). Blue mold was very low, and no differences were observed among the treatments.

In the commercial situation, a thermfog treatment applied to the fruit in a storage room may be delayed for 1-3 days after harvest until the room is filled with bins of fruit. In 2007, we also used inoculated fruit to look at whether delay of themfog treatment compromises fungicide efficacy for decay control. After harvest, apple fruit were inoculated with either Botrytis or Penicillium, and part of the fruit received the thermofogging treatment with pyrimethanil or fludioxonil at 0, 1, 2, and 3 days after inoculation. Delay of the thermofogging treatment significantly compromised the effectiveness of the treatments, particularly for blue mold control (data not shown).

It appears that thermfogging fungicides could be a promising option for control of blue mold and gray mold. However, in commercial operations, delay of thermfog treatment can compromise the efficacy of fungicides.

Table 7. Effectiveness of pyrimethanil and fludioxonil applied as a thermofog treatment in controlling decay originating from natural infections in Red Delicious apples

Treatment	Total Rot (%)	Gray mold (%)	Blue mold (%)
Nontreated	2.22 a	1.79 a	0.08 a
Fog with Fludioxonil	0.69 b	0.40 b	0.04 a
Fog with Pyrimethanil	0.63 b	0.27 b	0.02 a

Evaluation of natural compounds for control of blue mold incited by fludioxonil-resistant strains of Penicillium expansum

This work was done in collaboration with Bruce Campbell at the USDA ARS, Albany, CA. Two natural compounds, 2,5-dihydroxybenzoic acid (2,5-DHBA) and 2,3-dihydroxybenzaldehyde (2,3-DBAld), were tested as chemosensitizing agents to overcome fludioxonil resistance in *Penicillium expansum* on apple fruit. These two compounds have been shown effective as chemosensitizing agents to overcome fludioxonil resistance in *P. expansum* in an in-vitro test conducted by Bruce Campbell's lab.

It appeared that 2,5-DHBA and 2,3-DBAld were not able to overcome fludioxonil resistance of *P. expansum* on apple fruit, though these two compounds in combination with fludioxonil controlled a fludioxonil-resistant strain in an in-vitro test (Table 8). In 2008, additional compounds were screened by Campbell's lab for potential of chemosensitizing agents to overcome fludioxonil resistance. Campbell's lab found that octylgallate could be a promising chemosensitizing agent to overcome fludioxonil resistance in *P. expansum*. On the 2008 crop, we set up a trial to evaluate octylgallate in

combination with Scholar (fludioxonil) for control of blue mold caused by a fludioxonil-resistant strain. The fruit are currently in storage for decay development. Results will be forthcoming.

Table 8. Effectiveness of 2,5-dihydroxybenzoic acid (2,5-DHBA) and 2,3-dihydroxybenzaldehyde (2,3-DBAld) as chemosensitizing agents to overcome fludioxonil resistance in *Penicillium expansum* on apple fruit

	Fludioxonil-sensitive strain		Fludioxonil-resistant strain	
Treatment	% of fruit Infected	Lesion size (mm)	% of fruit Infected	Lesion size (mm)
Untreated Control	100.0	42.3	100.0	8.6
2,5-DHBA 18 mM	100.0	47.7	100.0	12.9
2,5-Dbald 1 mM	100.0	40.3	100.0	7.3
2,5-DHBA 18 mM + Scholar	0.0	0.0	100.0	12.4
2,5-DbAld 1 mM + Scholar	0.0	0.0	97.5	7.7
Scholar 230 SC 12 fl oz/100 gal	0.0	0.0	100.0	8.0

Executive Summary

This report is a summary of a one-year project. The goal of the project was to develop integrated programs using recently registered reduced-risk fungicides and a biocontrol agent to control major postharvest diseases in apples.

Blue mold and gray mold are major postharvest diseases of apples. In previous studies, we found that new reduced-risk fungicides Pristine, as a preharvest treatment, or Scholar and Penbotec, as a postharvest drench treatment, were effective in controlling these two diseases. In the current project, we evaluated various pre- and postharvest integrated programs or pre- and post-storage integrated programs for decay control. We found that residual activity of Pristine in apple fruit against *Penicillium expansum* (the cause of blue mold) was still evident five months after harvest. Preharvest Pristine in combination with postharvest biocontrol agent Bio-Save was more effective than Pristine alone in reducing blue mold at storage temperature. Similar to what we previously observed on Red Delicious, when Penbotec and Scholar were applied as drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Fuji fruit in CA storage conditions. Even after seven months in cold storage, the residues of Penbotec and Scholar in the drenched fruit still protected wounds from infection by *P. expansum*. It appeared that Penbotec had a better residual protection than Scholar. Scholar drench applied prior to storage in combination with Bio-Save applied at packing could be a viable option for blue mold control.

Pyrimethanil or fludioxonil applied as a thermfog treatment was able to reduce blue mold and gray mold. However, in commercial operations, a fungicide treatment applied by thermofogging to the fruit in a storage room may be delayed for 1-3 days after harvest until the room is filled with bins of fruit. We found that a 1- to 3-day delay of the thermofogging treatments significantly compromised the effectiveness of the treatmenst, particularly for blue mold control.

Sphaeropsis rot caused by the fungus *Sphaeropsis pyriputrescens* is another important postharvest disease of apples in Washington State. Although infections of apple fruit by *Sphaeropsis pyriputrescens* occurs during the fruit-growing season in the orchard, a preharvest spray with Pristine or Topsin reduced Sphaeropsis rot incidence in storage. A postharvest drench with one of the three apple-postharvest fungicides was highly effective in controlling Sphaeropsis rot.

Avoidance or management of resistance to new postharvest fungicides is important to postharvest decay control. In a previous study using laboratory-generated fungicide-resistant mutants, we found that pyrimethanil possesses a higher risk than fludioxonil in the development of resistance in *P. expansum*. In the current study, we evaluated whether DPA in the fungicide drench solution affects the effectiveness of fungicides against pyrimethanil- and fludioxonil-resistant strains of *P. expansum*. We found that although pyrimethanil reduced blue mold incited by pyrimethanil-resistant strains of *P. expansum*, use of DPA in combination with pyrimethanil can compromise the efficacy of pyrimethanil for control of pyrimethanil-resistant strains of *P. expansum*. In order to avoid the development of resistance to pyrimethanil in *P. expansum* populations, strategies for fungicide resistance management, such as rotation among postharvest fungicides or use of preharvest fungicides instead of postharvest drench with fungicides, should be implemented.

In collaboration with Bruce Campbell, we evaluated natural compounds as chemosensitizing agents to overcome fludioxonil resistance of *P. expansum*. We found that 2,5-DHBA or 2,3-DBAld was not able to overcome fludioxonil resistance of *P. expansum* on apple fruit, though these two compounds in combination with fludioxonil controlled a fludioxonil-resistant strain in an in-vitro test. More research is currently being conducted in Campbell and Xiao labs to evaluate natural compounds as chemosensitizing agents to overcome fungicide resistance in postharvest pathogens.

FINAL PROJECT REPORT

Project Title: Sprayable foam for trapping and killing codling moth larvae

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Cooperators: Lerry Lacey, Gary Judd

Other funding Sources - None

Total Project Funding: $62,300

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	$21,000	0	$21,700
Benefits	6,400	0	6,600
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	3,000	0	3,000
Travel	600	0	600
Miscellaneous	0	0	0
Total	$31,000	0	$31,000

ORIGINAL OBJECTIVES:

1. Develop, test, and select a biodegradable replacement, to be applied as a liquid or semi-solid to a tree trunk.

2. Evaluate pesticides and pathogenic nematodes in a candidate foam material to determine both larval recruitment, mortality and duration of effectiveness.

3. Compare cardboard banding and a biodegradable foam in apple orchards for efficacy and cost assessments.

RESULTS & DISCUSSION

SIGNIFICANT FINDINGS:

1. Initially, comparisons of polyurethane foam and cardboard banding showed a superiority of the foam in recruiting greater numbers of larvae that are seeking spin up sites.

However, it was considered that the polyurethane foam would be too expensive, and also is fairly indestructible and would require physical removal from trees and subsequent disposal. An inexpensive and biodegradable alternative was sought.

2. Laboratory evaluations of many alternative materials and formulations showed a clear connection between foam cell (bubble) size and efficacy in recruiting larvae to spin up, and superiority (low cost, ease of use) of several starch based materials over other base materials.

Base materials evaluated included aerated concrete, wheat starch, rice kernel waste material, sawdust and finely ground wood flour. Several adjuvants were included to enhance stickiness and topromote foaming action.

3. An industrial foam (texture sprayer) was modified and used for both mixing experimental materials and application to tree trunks in an orchard.

Field evaluations showed the need to reduce partical size to facilitate the use of the sprayer, the addition of a surfactant, and the need for some water repellency.

4. A set of candidate materials and a specific formulation were selected based on very low cost, biodegradability, and ease of field use.

This formulation was then further evaluated in laboratory assays using artificial trees, in the field on apple trees, and to assess the effectiveness of killing agents. The final material used for further study is a blended combination of 45 parts wood flour, 15 parts hardwood fiber, 28 parts amioca starch, 5 parts Celvol A 125, and 7 parts foamcell A-100, in water.

5. Addition of Permethrin over a range of dosages resulted in the death of larvae contacting the foam, rather than recruitment into the foam. Laboratory assays indicated a high rate of control of wandering larvae.

This suggests a different but effective strategy of using a durable lasting killing strip of material on the trunks of trees, rather than a “trap” such as the banding or foam. A precedent is in use to control climbing cutworms on grape vines.

6. Applications of entomophagous nematodes with foam were developed and tested by Lerry Lacey, with very good results against recruited codling moth larvae as a % kill.

7. The optimized biodegradable foam material yet does not hold up to direct hits from under-tree sprinkler systems.

Additional work is suggested in the following areas.

1. The addition of materials to provide water repellency to the foam may protect the material somewhat from direct hits by sprinklers, which tend to erode the band of foam from the tree.
2. Gary Judd has expressed interest in evaluating the material with the addition of the codling moth larval aggregation pheromone, to determine if recruitment into the foam can be enhanced by the presence of the pheromone. This might be done with microencapsulated pheromone mixed directly into the foam before it is applied.
3. Slower acting pesticides might be evaluated as toxicants to kill larvae entering foam. The advantage of using a slow acting pesticide would in part be to permit direct field evaluation of the effect, as larvae entering foam and dying could be counted. As it stands, larvae that contact the foam and die are not readily found and counted.
4. The use of entomopathogenic nematodes within foam to kill codling moth larvae appears to be effective. Further evaluations are required to determine control effects on a larger scale and to determine the longevity and durability of the nematodes as effective biological control agents.
5. The idea of using the foam material as an agent to apply a pesticide strip on tree trunks, rather than as a trap for larvae, is intriguing. A similar technique was developed by Doug Walsh and is recommended for use on grapes to protect vines from climbing cutworms.

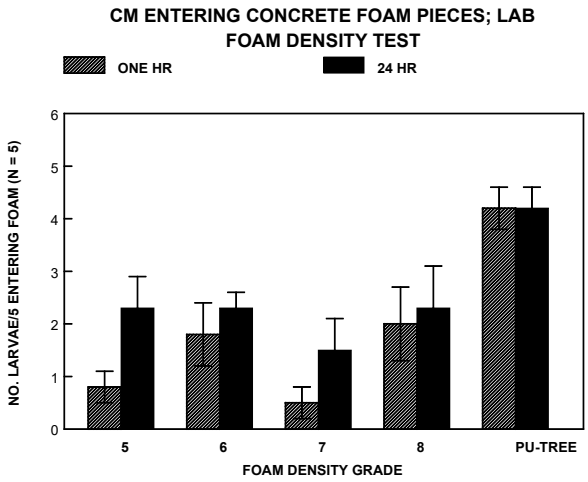
Best results in laboratory assays were with polyurethane foams, a fiber reinforced foam, a fiber roll, a straw/starch formulation, and cardboard. These results supported the hypotheses that efficacious materials facilitated codling moth entry by chewing through the material and by the presence and size of air pockets or cells. All materials that strongly recruited larvae were "chewable" and open in consistency.

Table 1. Percent of mature codling moth larvae entering piece of test material held in 16 oz plastic cup in laboratory. N = 10 to 20.

Material	% Larvae Entering Test Material	
	30 min	24 hours
Rice Straw/Starch Foam	20	100
Concrete Foam	0	60
Fiber Reinforced Concrete Foam		20
90		
Mearl 10 Cement Foam	0	20
Mearl 5 Cement Foam	0	0
Pressed Cork	0	25
Starch Fiber Foam	0	15
Pressed Board	0	10
Fiber Foam	20	70
Foam Freeze Starch	0	0
Polyethylene foam	0	50
Polyurethane Foam	10	90
Polystyrene Foam	10	65
Large Pore Starch Foam	90	90
Fiber Roll	100	100
Great Stuff®	50	100
Card Board	85	85

Comparisons of densities of ultra-light concrete did not show an improvement in efficacy with

decreasing density and all densities were inferior to cardboard banding. The lack of acceptance by some larvae may have been due to the toughness of the concrete, despite the presence of numerous small air pockets. It was surprising nonetheless to see codling moth larvae bore into soft forms of concrete and spin up cocoons within the concrete.



Assessments of formulations of milled wheat straw were efficacious in laboratory assays, and the series of alterations made in the formulation were intended to improve water repellency, stickiness and maintenance of depth, and ease of application to the tree trunk.

A milled wheat straw sprayable foam applied to apple tree trunks in autumn of 2007 was successfully applied through a texture applicator air gun, to a depth of about ½ inch. This material remained intact on the trees through December, but was readily knocked off at that time.

Plans and Time Line for 2008.
January to April. Additional foams will be laboratory-tested for acceptability to codling moth larvae, in Wapato. These assays will further evaluate the milled wheat straw mixtures, altered to provide greater foaming action after application to the tree.

May/June. One or more candidate materials will be evaluated in the field, using the commercial foamer applicator, to determine the acceptability of such applications to codling moth larvae when applied to tree trunks. These treatments will be directly compared to cardboard banding. Applications to apple tree trunks will be made in early June, and counts made of cocoons in early July.

May to August. Formulation alterations will be made in Albany to provide better foaming action and larger cell sizes within the material applied to trunks. A second generation milled wheat starch foam will then be tested in the laboratory in Wapato to determine if changes in the formulation impacted acceptability to larvae. In addition, preliminary attempts will be made in the laboratory to test a pesticide and nematodes in the foam formulation. These materials will be evaluated in the laboratory, using the arena bioassay, in comparison to foam without pesticide or nematodes. Data will be obtained on recruitment of larvae into the foam (to test the hypothesis of no repellency of the treatments) and on mortality and survival of larvae within the foam.

August /September. Field trials will evaluate the second generation foam, in comparison to cardboard banding , to evaluate efficacy in recruiting larvae in the field, but also to durability when exposed to irrigation sprinklers.

September 2007 into January 2008. It is anticipated that a series of laboratory assays will need to be done to evaluate and compare several pesticides at different dosages, and different dosages of nematodes, to select dosages that provide optimum results in anticipation for field testing in 2008. In addition, information obtained from the two field trials may indicate the need for additional fine tuning of the foam formulation to provide durability and rain fastness. Any changes to the formulation would necessitate additional laboratory testing before the next field season.

EXECUTIVE SUMMARY

The original vision of the project was to develop a material to mechanically apply to apple and pear tree trunks that might replace cardboard banding as a trap for codling moth larvae seeking spin up sites. This material needs to be biodegradable and incorporate a toxicant or biocontrol agent to eliminate the need for removal, as banding is presently removed and destroyed. Additionally, a formulation that can be mechanically applied might be suitable as a carrier for the larval aggregation pheromone under development by Simon Fraser University personnel. Replacement of cardboard banding by a sprayable, biodegradable foam with toxicant and pheromone incorporated might reduce costs of application and subsequent removal and destruction of banding. Specific objectives then were to:

1. Develop, test, and select a biodegradable replacement, to be applied as a liquid or semi-solid to a tree trunk.
2. Evaluate pesticides and pathogenic nematodes in a candidate foam material to determine both larval recruitment, mortality and duration of effectiveness.
3. Compare cardboard banding and a biodegradable foam in apple orchards for efficacy and cost assessments.

Objective 1 was largely accomplished with the evaluation in laboratory assays of a number of base materials and formulations. A formulation based on finely ground wood flour was then developed for testing with pesticide, with nematodes, in the laboratory and on apple trees. Several trials were conducted on apple trees to evaluate mechanical application and durability. Durability was considered to be suitable, but with problems encountered when irrigation sprinklers directly hit foam on trees.

Objective 2 was largely met. Pesticide (Permethrin) was evaluated as an adjuvant to the foam, as well as pathogenic nematodes. These evaluations were made both in the lab and in the field. Both were highly effective in killing codling moth larvae. However, in the laboratory assays it was clear that larvae were killed by contact with the foam, even at very low pesticide concentrations, and without the opportunity to tunnel into the foam. Hence, we were not able to evaluate larval recruitment into foam in the field test. Good results were obtained in the lab and in the field with nematodes because larvae were attacked by nematodes following entry into the foam.

Objective 3 was not well met. Direct comparisons of foam and cardboard were successful without pesticide, but did not provide meaningful results when Permethrin was used, presumably because larvae were toxified upon contact and without tunneling into foam. Counts of larvae in foam with pesticide were then very low.

The final results of the project provide both intriguing developments and constraints that yet need to be overcome for commercial application and availability. We do feel that this approach provides a cheap alternative to the current practice of banding trees, with considerable flexibility for added treatments to enhance efficacy. Cost savings would be with 1) the replacement of labor with a mechanical application, 2) no need to physically remove material from trees, 3) no need to destroy larvae in the material, and 4) possible reduction in most of materials. Treatment options that are possible include 1) use of pathogens such as entomopathogenic nematodes for an organic option, 2) use of a larval aggregation pheromone (not tested here), and 3) other pesticide treatments to kill larvae. Constraints or further improvements suggested include 1) additional water-proofing to resist erosion from direct contact by sprinklers, 2) possibly a slower acting pesticide to permit larvae to enter foam, and 3) stronger foaming action which may further promote larval entry. It is acknowledged that the use of this material with pesticide added and sprayed as a band onto tree trunks provides a possible different treatment as a potentially equally effective treatment. That is, it seems possible that a pesticide-treated foam sprayed in a band onto an apple trunk may kill all larvae moving along the trunk without requiring larval burrowing or entry into the material. It is our intention to investigate this possibility further.

FINAL PROJECT REPORT

Project Title: Molecular characterization of taste, smell and feeding in codling moth

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Cooperators: Drs. Tom Unruh, Pete Landolt and Kevin Wanner

Other funding Sources

Agency Name: USDA-CSREES NRI
Amount awarded: $189,804 for 4/2008 - 3/2010
Notes: This award was obtained using data generated with the funds provided by WTFRC. For that we are most grateful

Total Project Funding: $80,000

Budget History:

Item	Year 1: 2007	Year 2: 2008	
Salaries			
Benefits			
Wages	6,240	6,490	
Benefits	150	156	
Equipment			
Supplies	28,110	27,854	
Travel	500	500	
Miscellaneous	5000	5000	
Total	40,000	40,000	

ORIGINAL OBJECTIVES

1) Construct cDNA libraries from codling moth sensory organs and neuroendocrine tissues.
2) Sequence cDNA libraries and perform searches to identify target receptors.
3) Clone target receptors into expression systems suitable for analysis in insect cell lines.
4) Initiate assays to identify receptors for pheromones and kairomones used for codling moth control.
5) Convert cell based assays for practical use in high-throughput screening programs.

Revised Objectives converting original objectives from a 3 year to 2 year time line as requested by Jim McFerson.

Year 1
1) Prepare cDNA from male and female codling moth antenna.
2) Generate antennal ESTs via pyrosequencing services offered by 454 Life Sciences Corp.
3) Generate cell lines expressing codling moth proteins involved in odorant signal transduction.
4) Clone full length cDNAs encoding odorant receptors identified from EST sequences

Year 2
1) Validate cell-based assays that will be used to identify codling moth pheromone receptors.
2) Clone potential codling moth pheromone receptors into cells used for assays.
3) Initiate assays to identify receptors for codling moth-active pheromones and kairomones.

SIGNIFICANT FINDINGS

1) Discovered conserved amino acid sequence and developed a procedure that could be used to identify and clone chemosensory receptors from the codling moth. This was the first time this had been accomplished for an insect without a sequenced genome. Furthermore the procedure is applicable to almost all insects of the Order Lepidoptera (moths and butterflies) as evidenced by our ability to identify and clone "pheromone" receptors from other insect pests of tree fruit including Obliquebanded leafroller, Light Brown Apple Moth, and Apple Clearwing Moth. Additionally, the value of the technique developed here has transcended tree fruit pests and been used to identify and clone "pheromone" receptors from lepidopteran pests of corn and cotton (European corn borer and the corn earworm/cotton bollworm).

2) Nine full-length odorant receptors have been cloned from codling moth, including five "pheromone" receptors, one ubiquitous receptor, and three general odorant receptors. These receptors have been cloned into cell expression vectors and are being prepared for use in cell-based assay screens.

3) One antennal specific and one nervous system specific G-Protein have been cloned and are being assayed for use as a potential target for new pesticide development. The antennal specific G-protein is a critical component for use in high-throughput assays to determine odorant receptor ligands.

4) cDNA has been prepared from male and female chemosensory organs (antennae, legs and mouthparts) and neonate and 5th instar larvae and is being sequenced by Dr. Amit Dhingra at Washington State University. The results from the sequencing will allow us to identify other chemosensory receptors expressed in those codling moth life stages.

RESULTS

Because the genome sequence of the CM is not yet available, three different approaches were proposed to identify chemosensory receptors: A) PCR amplification of chemosensory receptors using degenerate primers designed from conserved regions of previously identified receptors in other insects from cDNA prepared from mRNA derived from CM chemosensory organs of newly emerged adults; B) direct pyrosequencing of cDNA prepared from male and female chemosensory organs; and C) screening libraries constructed from cDNA prepared as above with DNA probes generated from receptors cloned from other lepidopterous insects.

A) PCR amplification of chemosensory receptors using degenerate oligonucleotide primers.

The main focus of this part of the project has been to identify pheromone receptors expressed in antennae from CM males. The results presented in the original proposal remain unchanged and to date we have identified and cloned sequences encoding five full length members of the pheromone receptor family. Additionally, CM cDNA sequences encoding receptors corresponding to Ors 2, 10, 20, 21 and 35 from *Bombyx mori* and *Heliothis virescens* have also been identified and cloned. Or2, the ubiquitous receptor, will be used in developing the high-throughput cell-based assay in objectives 2 and 3.

A major benefit of the degenerate oligonucleotide approach to identify members of the pheromone receptor family from CM is its applicability to other lepidopteran pest species. The degenerate primers developed for this project were used in 3' RACE reactions to amplify cDNAs encoding members of the pheromone receptor subfamily from total RNA extracted from male antennae from *Cydia pomonella*, *Choristoneura rosaceana*, *Epiphyas postvittana*, *Helicoverpa zea*, *Manduca sexta*, *Ostrinia nubilalis*, and *Trichoplusia ni*. PCR products of ~ 200 - 700 bp were visualized on agarose gels stained with EtBr and bands were excised and TA cloned (data not shown). Twenty five transcripts encoding putative members of the pheromone receptor family were identified, five from *H. zea*, two from *M. sexta*, two from *C. rosaceana*, four from *E. postvittana*, five from *O. nubilalis*, two from *T. ni*, and five from *C. pomonella*. The 25 transcripts ranged in size from 238 - 642 bp (data not shown) and encoded 49 - 54 amino acids with high levels of similarity to the lepidopteran pheromone receptor subfamily (Figure 1). With the exception of *M. sexta*, the transcripts from the other species appear to be from individual genes based upon the uniqueness of the DNA sequences of their 3' untranslated regions (UTRs; data not shown). Further characterizations, including cloning full length transcripts and the genes encoding them, will be needed to determine the actual number of unique pheromone receptor genes.

The deduced amino acid sequences from the 25 transcripts cloned from seven different lepidopteran species were used along with the C-terminal regions of reported lepidopteran Ors to construct a phylogenetic tree. Significantly, all 25 sequences cloned using degenerate PCR primers grouped with other members of the pheromone receptor subfamily and not with other general Ors such as female-biased silkworm Ors19, 30, 45,46,47 and 48 (Figure 2). These results indicate that the degenerate primers are specific to the pheromone receptor subfamily and that the C-terminal region yields sufficient sequence information to assign the peptides to this subfamily. To determine the utility of the 3' RACE approach to identify Ors, degenerate PCR primers were designed against a region conserved between BmOrs 35, 37 & 38. An Or transcript was amplified from *Cydia pomonella* antennae, termed CpOr38, and the deduced amino acid sequence of CpOr38 groups with BmOrs 35, 37 & 38 and not with the pheromone receptor subfamily or other Ors (Figure 2), a further demonstration of the utility of this approach. However, these results will need to be verified using amino acid sequences of full-length receptors allowing for more detailed analyses of the phylogenetic relationships.

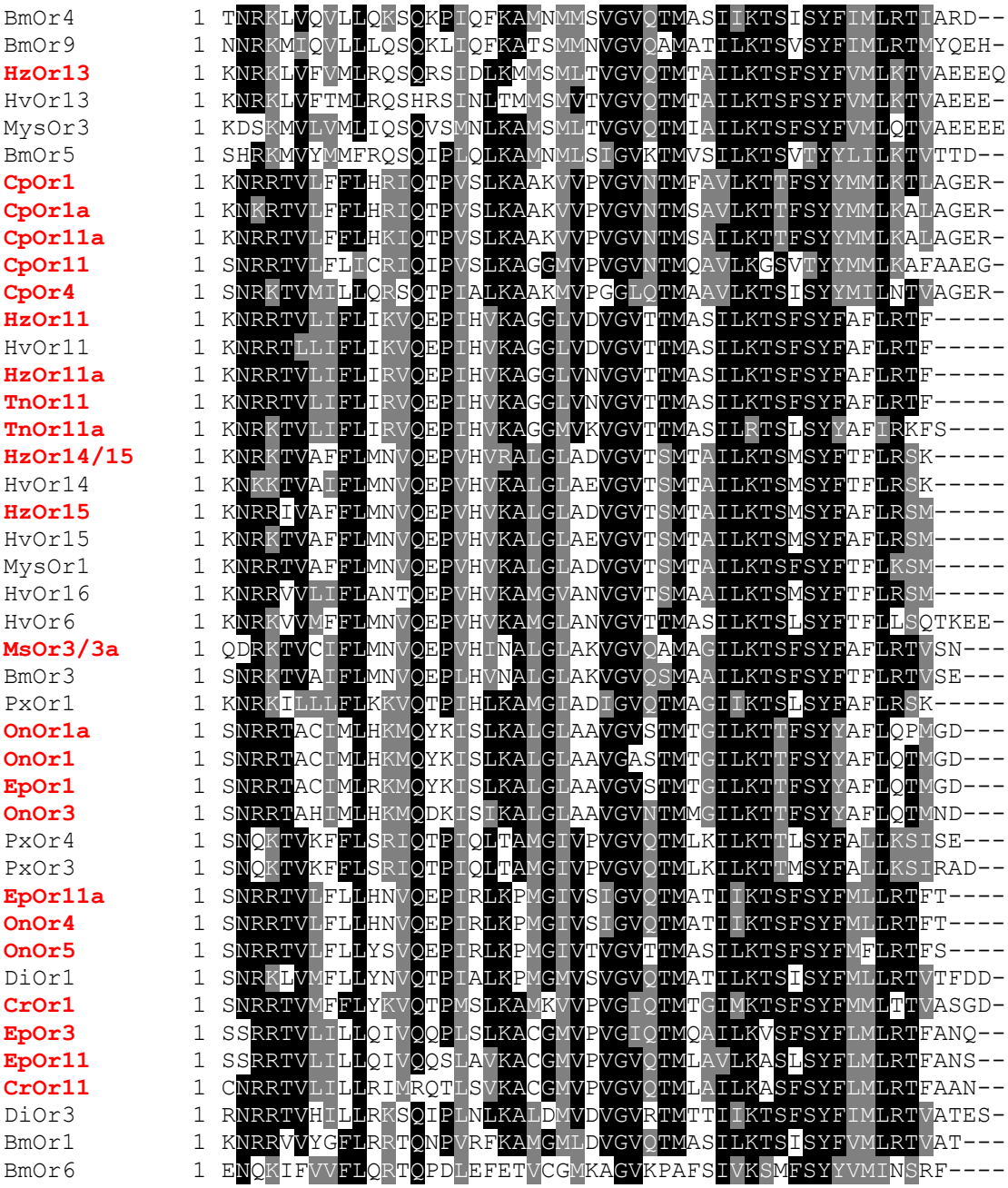

Figure 1. Boxshade of Clustal alignment of the carboxy terminal amino acids of 43 putative members of the lepidopteran pheromone receptor family of proteins. Black background indicates majority of the amino acids are identical; grey background majority conserved. Bm = *Bombyx mori*, Cp = *Cydia pomonella*, Cr = *Choristoneura rosaceana*, Di = *Diaphania indica*, Ep = *Epiphyas postvittana*, Hv = *Heliothis virescens*, Hz = *Helicoverpa zea*, Ms = *Manduca sexta*, Mys = *Mythimna separata*, On = *Ostrinia nubilalis*, Px = *Plutella xylostella* and Tn = *Trichoplusia ni*. Sequence names in red were generated in this study.

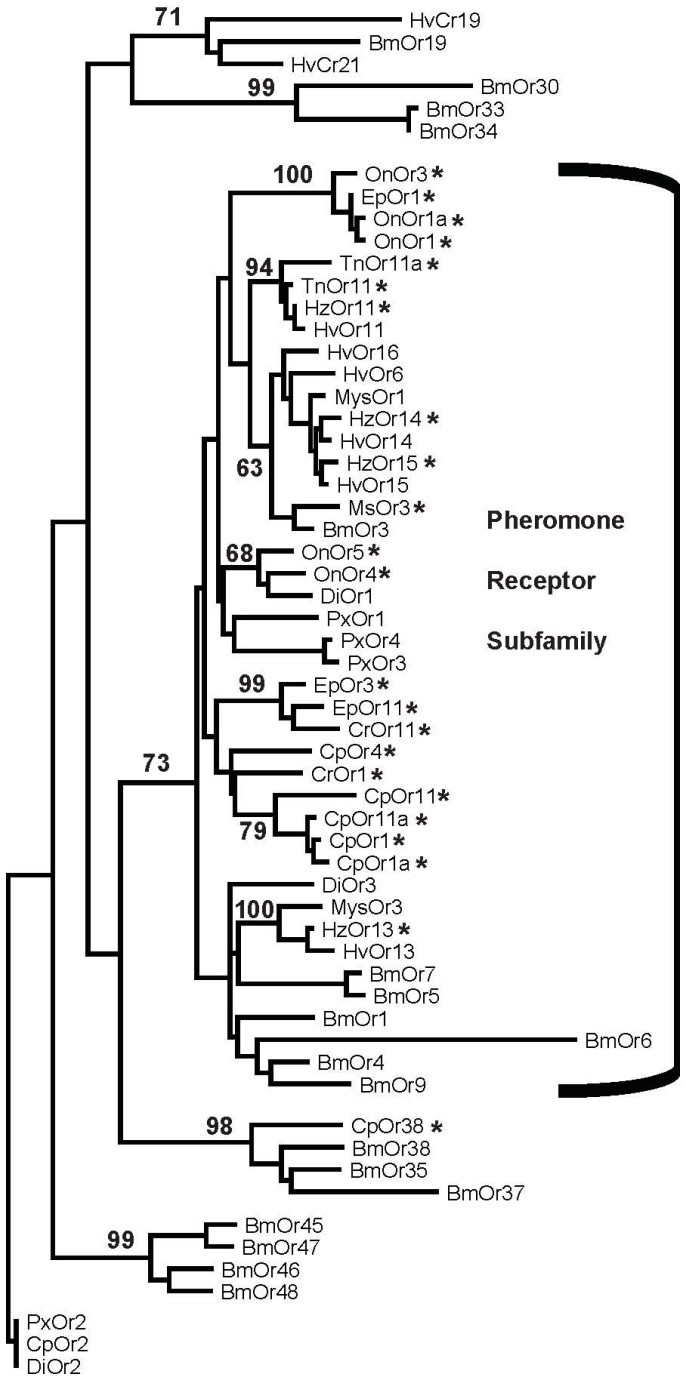

Figure 2. Phylogenetic tree illustrating the relationship of the 25 putative pheromone receptors reported herein (indicated by an *) and 19 previously published pheromone receptors. The tree is rooted using *Drosophila melanogaster* Or83b orthologs PxOr2, CpOr2 & DiOr2 (see Wanner *et al*., 2007). CpOr38 was identified using degenerate PCR primers designed against BmOr38. Bootstrap values are indicated at significant branch points as a percentage of 10 000 replicates. Bm = *Bombyx mori*, Cp = *Cydia pomonella*, Cr = *Choristoneura rosaceana*, Di = *Diaphania indica*, Ep = *Epiphyas postvittana*, Hv = *Heliothis virescens*, Hz = *Helicoverpa zea*, Ms = *Manduca sexta*, Mys = *Mythimna separata*, On = *Ostrinia nubilalis*, Px = *Plutella xylostella* and Tn = *Trichoplusia ni*.

B) Direct pyrosequencing of cDNA prepared from male and female chemosensory organs.

Four cDNA pools prepared from CM chemosensory organs (antennae and pooled legs and mouthparts from males and females) have been sent to Dr. Amit Dhingra (Washington State University) for 454 type pyrosequencing. The pyrosequencing runs will be completed soon and then the sequences will be assembled and annotated. Once the assembly and annotation are complete, the sequences that encode chemosensory receptors will be cloned from CM and readied for ligand determination once the cell-based assay system is fully developed. Annotated sequences will be deposited with GenBank with unidentified single read sequences deposited in dbEST, an expressed sequence tag database.

DISCUSSION

The results from this study have provided not only a method to identify chemosensory receptors in insects without a sequenced genome (which is the majority of insect species), but also has laid the foundation for tools to elucidate the mode of action on pheromones. While these results do not make any immediate products for orchardists to use, we anticipate that in the targets identified in this study will have potential impact on the future discovery of new agrochemicals that will be specific to codling moth control.

EXECUTIVE SUMMARY

Pheromone and kairomone communication, and more generally odor and taste perception, are crucial aspects of codling moth biology and the basis for mating disruption, attract and kill, and monitoring strategies used in the orchard. The codling moth's ability to perceive odors (including pheromones and kairomones), taste food sources, and produce hormones that regulate feeding, digestion and reproduction are all controlled by the brain. Specifically, the senses of smell and taste often regulate feeding and reproductive behaviors. The nerve receptor networks that are involved in the regulation of feeding and reproductive behaviors have been characterized only in insect systems where the genome of that organism has been sequenced (this does not include the codling moth). Therefore, the *goal of our project* was to gain an understanding of how the senses of smell and taste in codling moths lead to the regulation of host finding, feeding, and reproductive behaviors. This fundamental study should provide insight into the molecular basis and the components involved in the codling moth's ability to perceive odorants and enhance our understanding of mating disruption and attraction technologies currently used in the orchard. Identification of the molecular components involved in odor and taste perception will also provide targets and assays that will allow for the rapid screening of potential stimulants and inhibitors of codling moth sensory receptors allowing for the development of more potent lures or disruptants of codling moth's ability to perceive pheromones, kairomones, or feeding stimulants.

Significant progress was made on this project in which a conserved amino acid sequence was identified and a procedure developed that could be used to identify and clone chemosensory receptors from the codling moth. This represents the first time odorant receptors have been identified for an insect without a sequenced genome. The procedure developed using codling moth as a model system was found to be applicable to almost all insects of the Order Lepidoptera (moths and butterflies) as evidenced by our ability to identify and clone "pheromone" receptors from other insect pests of tree fruit including Obliquebanded leafroller, Light Brown Apple Moth, and Apple Clearwing Moth. Additionally, the value of the technique developed here has transcended tree fruit pests and been used to identify and clone "pheromone" receptors from lepidopteran pests of corn and cotton (European corn borer and the corn earworm/cotton bollworm).

The tools generated in this project have also laid the groundwork for development of a high-throughput cell based assay system that will enable us to determine the pheromone or kairomone that interacts with each of the receptors identified from codling moth. The funding obtained from the WTFRC has enabled us to generate the preliminary data to secure enough outside funding to complete this ambitious project. We hope that the results generated from the cell assay system will again make the codling moth a model for studies in other lepidopterous pest insects.

Future directions for this project include the development of the cell based assay system mentioned above, and adapting that system so that it will be able to be used by researchers and chemical companies as an inexpensive tool for the discovery of new semiochemicals that can be used to control codling moth in the orchard. We also hope to these advances to help make codling moth recognized as a model organism for the development of biorational means of pest control.

FINAL PROJECT REPORT

Project Title: Identification of Bt toxin targets in codling moth larvae

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State/Zip:	WA 98951	**State/Zip**:	WA 99164-6382

Other funding Sources - none

Total Project Funding:

Budget History:

Item	Year 1: 2007	Year 2: 2008	Year 3:
Salaries			
Benefits			
Wages	6,240		
Benefits	150		
Equipment			
Supplies	28,110		
Travel	500		
Miscellaneous	5000		
Total	40,000	0	

ORIGINAL OBJECTIVES

The specific objectives of this proposal include:

1) Determine the potencies of 10 Bt toxins against codling moth larvae and cell line.
2) Determine the mode of action of the most potent Bt toxins.
3) Identify key molecules affected by the bioactive Bt toxins
4) Clone transcripts encoding the key molecules affected by Bt toxins
5) Develop a cell-based assay system to search for novel insecticides that alter key molecules affected by Bt toxins

Revised Objectives converting original objectives from a 3 year to 2 year time line as requested by Jim McFerson.

Year 1

1) Determine the toxicity of Bt toxins against codling moth larvae and a codling moth cell line
2) Using the codling moth cell line as a model, determine Bt toxin effects on signal transduction pathways using established assays that monitor chemical signals and cell response.
3) Determine Bt toxin membrane receptors in codling moth larvae and cell line.

Year 2

1) Determine the effects of Bt toxins on signal transduction pathways in codling moth larvae.
2) Identify the key molecules affected by the bioactive Bt toxins.
3) Clone genes encoding key signal transduction proteins affected by the Bt toxins.

SIGNIFICANT FINDINGS

This project got off to a slow start and a no cost extension was requested last year. The findings of this project regarding codling moth are minimal. We have spent much of the past year developing procedures to 1) extract and purify Bt toxins and 2) determine cell line toxicity. We will be using the remaining funds to complete work on the codling moth over the next year.

While the above procedures were being developed, we used our time to characterize a phenomenon observed in a *Helicoverpa zea* colony (corn earworm/cotton bollworm) that is resistant to the affects of Bt toxin. Because our hypothesis is that Bt toxins exert their affects on signal transduction pathways, we started to explore the resistance mechanism of the Bt resistant H. zea colony. The following findings have been made:

1) Males of the Bt resistant *H. zea* line do not recognize females. We have verified this observation using synthetic pheromones in the flight tunnel. This study is ongoing.

2) A member of the pheromone receptor family expressed in Bt susceptible *H. zea* has not yet been detected in the Bt resistant colony.

3) The G-protein that mediates signal transduction of odorant receptors in the antenna has not yet been detected in the Bt resistant colony.

RESULTS AND DISCUSSION

Currently we do not have completely analyzed data for this project. We would respectfully request an extension for submission of this section and the EXECUTIVE SUMMARY until June of 2009, to give us the opportunity to verify, by repetition, the results that we do have and to finish the work on analyzing the affects of Bt toxins on signal transduction molecules.

FINAL REPORT **DURATION: 1 YEAR**

Project Title: Apple maggot host attractants

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State/Province/Zip	WA/98951	**State/Province/Zip:**	NY/14456

Cooperators: Various homeowners, orchard owners, county parks personnel

Total Project Request: **Year 1:** $30,000

Other funding Sources

Agency Name: Washington State Commission on Pesticide Registration (WSCPR)
Amount requested or awarded: $26,130

WTFRC Collaborative expenses: None

Budget 1

Organization: USDA-ARS	**Contract Administrator:** Bobbie Bobango		
Telephone: 509-454-6575	**Email:** Bobbie.Bobango@ARS.USDA.GOV		
Item	**2008**		
Salaries	0		
Benefits	0		
Wages	$11,000[1]		
Benefits	$1,100		
Equipment	0		
Supplies	$1,500[2]		
Travel	$1,400[3]		
Miscellaneous	0		
Total	$15,000		

[1] One GS-5 technician; [2]Traps and components for lures; [3]Fuel for 2 personal car for travel to field sites to collect fruit/pupae and to conduct trapping experiments.

Budget 2:

Organization: Cornell University	**Contract Administrator:** Donna Loeb		
Telephone: 315-787-2325	**Email:** drr2@cornell.edu		
Item	**2008**		
Salaries	$9,840		
Benefits	$5,160		
Supplies	0		
Travel	0		
Miscellaneous	0		
Total	$15,000		

RECAP OF ORGINAL OBJECTIVES

1. To expand the survey of fly responses to traps baited with apple and other fruit volatiles at strategic host localities in central and western Washington.
2. To complete the identification of volatiles from black hawthorn, ornamental hawthorn, snowberry, and apple fruit.
3. To conduct extensive behavioral tests of the responses of apple maggot from Washington to volatile blends identified from black hawthorn, ornamental hawthorn, snowberry, and apple.

SIGNIFICANT FINDINGS

● Washington apple maggot (AM) flies reared from apple were more attracted in a flight tunnel to a newly identified apple blend (WA apple) than to the previously identified eastern apple blend.

● AM flies reared from black hawthorn fruit responded in a flight tunnel in much greater numbers to a newly identified WA black hawthorn blend (haw blend) than to eastern hawthorn, WA apple, and eastern apple blends.

● AM flies reared from ornamental hawthorn fruit did not respond in a flight tunnel in high numbers to any of the blends.

● Field tests showed that AM flies are attracted to the eastern apple blend and the WA apple blend in approximately equal numbers in apple, ornamental hawthorn, and black hawthorn trees, and generally were more attracted to them later than earlier in the season. On isolated apple trees, apple blends performed better than on apple trees in sites with hawthorn trees.

● On central WA black hawthorn trees, fruit volatiles attracted few flies, indicating that the fruit volatiles tested may be habitat, host, or population specific.

● However, in central Washington, fruit volatile-baited sticky red spheres were much more selective than ammonia-baited red spheres, as fruit volatiles attracted AM flies almost exclusively, whereas ammonia attracted both AM flies and high numbers of snowberry maggot flies, making fly identifications difficult.

RESULTS AND DISCUSSION

Objective 1. Expand Survey of Fly Responses to Traps Baited with Apple and Other Fruit Volatiles in Central and Western Washington. Trapping using new fruit volatiles identified and tested in objectives 2 and 3 (below) was conducted on three host trees: (1) apple; (2) ornamental hawthorn (*Crataegus monogyna*); (3) black hawthorn (*Crataegus suksdorfii* and *Crataegus douglasii*). Trees were trapped in three regions in Washington - Puyallup, Vancouver/Skamania, and Wenas. On apple in Puyallup, flies were attracted equally (statistically) to the eastern apple blend, newly identified WA apple blend, and AC treatments (**Table 1**).

On apple (**Table 1**) in Skamania, flies were attracted equally to the eastern apple and WA apple blends, although less than to AC. The differences between the control and eastern and WA apple blends at Puyallup were greater than those at Skamania. At Puyallup, there were 9.8 times more flies in the apple blend treatments than in the control, whereas at Skamania, there were only 1.9 and 1.3 times more. A likely explanation is that at Puyallup, an isolated apple orchard was trapped, whereas at Skamania, mixed stands of apple and hawthorn trees were trapped. Apple blend odors may have stood out more when the background odors were from apple only as opposed to being from apple and hawthorns combined, which may have an antagonistic effect on one another and make detection of apple blend odors from lures more difficult for flies to detect. On apple at WSU, flies were not more

attracted to the eastern apple blend than to the control. The population at this site was low, which may have contributed to the inability to detect a significant difference. The test here was conducted early in the season, before the WA apple blend was available.

Table 1. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in apple trees in Washington, 2008.

On Apple Trees					
Treat	Site 1 (Puyallup)	Treat	Site 2 (Skamania)	Treat	Site 3 (WSU)
Control	13.0B	Control	157.8C	Control	43.0B
AC	168.8A	AC	640.8A	AC	121.4A
East. Apple	122.0A	East. Apple	297.2B	East. Apple	63.2B
WA Apple	128.0A	WA Apple	213.0BC		
$F = 21.6$; df = 3, 12; $P < 0.0001$		$F = 23.7$; df = 3, 12; $P < 0.0001$		$F = 8.8$; df = 2, 8; $P = 0.0094$	

[b]From 21 August to 9 October.

On ornamental hawthorn trees (**Table 2**) at Puyallup, flies were equally attracted to the eastern apple and WA apple blends, at 5.1 and 3.7 times more than the control. On ornamental hawthorn at Skamania, the eastern and WA apple blends were no more attractive than the control. This was also true on ornamental hawthorn at WSU. These results suggest that the host tree species affects responses by flies to apple blends. Flies that develop in ornamental hawthorn at some sites apparently are not attracted to the current apple blends that attract flies that develop in apples.

Table 2. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in ornamental hawthorn trees in Washington, 2008.

On Ornamental Hawthorn Trees					
Treat	Site 1 (Puyallup)	Treat	Site 2 (Skamania)	Treat	Site 3(WSU)
Control	20.0C	Control	18.6B	Control	3.0A
AC	142.8A	AC	42.6A	AC	15.8A
East. Apple	101.2B	East. Apple	18.4B	East. Apple	10.2A
WA Apple	73.4B	WA Apple	15.2B		
$F = 32.2$; df = 3, 12; $P < 0.0001$		$F = 3.5$; df = 3, 12; $P = 0.0488$		$F = 3.4$; df = 2, 8; $P = 0.0831$	

On western black hawthorn trees (**Table 3**) at Saint Cloud, flies were 2.1 times more attracted to the black haw blend than to the control. The black haw blend lacked a component that needed to be synthesized in the laboratory, so the complete blend needs to be tested. By the time the black haw blend was identified in the lab (objective 2), the field season was underway and there was insufficient time to synthesize this component. On black hawthorn, flies were more attracted to the eastern apple than WA apple blend, but the WA apple blend was still 2.6 times more attractive than the control. On black hawthorn at WSU, the black haw blend did not attract more flies than the control.

Table 3. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in western black hawthorn trees in Washington, 2008.

On Western WA Black Hawthorn Trees			
Treat	Site 1 (Skamania)	Treat	Site 2 (WSU)
Control	40.2D	Control	8.6B
AC	518.8A	AC	18.0A
Black Haw	86.4C	East. Apple	4.4B
East. Apple	185.8B		
WA Apple	106.0C		
$F = 41.7$; df = 4, 6; $P < 0.0001$		$F = 12.2$; df = 2, 8; $P = 0.0037$	

On central WA black hawthorn trees (**Table 4**), flies were not attracted to the eastern apple and black hawthorn blends. Results suggest that responses by flies at different sites and from different hosts differed, as alluded to before. Because all apple maggot fly populations tested to date respond to fruit volatiles, it is likely that these flies will be attracted to the complete black haw blend that has yet to be tested. Importantly, however, 0% and 26.3% of flies caught on spheres baited with the eastern and WA apple blends, respectively, were snowberry maggot flies, whereas 76.8% of flies caught on ammonia-baited spheres were snowberry maggot flies, indicating much greater specificity of the apple blends for apple maggot flies. Because apple maggot and snowberry maggot flies are indistinguishable without laboratory examination, use of fruit volatiles that target apple maggot flies has a clear advantage over the use of ammonia.

Table 4. Mean total numbers of apple maggot flies caught over the season on sticky red sphere traps baited with various fruit volatiles in central black hawthorn trees in Washington, 2008.

On Central WA Black Hawthorn Trees			
Treat	Site 1 (Wenas)[a]	Treat	Site 1 (Wenas)[b]
Control	0.60B	Control	0.13C
AC	3.27A	AC	3.13A
East. Apple	0.07B	East. Apple	1.20AB
Black Haw	0.20B	WA Apple	0.93BC
$F = 11.9$; df = 3, 56; $P < 0.0001$		$F = 6.7$; df = 3, 56; $P = 0.0006$	

[a]From 17 July to 21 August.

On apples, there were changes in attractiveness of the volatiles over the season (**Fig. 1**). At Puyallup (**Fig. 1A**), the eastern apple blend was slightly more attractive than the WA blend early in the season, but later they were equally attractive and on some dates the WA apple blend was more attractive. On apples in Skamania County (**Fig. 1B**), the WA apple blend was slightly more attractive than the eastern apple blend in early August, but the trend was reversed later in the month and into early September. Overall, both apple blends appeared less attractive at Skamania than at Puyallup, perhaps because there were mixed stands of apples and hawthorns in Skamania, whereas in Puyallup the apple orchard was isolated.

On ornamental hawthorn in Puyallup (**Fig. 2A**), the eastern apple blend was more attractive than the WA apple blend during mid season, but the WA apple blend was equally attractive later in the season. On black hawthorn at Skamania (**Fig. 2B**), the eastern apple blend was generally more attractive than the WA apple blend throughout the season, and the black haw blend was attractive late in the season.

Overall trapping results indicate that apple volatile blends identified as attractive in the flight tunnel (objectives 2 and 3 below) are also attractive in the field. Their effectiveness, however, seems to depend on the host and site where they are used. There may not be a general fruit volatile blend that is equally effective across all host trees, so several specific blends, specifically one from apple and one or two from hawthorns, may need to be identified for use in field detection surveys in the field. The inconsistencies in attractiveness of apple blends suggest that flies which develop in apple, ornamental hawthorn, and black hawthorn in Washington are genetically and behaviorally different from one another.

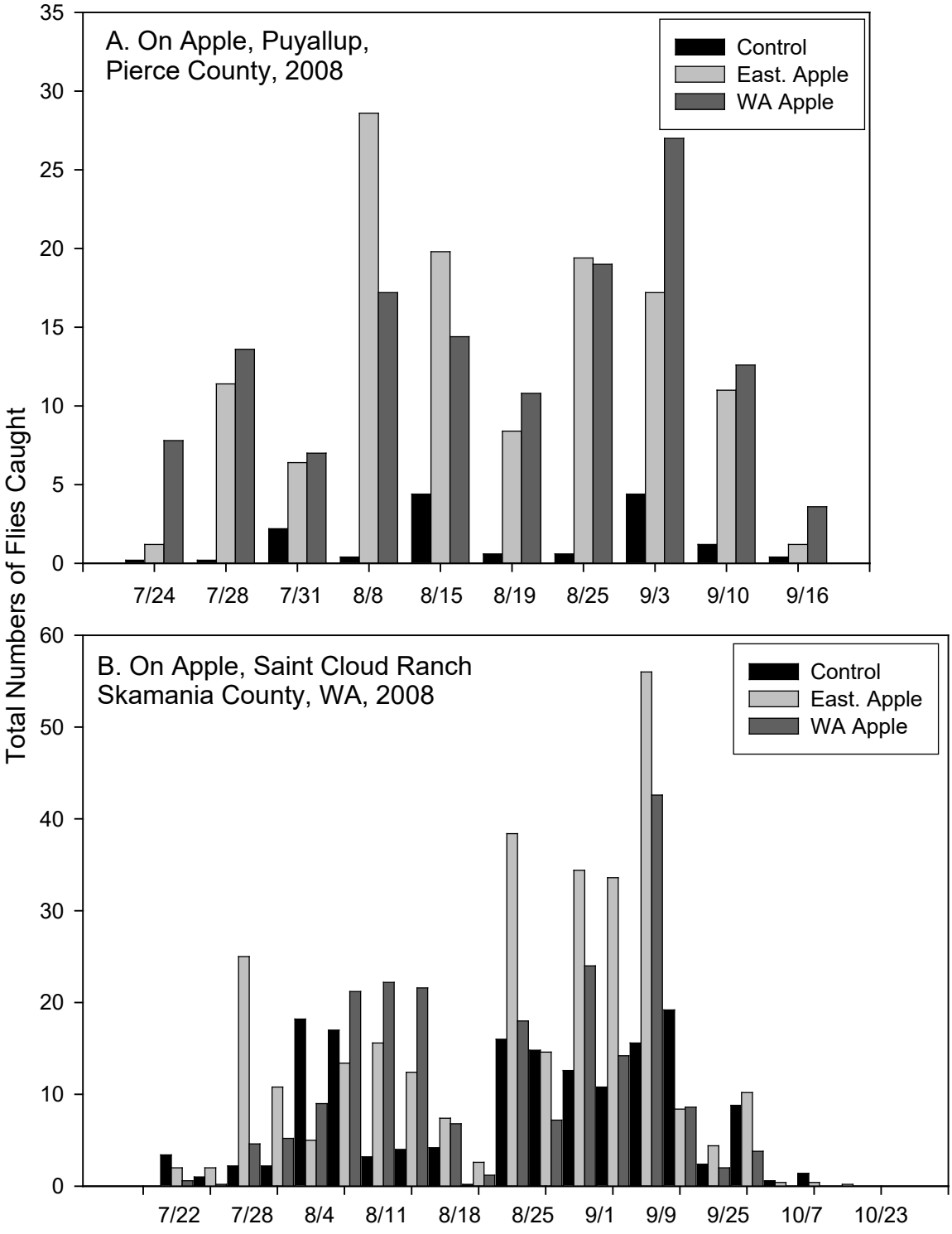

Fig. 1. Seasonal captures of apple maggot flies on sticky red spheres baited with different attractants on apple trees in 2008

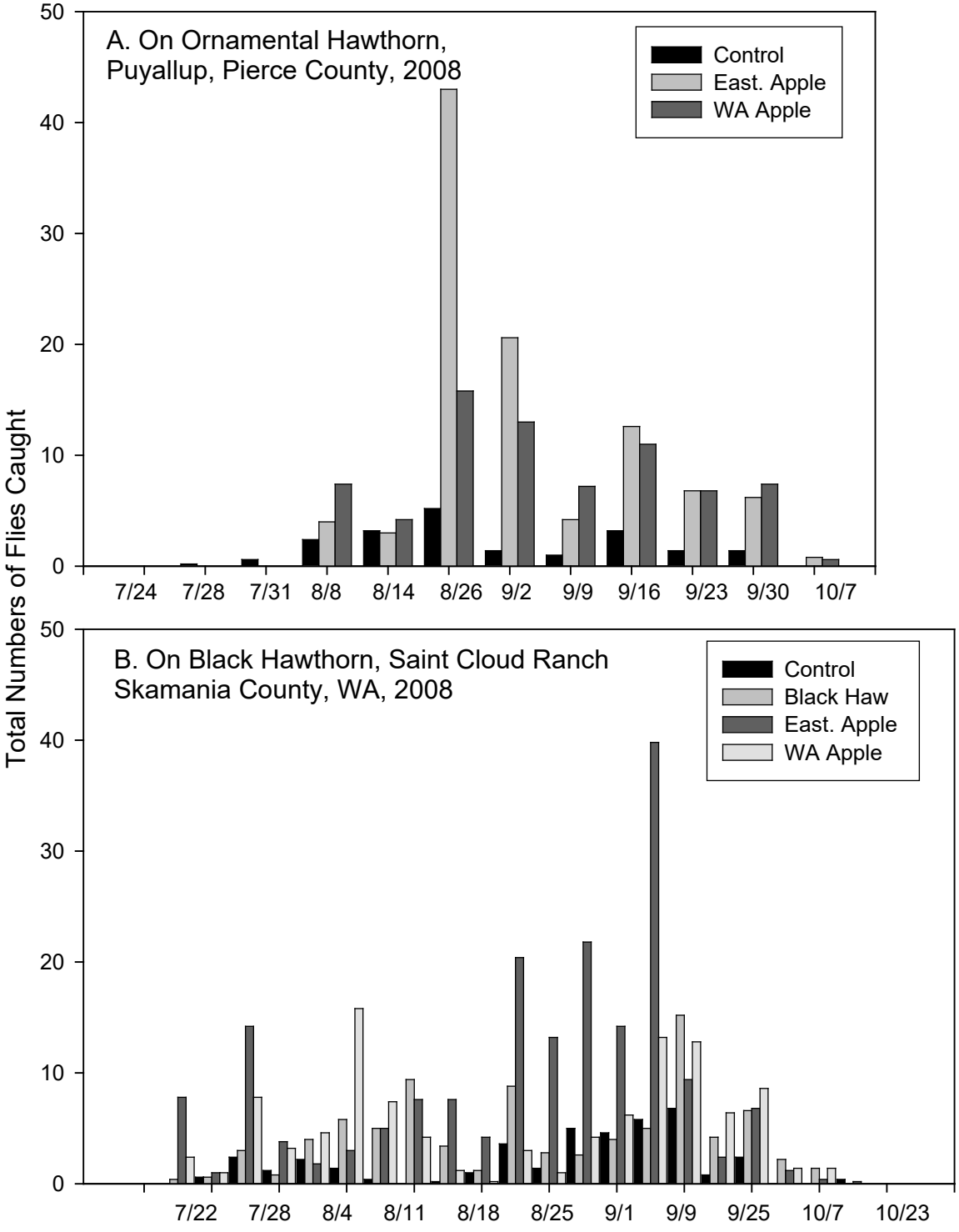

Fig. 2. Seasonal captures of apple maggot flies on sticky red spheres baited with different attractants on ornamental and black hawthorn trees in 2008

Objective 2. Complete Identification of Volatiles From Black hawthorn, Ornamental Hawthorn, Snowberry, and Apple Fruit. Characterization of fruit volatiles were performed using a combination of gas chromatography (GC) / electroantennagram (EAG) analysis of solid phase microextraction (SPME) samples from fruit. Synthetic blends of active compounds were iteratively tested in the flight tunnel to induce similar responses in flies as whole fruit extracts. Through repeated testing, a promising 9-component blend from apples and a 10-component blend from central WA black hawthorn fruit were isolated, which were then tested in the flight tunnel (objective 3).

Objective 3. Conduct Extensive Behavioral Tests of the Responses of Apple Maggot From Washington. In 2008 flight tunnel tests, apple maggot flies responded to the new blends of volatiles, which was the basis for the fruit volatile tests in the field (objective 1). Fly responses varied, but several clear patterns emerged (**Figs. 3, 4, 5, 6**): whereas NY apple maggots reared from apple were attracted to the eastern apple and WA apple blend equally (**Fig. 3**), Washington apple maggot flies reared from apple from St. Cloud (Skamania) (**Fig. 4**) and Puyallup (**Fig. 5**) were much more attracted to the WA apple blend than eastern apple blend. Apple maggot flies reared from black hawthorn fruit (**Fig. 6**) responded in the flight tunnel in much greater numbers to the newly identified WA black hawthorn blend than to eastern hawthorn, WA apple, and eastern apple blends. However, apple maggot flies reared from ornamental hawthorn from Puyallup (**Fig. 7**) did not respond in the flight tunnel in high numbers to any of the blends. Even though flies from apples tended to respond to apple blends and flies from hawthorns to the haw blend, some flies responded to several blends **(Fig. 8)**. For example, four flies from apple from Washington responded to the eastern apple blend, WA apple blend, eastern hawthorn blend, and western hawthorn blend (BH). This suggests that within a fly population, some flies inherently are general responders, whereas other flies are more specific. Thus, any new attractant would be expected to attract some flies from apple and some from hawthorn. However, it remains true that flies from a particular host are most attracted to odors from that host. Snowberry fruit volatiles were isolated, but flies did not respond to them.

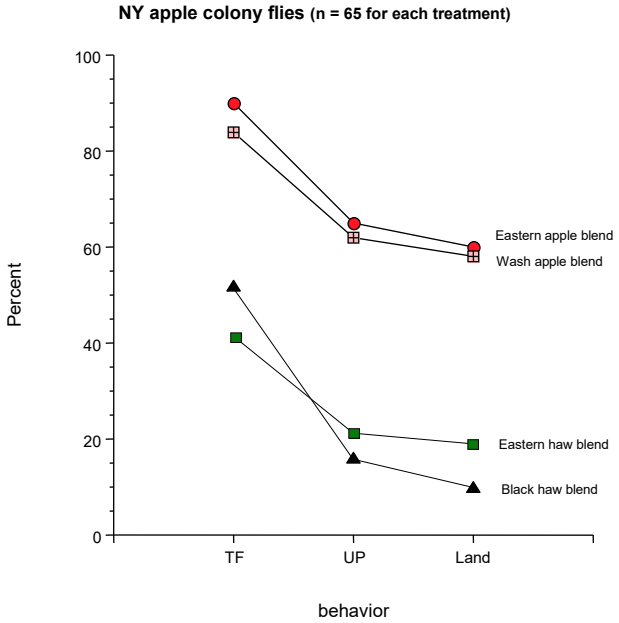

Fig. 3. Responses in a flight tunnel of NY apple maggot flies reared from apple to attractants.

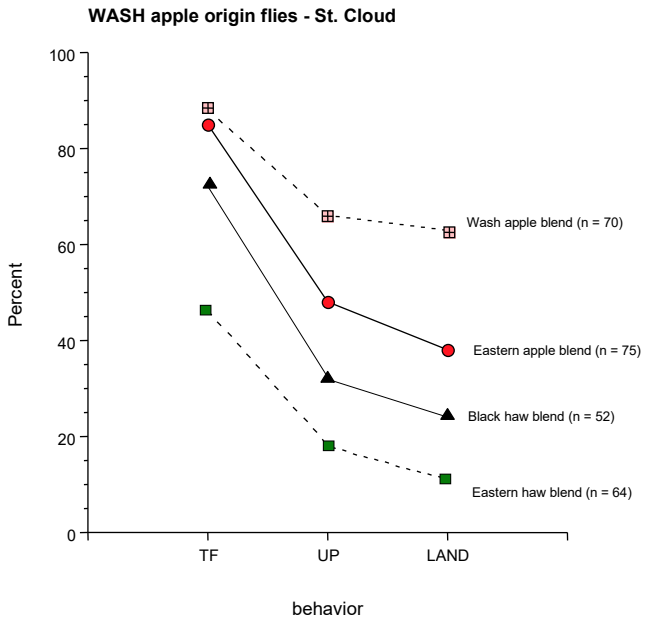

Fig. 4. Responses in a flight tunnel of WA (Skamania County) apple maggot flies from apple to attractants.

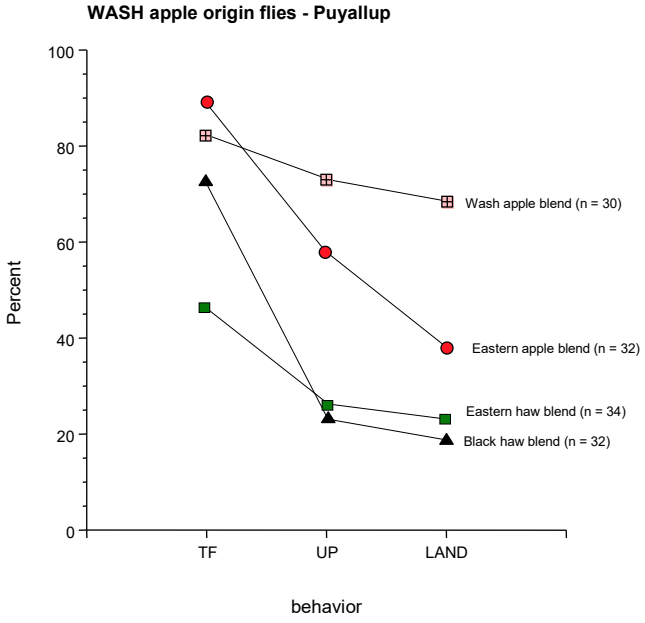

Fig. 5. Responses in a flight tunnel of WA (Puyallup) apple maggot flies reared from apple to attractants.

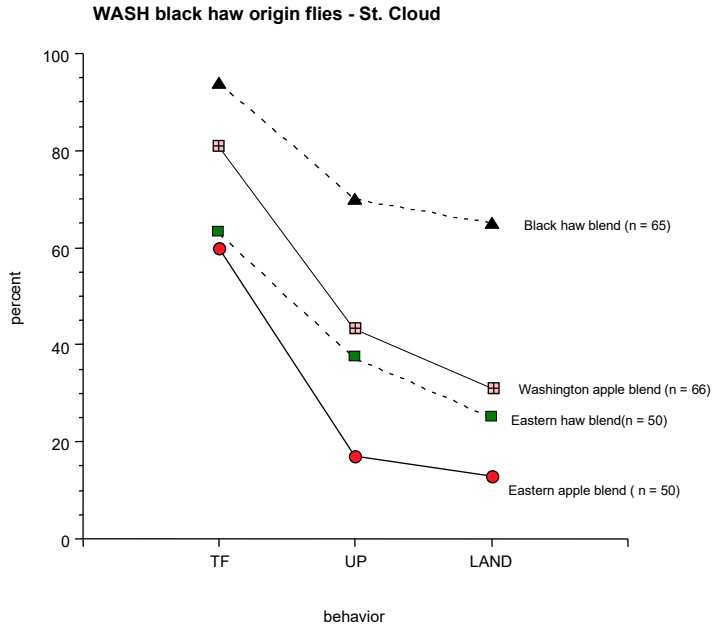

Fig. 6. Responses in a flight tunnel of WA (Skamania County) apple maggot flies reared from western WA black hawthorn to attractants.

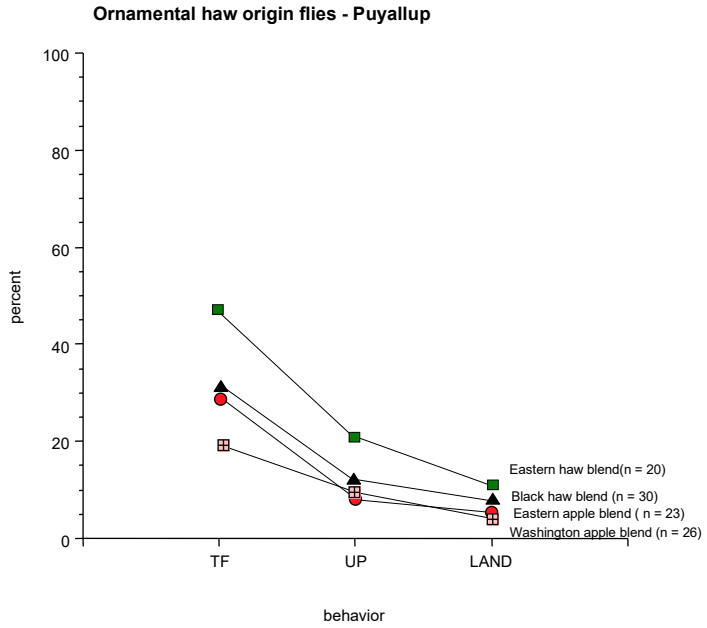

Fig. 7. Responses in a flight tunnel of WA (Puyallup) apple maggot flies reared from ornamental hawthorn to attractants.

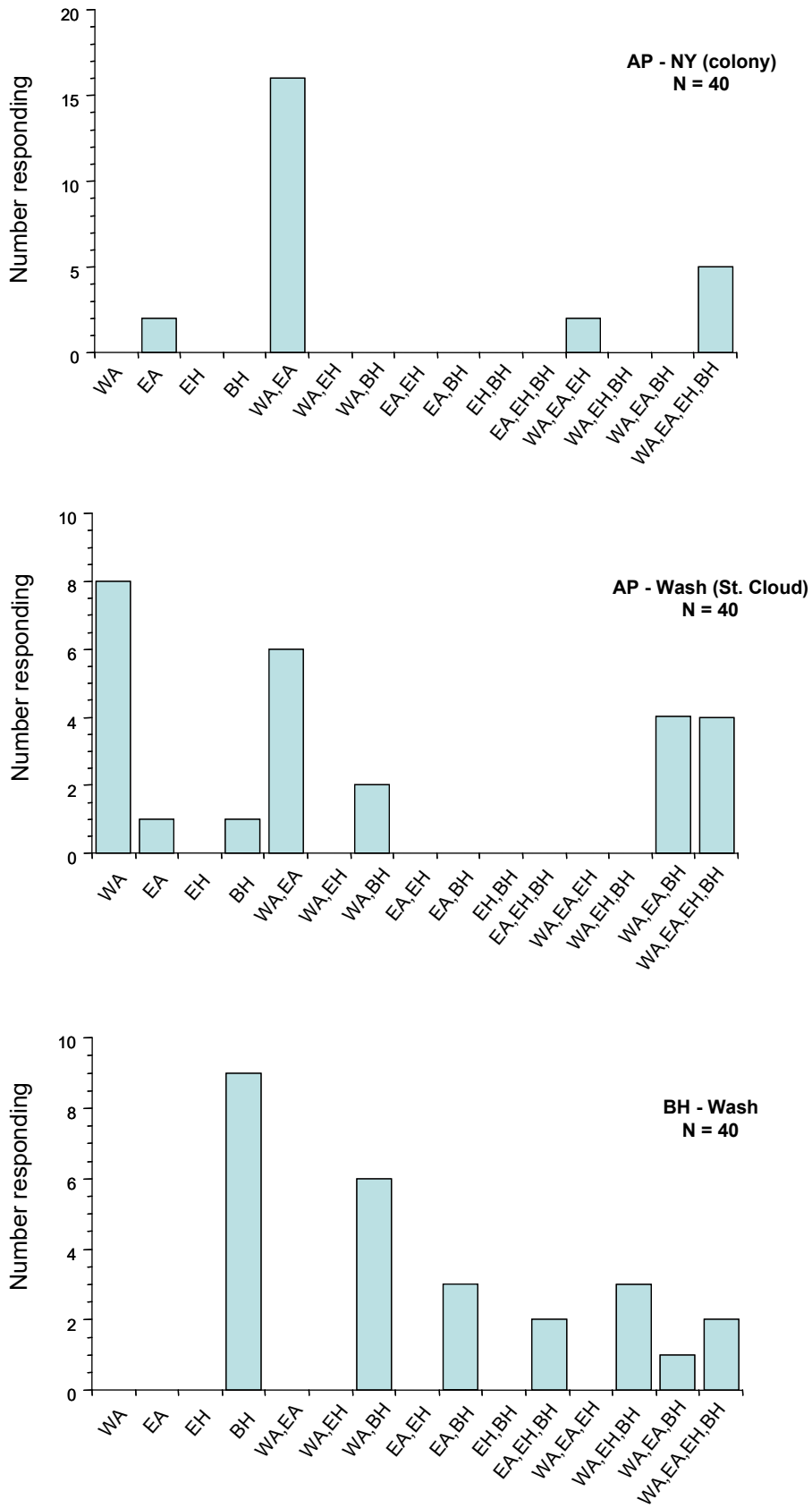

Fig. 8. Responses of NY and WA apple maggot flies to different fruit volatile blends.

Differences between field and laboratory results may be caused in part by the release rate of volatiles. In a flight tunnel, the flies need to fly only 1 m from the release point to the odor source. The amount of odor reaching the flies may be different than in the field, where many flies may be greater than 1 m away from the vials that contained the new blends. Thus, release rates may need to be increased in the field to elicit greater responses. Also, there are no competing host odors in a flight tunnel that could interfere with attractiveness, whereas these are present in the field and some odors may even be antagonistic with the newly identified blends. If so, blends that are not antagonized by other volatiles should be more attractive than current blends. Another possibility that may explain differences in the laboratory and field is that flies differed in their physiological state, i.e., their age, nutritional background, nutrient levels, prior experience with host odors, and mating status differed.

Significance to the Industry and Potential Economic Benefits

A highly effective attractant can be used to determine where apple maggot flies are located, and can document changes in their distribution, which is important because flies appear to be spreading into new regions in Washington. An insect population cannot be controlled if its distribution is unknown. A sensitive trap that can detect flies may help prevent their spread within the apple-growing regions. Keeping flies out of apple orchards can be accomplished if they are detected first and the positive sites are treated. Inaction because apple maggot flies are not detected due to insensitive traps may result in infested orchards and economic losses. There is no tolerance for larvae in fruit and shipments from any infested orchards likely would be banned.

A highly attractive and specific attractant can result in reduced labor costs needed to identify snowberry maggots caught on traps baited with ammonia lures that are intended to capture apple maggot flies. Costs can instead be re-directed to more in-field efforts of fly trapping in high-risk areas.

Present and future export markets will want to know if an area is apple maggot-free before accepting apples from the area. A highly attractive volatile that can detect low populations of flies can be used to provide evidence that an area is free of flies. This may help the industry gain access to markets that require areas be pest-free (fly-free) or that are considered to be low in pest prevalence.

EXECUTIVE SUMMARY

The presence of wide-spread breeding populations of the apple maggot fly in central Washington represents a serious threat to the apple industry, as there is a zero tolerance for larvae in exported apples. The fly continues be to found at new sites each year, suggesting the fly population is spreading. A major advance in managing the fly would be the development of a highly effective attractant that can be used to determine where the fly is located, and that can document changes in its distribution in central Washington. However, field survey tests using the fruit volatile attractants that are very effective in the eastern U. S. suggest they are not as effective for detecting the fly in Washington. An additional problem in developing an effective monitoring system is that we do not completely understand the identity of the various fly populations and their host/odor preferences in Washington. In 2007, with funding from the WTFRC, studies were initiated to establish whether fruit and flies from Washington displayed different volatile profiles/preferences. In a continuation of 2007 work, a project was conducted in 2008 to (1) to expand the survey of fly responses to traps baited with apple and other fruit volatiles at strategic host localities in central and western Washington; (2) to complete the identification of volatiles from black hawthorn, ornamental hawthorn, snowberry, and apple fruit, and (3) to conduct extensive behavioral tests of the responses of apple maggot from Washington to volatile blends identified from black hawthorn, ornamental hawthorn, snowberry, and apple. Washington apple maggot flies reared from apple were more attracted in a flight tunnel to a newly identified apple blend than to the previously identified eastern apple blend. AM flies reared from black hawthorn fruit responded in the flight tunnel in much greater numbers to a newly identified WA black hawthorn blend than to eastern hawthorn, WA apple, and eastern apple blends. AM flies reared from ornamental hawthorn fruit did not respond in the flight tunnel in high numbers to any of the blends. Snowberry fruit volatiles were isolated, but flies did not respond to them. Field tests showed that AM flies are attracted to the eastern apple blend and the newly identified WA apple blend in approximately equal numbers in apple, ornamental hawthorn, and black hawthorn trees, and generally were more attracted to them later than earlier in the season. On isolated apple trees, fruit volatiles performed better than on apple trees in sites with hawthorn trees. On central WA black hawthorn trees, fruit volatiles did not appear to be highly attract flies, indicating that the fruit volatiles tested may be habitat specific. Importantly, however, 0% and 26.3% of flies caught on spheres baited with the eastern and WA apple blends, respectively, were snowberry maggot flies, whereas 76.8% of flies caught on ammonia-baited spheres were snowberry maggot flies, indicating much greater specificity of the apple blends for apple maggot flies. Because apple maggot and snowberry maggot flies are indistinguishable without laboratory examination, use of fruit volatiles that target apple maggot flies has an advantage over the use of ammonia. There are other potential benefits of identifying attractive fruit volatiles. A sensitive trap that can detect flies may help prevent their spread within the apple-growing regions. Inaction because apple maggot flies are not detected due to insensitive traps may result in infested orchards and economic losses. There is no tolerance for larvae in fruit and shipments from any infested orchards likely would be banned. Finally, present and future export markets may want to know if an area is apple maggot-free before accepting apples from the area. A highly attractive volatile that can detect low populations of flies can be used to provide evidence that an area is free of flies. Results from the present flight tunnel tests show fruit volatiles are very promising but that further testing is needed to identify even more attractive blends for use in the field.

FINAL REPORT **DURATION: 1 YEAR**

Project Title: DNA and morphometric diagnostics for apple and snowberry maggot flies

PI:	Wee Yee	**Co-PI(2):**	Jeff Feder
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Total Project Request: Year 1: $25,000

Other funding Sources: none

Budget 1:

Organization: USDA-ARS	**Contract Administrator:** Bobbie Bobango		
Telephone: 509-454-6575	**Email:** Bobbie.Bobango@ARS.USDA.GOV		
Item	**2008**		
Salaries	0		
Benefits	0		
Wages	$12,700		
Benefits	$1,270		
Equipment	0		
Supplies	$800		
Travel	$230		
Miscellaneous	0		
Total	$15,000		

Budget 2:

Organization: Univ. Notre Dame	**Contract Administrator:** Rick Hilliard		
Telephone: 574-631-5386	**Email:** Hilliard.1@nd.edu		
Item	**2008**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$9,000		
Travel	$1,000		
Miscellaneous	0		
Total	$10,000		

RECAP OF ORIGINAL OBJECTIVES

1. Increase sample sizes for morphometric diagnostics between apple and snowberry maggot.
2. Determine if host races of apple maggot exist in Washington using microsatellite variation.

SIGNIFICANT FINDINGS

● Wing shape identified female apple maggot flies with 97% accuracy.

● Wing shape misidentified 3% of apple maggot flies as snowberry maggots flies.

● Wing shape identified 97–100% of female snowberry maggot flies.

● Using wing shape, apple maggot flies are more likely to be misidentified as snowberry maggot flies than are snowberry maggot flies to be misidentified as apple maggot flies.

● Wing shape analysis correctly identified 100% of flies whose identities were questionable based on ovipositor lengths and therefore is a useful method for pest detection and management.

● Different populations of apple maggot flies may have slightly different wing shapes, but apple maggot flies almost always separate out from snowberry maggot flies.

● Microsatellite genetic markers suggest that significant differences may exist between flies reared from apple and black hawthorns in Washington, and that host races of the flies exist.

● None of eight loci analyzed possessed a high frequency allele for the Washington/Oregon fly populations at any of the eight microsatellites that was also not found in the East, implying the eastern and western apple maggot populations are fairly closely genetically related.

● Microsatellite genetic markers suggest that significant differences between apple maggot and snowberry maggot flies in Washington may are detectable.

RESULTS AND DISCUSSION

Objective 1. Increase Sample Sizes for Morphometric Diagnostics Between Apple and Snowberry Maggot.

More flies were added to the analyses that were started in 2007, for totals of 140 female apple maggots and 71 snowberry maggot flies. Addition of these flies resulted in more rigorous results than those obtained in 2007, and in more solid conclusions.

Visual inspection indicated that the wing of the female apple maggot fly is longer and tapers more at the end than that of the snowberry maggot fly, which is more rounded at the tip and stubbier in general appearance (Fig. 1). (Wings of male flies are similar.) However, there are variations in wing shape. For analyzing variation in wing shapes, 18 landmarks were added to the wings of the flies (Fig. 1), an increase from the 14 utilized in 2007, and subjected to canonical variates analysis (CVA). CVA separated western WA flies into two groups, one for apple maggot and one for snowberry maggot fly (Fig. 2). Based on wing shape alone, the assignments test from CVA correctly identified 97% of 140 apple maggots, and correctly identified 100% of 71 snowberry maggots (Table 1). (Central WA flies from black hawthorn were not included because of low sample size.)

When CVA was performed on different fly groups based on host fruit and collection area, including from central WA (Fig. 3), there was evidence that wing shapes of some populations of apple maggot flies differed from one another and that wings of the two populations of snowberry maggot also differed. The assignments test from CVA misclassified many apple maggot flies across

groups, but 95% of apple maggots were correctly identified to species, and 97% of snowberry maggots were correctly identified to species (Table 2). Addition of flies to an analysis can change the outcome because variables from the different observations are interrelated.

To test the validity of CVA of wing shape, wings of flies from apple and hawthorns that had intermediate ovipositor lengths (and therefore cannot be distinguished as apple or snowberry maggot flies without host information) were analyzed. A program that compares known flies with unknowns was used for analysis. The program classified unknown specimens into one or the other group based on wing shape variables. The 18 apple maggots entered as "unknowns" were all grouped with apple maggots (Fig. 4) and the assignments test correctly identified 100% of them (Table 3). Of the known apple and snowberry maggots, 99% and 100%, respectively, were correctly identified.

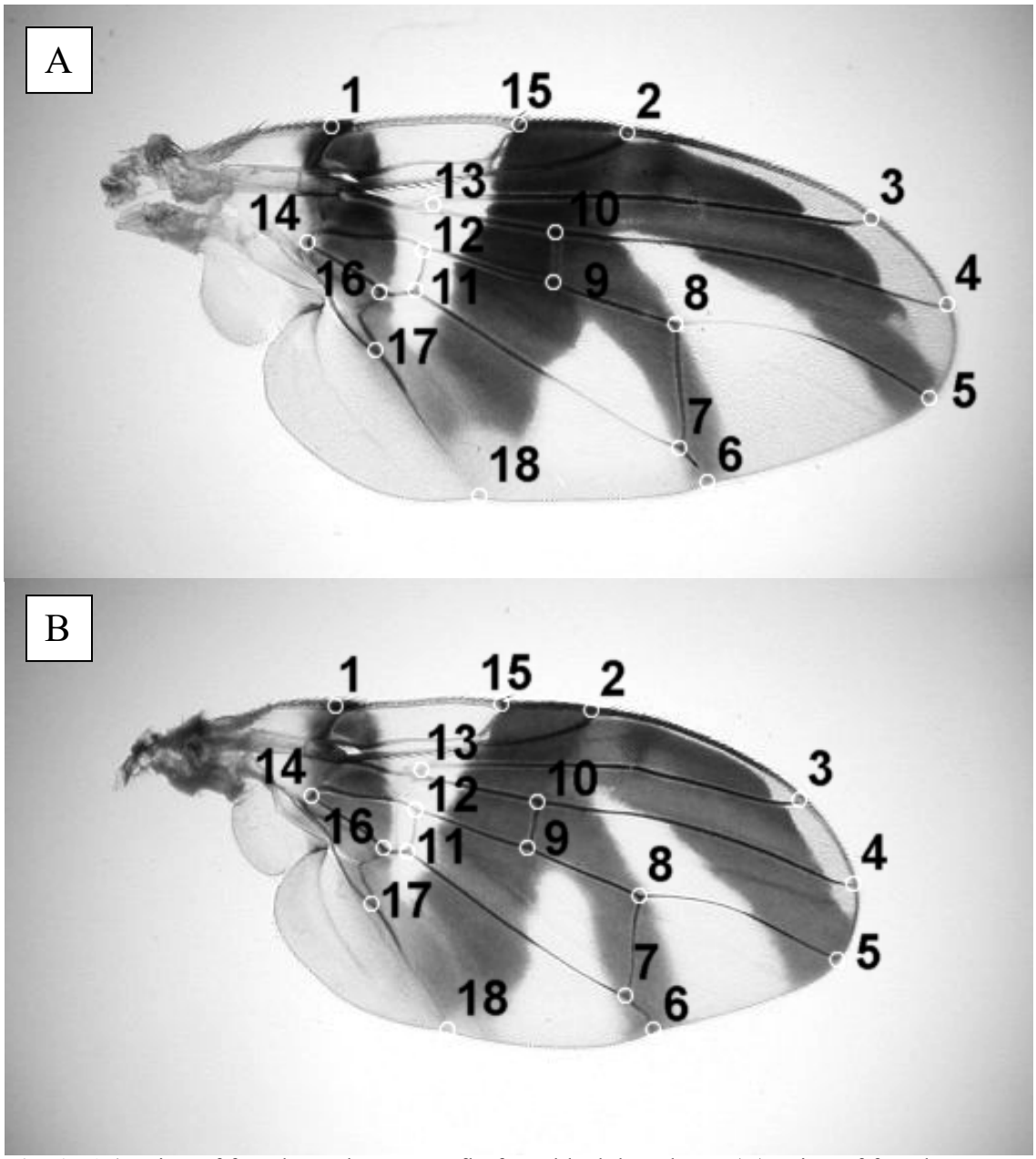

Fig. 1. (A) Wing of female apple maggot fly from black hawthorn; (B) Wing of female snowberry maggot fly from snowberry. Numbers are landmark positions on the wings.

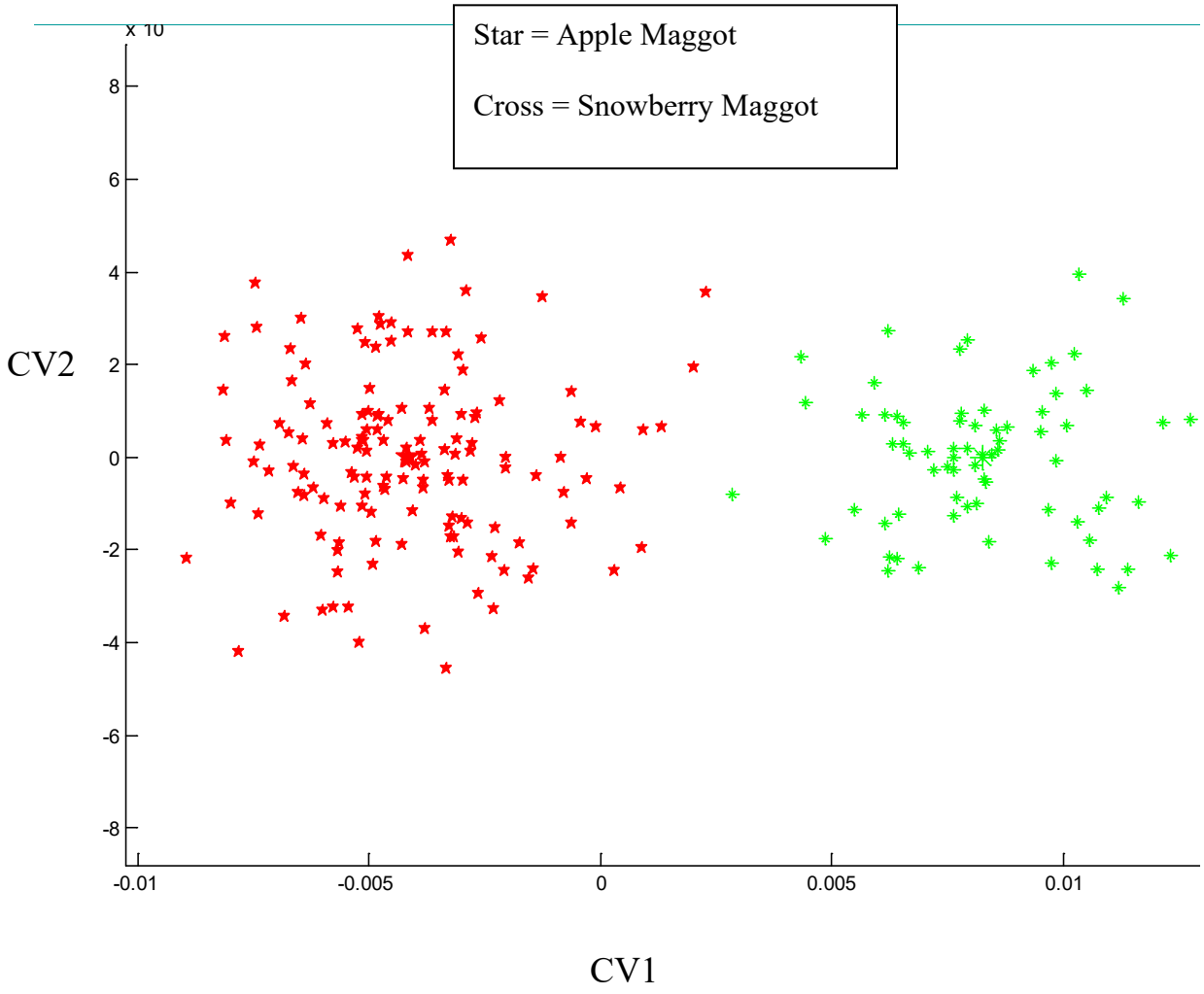

Fig. 2. Separation of female apple maggot and snowberry maggot flies into two groups based on CVA of wing shape. Flies from different hosts and from different sites were combined.

Table 1. Assignments test (numbers, % in parentheses) based on CVA of all WA apple and snowberry maggot flies (jackknifed data). Misclassified flies in bold.

	Classified as:		
Known Species	Apple Maggot	Snowberry Maggot	Totals
Apple Maggot	136 (97)	**4 (3)**	140
Snowberry Maggot	0 (0)	71 (100)	71

1 x = Apple Maggot from apple, Vancouver/Skamania
2 Star = Apple Maggot from apple, Puyallup
3 * = Apple Maggot from ornamental hawthorn, Vancouver/Skamania
4 + = Apple Maggot from ornamental hawthorn, Puyallup
5 Square = Apple Maggot from black hawthorn, Vancouver/Skamania
6 Black Triangle = Apple Maggot from black hawthorn, Wenas (central WA)
7 Gray Triangle = Snowberry Maggot from snowberry, Vancouver/Skamania
8 Black Circle = Snowberry Maggot from snowberry, central WA

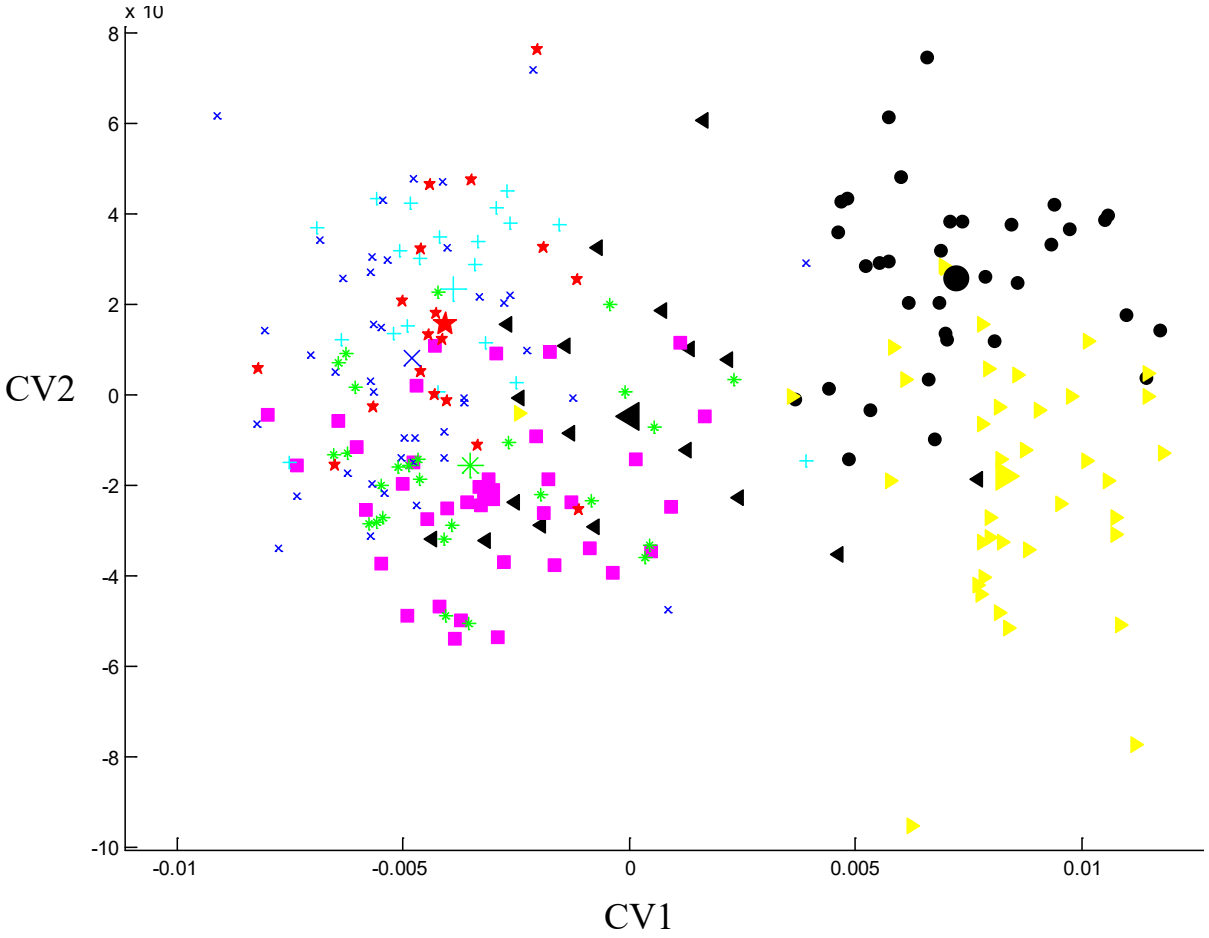

Fig. 3. Separation of female apple maggot and snowberry maggot flies from different host fruit and collection areas in Washington.

Table 2. Assignments test (numbers, % inside parentheses) based on CVA of 8 groups of apple and snowberry maggot flies (jackknifed data). Misclassified flies in bold.

Known Group	Classified as:								
	1, AM	2, AM	3, AM	4, AM	5, AM	6, AM	7, SB	8, SB	Totals
1, AM	16 (38)	5 (12)	4 (10)	7 (17)	6 (14)	3 (7)	**1 (2)**	0	42
2, AM	3 (17)	5 (28)	3 (17)	2 (11)	3 (17)	2 (11)	0	0	18
3, AM	6 (22)	0	5 (18)	3 (11)	10 (37)	2 (7)	0	**1 (4)**	27
4, AM	4 (20)	3 (15)	2 (10)	10 (50)	0	0	0	**1 (5)**	20
5, AM	3 (8)	2 (5)	10 (29)	2 (5)	15 (44)	2 (5)	0	**2 (5)**	34
6, AM	0	2 (11)	1 (6)	1 (6)	1 (6)	10 (55)	**2 (10)**	**1 (6)**	18
7, SB	0	0	0	0	**1 (3)**	**1 (3)**	28 (73)	8 (21)	38
8, SB	0	0	0	0	0	0	9 (26)	25 (74)	34

Numbers of 1–8 same as key in Figure 3; AM, apple maggot; SB, snowberry maggot.

Star = Apple Maggot
Cross = Snowberry Maggot
Triangle = Apple Maggot Flies (based on known rearing host) with intermediate ovipositor lengths entered as unknowns

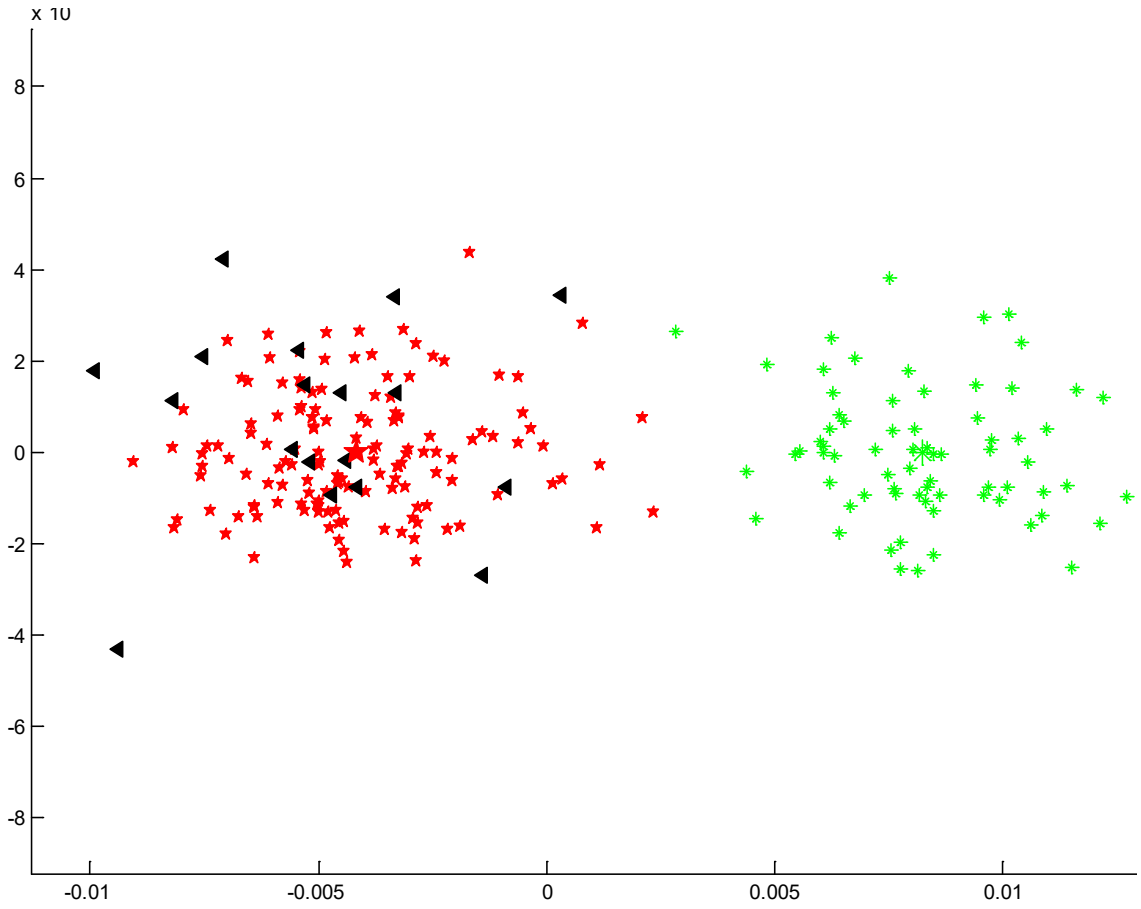

Fig. 4. Classification of 18 female apple maggots (triangles) with intermediate ovipositor lengths into the apple maggot group based on wing shape analysis. Stars, known apple maggot; asterisks, known snowberry maggot.

Table 3. Assignments test (numbers, % inside parentheses) based on CVA of 18 apple maggots called 'unknown flies' (intermediate ovipositor length) into known groups. Misclassified flies in bold.

	Classified as:		
Actual Species	Apple Maggot	Snowberry Maggot	Totals
Apple Maggot	138 (99)	**2 (1)**	140
Snowberry Maggot	0	71 (100)	71
'Unknown Flies'	18 (100)	0	18

Overall results of the wing shape analyses reveal that wing shape is a good character to separate the species. Wings of the two species usually have different shapes, with only about a 3% overlap, which is an improvement over the use of older criteria. In practice, a wing of a questionable fly with intermediate ovipositor length can be digitized with landmarks and entered as an observation in a 'training data set' generated from known flies. The data set can be saved on a computer and used

repeatedly for classification purposes. Based on the wing shapes of the other flies, the shape of the unknown is compared and is then placed into one of the known groups by the computer.

Wing shape is good at classifying flies, but a goal should be to eliminate even a 3% probability of misdiagnosis, which might be achieved if shapes of other structures are included in the identification process. Preliminary work in 2008 showed that in addition to wing shape, ovipositor shape may also differ between species. Furthermore, the shape of the male genitalia (specifically the claspers) may be diagnostic of species. Use of a combination of measures, including wing shape, should enable us to positively identify virtually all problematic flies.

Objective 2. Determine if host races of apple maggot exist in Washington using microsatellite variation.

In work partially funded by the WTFRC in 2007 and 2008, we found that microsatellite genetic markers suggest that significant differences may exist between flies reared from apple and hawthorns in Washington, as well as snowberries. We found that all of the 80 PCR primers developed to amplify microsatellite loci for eastern populations of apple maggot, also worked for western apple maggot flies from Washington state. A subset of eight loci has now been scored for approximately 30 flies each collected from two sympatric apple, black hawthorn, ornamental hawthorn and snowberry sites in the Vancouver/Portland area in Happy Valley (Skamania County) and Devine (Clark County) (These eight loci are designated p71, p50, p80, p37, p27, p11, p29, and p60). Six of the eight loci (all except p11 and p29) displayed significant allele frequency differences among the apple and the hawthorn populations ranging on the order of from 10 to 25%; results from four representative loci are shown in **Table 4.** These data are consistent with the existence of host races in the state. Washington flies from the two sites did not show substantial genetic differences from flies from the eastern United States (Illinois, Indiana, and Michigan). None of the eight loci possessed a high frequency allele for the Washington fly populations at any of the eight microsatellites that was also not found in the East. This result implies that the eastern and western apple maggot populations may be fairly closely genetically related. At face value, these current findings are consistent with an introduction of the apple maggot fly from the East to the West. However, more genetic screening from additional loci is needed to confirm the preliminary data.

Table 4. Allele frequencies of four representative microsatellites (gene loci) of two apple maggot populations from apple and black hawthorn and one from snowberry in Washington. Most common allele within a population is in **BOLD**

		Home Valley Population		Devine Site Population		Home Valley
		Apple Maggot		Apple Maggot		Snowberry M.
Locus	Allele No.	Apple	Black Haw	Apple	Black Haw	Snowberry
p29	1	0	0	0	0	0
	2	0	0.0385	0.1429	0.1304	0
	3	0.0227	0	0	0	0
	4	0	0.0769	0.0714	0.0217	0
	5	0	0	0	0	0
	6	0.1818	0.1154	**0.2857**	**0.3696**	0.1250
	7	0	0	0	0	0
	8	0.0227	0.0769	0	0	0
	9	0.1364	0.2308	0.0952	0.0652	0.1250
	10	0	0	0	0.0435	0
	11	**0.3409**	**0.4231**	0.2381	0.1304	0
	12	0.0909	0	0.0476	0.0217	0
	13	0	0	0	0	0
	14	0	0	0	0	0

	15	0.0909	0	0.0238	0.1087	**0.5000**
	16	0.0227	0	0.0238	0	0
	17	0	0	0	0	0
	18	0.0227	0	0.0238	0	0
	19	0.0682	0.0385	0.0476	0.1087	0
	20	0	0	0	0	0.2500
	21	0	0	0	0	0
	22	0	0	0	0	0
Sample Size		22	13	21	23	4
		Home Valley Population		Devine Site Population		Home Valley
		Apple Maggot		Apple Maggot		Snowberry M.
Locus	Allele No.	Apple	Black Haw	Apple	Black Haw	Snowberry
p37	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0.1053	0.1111	0.1667	0.0690	0
	6	0	0	0	0	0
	7	0	0	0	0.0172	0
	8	0	0.0278	0	0	**0.3889**
	9	0.0789	0.0278	0	0.0172	0
	10	0	0.0278	0	0.0345	0.0556
	11	0	0	0	0	0.1111
	12	0	0	0	0	0
	13	**0.1579**	0.0833	0.1500	**0.2069**	0
	14	0.1053	**0.2778**	**0.2000**	0.1207	0
	15	0	0.0278	0.0333	0.0172	0.3333
	16	0.0263	0	0	0.0690	0
	17	0	0	0	0	0
	18	0	0	0.0167	0	0.0556
	19	0.0526	0.0556	0.1667	0.0862	0
	20	0.0789	0.1389	0.0500	0	0
	21	0	0	0.0167	0	0
	22	0	0.0556	0	0.0172	0
	23	0.0263	0	0	0.0517	0.0556
	24	0.0526	0.0556	0.0500	0.1724	0
	25	**0.1579**	0.0556	0.100	0.0690	0
	26	0	0	0	0	0
	27	0	0	0	0	0
	28	0.0526	0	0	0	0
	29	0.0263	0.0278	0	0	0
	30	0	0	0	0	0
	31	0	0	0	0	0
	32	0	0	0	0	0
	33	0	0	0	0	0
	34	0	0	0	0	0
	35	0	0	0	0	0
	36	0.0526	0.0278	0.0167	0	0
	37	0	0	0.0167	0.0172	0

	38	0.0263	0	0.0167	0.0345	0
	Sample Size	19	18	30	29	9
		Home Valley Population		Devine Site Population		Home Valley
		Apple Maggot		Apple Maggot		Snowberry M.
Locus	Allele No.	Apple	Black Haw	Apple	Black Haw	Snowberry
p60	1	0	0.0278	0	0	-----
	2	0.3529	0.4444	0.4655	**0.6875**	-----
	3	**0.6471**	**0.5278**	**0.5345**	0.3125	-----
	4	0	0	0	0	-----
	5	0	0	0	0	-----
	6	0	0	0	0	-----
	Sample Size	34	18	29	32	0
		Home Valley Population		Devine Site Population		Home Valley
		Apple Maggot		Apple Maggot		Snowberry M.
Locus	Allele No.	Apple	Black Haw	Apple	Black Haw	Snowberry
p71	1	0	0	0	0	0
	2	0	0	0	0	0.0740
	3	0	0	0	0	0
	4	0.0294	0	0	0	0
	5	0.0147	0.0750	0.0938	0.2143	0
	6	0.2500	0.1750	0.1250	0.0893	0.1429
	7	0.2353	0.1500	0.1875	0.2321	0.1429
	8	0.0882	0.0250	0	0	0
	9	**0.3235**	**0.5250**	**0.5625**	**0.4107**	0.0714
	10	0.0441	0	0.0312	0.0536	0.1429
	11	0	0.0500	0	0	**0.3571**
	12	0	0	0	0	0.0714
	13	0.0147	0	0	0	0
	Sample Size	34	20	16	28	7

We also found that all eight of the loci displayed substantial frequency differences between the apple maggot populations attacking apple and hawthorn and snowberry maggots infesting snowberry; representative data are shown on **Table 4.** Generally speaking, common alleles in snowberry flies at each of the eight loci ranging in frequency from 0.25 to 0.5 were rare (~0.03) and in some cases (locus 29) may be absent from apple maggot populations. In addition, for the loci 27 and 29 we found high frequency alleles in apple maggot (~0.30) apparently not present in snowberry maggot. Additional genetic surveys from more sites are needed to confirm these findings, however, they suggest that it may be possible to score field collected flies for several microsatellite loci and statistically distinguish apple from snowberry flies with high accuracy.

Based on the encouraging genetic results, we concentrated our effort in the summer and fall of 2008 on collecting an extensive number of sympatric fly populations on the four major host plants throughout the states of Washington and Oregon. The major hub of collecting was centered in the Vancouver/Portland area where the apple maggot was believed to have initially been introduced in the 1970s. Extending from Vancouver/Portland, we have now collected a total of 16 sites with multiple host plants in three transects running north to Seattle, south to Eugene and east through the Columbia river gorge. These samples are currently being reared in the Washington State University Experimental facility in Vancouver. Our plan is to utilize a portion of the samples for genetic analysis, a portion for morphological analysis, and a portion for controlled rearing to assess the populations for possible host and regional differences in their adult eclosion times.

Significance to the Industry and Potential Economic Benefits

Methods that can be used to accurately distinguish apple and snowberry maggot flies are crucial to the apple industry because identifications are the basis for quarantine-related measures such as certification trapping (mass trapping around orchards) imposed by the WSDA, as well as insecticide spraying and tree removal conducted by county pest boards. These all incur costs, which are wasted if snowberry maggot flies are misidentified as apple maggot flies. Correct identification will determine where apple maggot flies are located, which will result in more effective control measures to prevent their spread into new regions and commercial orchards. Inaction because apple maggot flies are identified as a snowberry maggot flies could result in economic losses because there is no tolerance for any larvae in fruit and shipments from an infested orchard likely would be banned. In addition, in the future, export markets may require more rigid identification methods, in part for political gain, in addition to protection of the market's agricultural industry. These markets have access to older literature showing the difficulty in separating apple maggot and snowberry maggot flies, and to this point there is no way of identifying all flies with 100% accuracy. Preparation for this by generating rigid scientific data and identification methods now will be useful if this happens.

Identification of hawthorn host races may support arguments that certain populations of flies are low threats to the apple industry because these populations prefer hawthorns to apples.

Flies that cannot be easily distinguished can be sent to an agency such as WSDA that potentially will have a data base with shape data from known flies. Data from unknown flies can be entered into a computer program that will classify the fly. Processing flies and the use of the program can be conducted by competent personnel with minimal training and expense.

EXECUTIVE SUMMARY

The apple maggot fly, endemic to eastern North America, is now breeding in central Washington near major apple-growing areas. In 2008, flies were detected at new sites in Okanogan, Kittitas, Yakima, Franklin, Benton, and Walla Walla Counties. There is a zero tolerance for larvae of apple maggot in apples, and to prevent further spread of flies within apple-growing areas, local regulatory agencies rely on early detection and immediate control programs. Apple maggot flies caught near apple orchards pose a quarantine problem for apple export to California as well as virtually all of our export markets abroad. A major problem with detection programs, however, is that flies caught on traps cannot always be identified to species, so quarantines or control measures potentially may not be justified.

Apple maggot fly is almost indistinguishable from the snowberry maggot fly, a native species that attacks snowberry fruit and is caught on the same traps as are apple maggots, usually in much higher numbers. Morphological criteria used in the past to discriminate the two species are continuous and show overlap in both female and male flies. In particular, ovipositor length is used to separate females one from the other species, as apple maggots usually have longer ovipositors than snowberry maggots. Work funded by the WTFRC in 2007 showed that wing shape analysis is potentially an effective method for separating the two species independent of the other morphological data. A project was conducted in 2008 to (1) determine if wing shape is diagnostic of apple and snowberry maggot flies, with emphasis on increasing sample sizes for analysis, and to (1) determine if host races of apple maggot exist in Washington using microsatellite variation. Results showed that wing shape analysis identified female apple maggot flies with 97% accuracy, it misidentified 3% of apple maggot as snowberry maggots, and that it identified 97-100% of female snowberry maggot flies. Apple maggot flies are more likely to be misidentified as snowberry maggots than are snowberry maggot flies to be misidentified as apple maggot flies. Importantly, wing shape analysis correctly identified 100% of flies whose identities were questionable based on ovipositor lengths and therefore is a useful method for pest detection and management.

DNA analysis using microsatellite genetic markers suggest that significant differences may exist between flies reared from apple and hawthorns in Washington, as well as snowberries. We found that all of the 80 PCR primers developed to amplify microsatellite loci for eastern populations of apple maggot, also worked for western apple maggot flies from Washington state. A subset of eight loci has now been scored for flies collected from two sympatric apple, black hawthorn, ornamental hawthorn and snowberry sites in the Vancouver/Portland area. Six of the eight loci displayed significant allele frequency differences among the apple and the hawthorn populations. These data are consistent with the existence of host races in the state. Flies from the two sites did not show substantial genetic differences from flies from the eastern United States. None of the eight loci possessed a high frequency allele for the Washington fly populations at any of the eight microsatellites that was also not found in the East. This result implies that the eastern and western flies may be fairly closely genetically related, suggesting apple maggot fly in the West was introduced from the East. However, more genetic screening from additional loci is needed to confirm the preliminary data. We also found that all eight of the loci also displayed substantial frequency differences between the apple maggot flies infesting apple and hawthorn and snowberry flies infesting snowberry.

More studies are needed to complete the morphometric and DNA diagnostics work for apple and snowberry flies. A future direction is to eliminate even a 3% probability of misdiagnosis using wing shape, which can probably be achieved if shapes of other structures are included in the identification process. Ovipositor shape and male genitalia shape need to be examined in addition to wing shape. Using a combination of measures should enable us to positively identify virtually all problematic flies. The genetic survey of flies needs to be continued and completed to assess the potential genetic source (introduced vs. native) for apple and hawthorn flies in Washington. They will also substantiate whether a subset of microsatellites can diagnostically distinguish apple maggot from snowberry maggot.

FINAL REPORT **DURATION**: 2 years (2007-2008)

Project Title: Fate of codling moth in apples after harvest

PI: Lisa G. Neven
Organization: USDA-ARS
Telephone/email: (509) 454-6556
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State/Province/Zip WA, 98951

Cooperators: Michael Willett, NHC

Total funding request: $113,515

Other funding sources: None

Budget:

Year 1: $58,815 **Year 2:** $55,000 **Total Funding: $113,815**

Item	Year 1: 2007	Year 2: 2008
Salaries	$31,312	$32,250
Benefits	$9,393	$9,675
Wages		
Benefits		
Equipment		
Supplies	$18,110	$13,075
Travel		
Miscellaneous		
Total	$58,815	$55,000

Original Objectives:
The overall objective of this project is to develop information regarding the fate of codling moth in apples destined for export to Asian Pacific countries. The specific objectives were:

Objectives:
1) Determine the critical duration of chilling needed for diapause-destined larvae needed to break diapause.
2) Determine the fate of diapause-destined larvae under tropical environments (short photoperiod, elevated temperatures, high chilling temperatures, short chilling period).
3) Determine the proportion of field codling moth population entering diapause at each harvest date.
4) Determine the proportion of both field and laboratory codling moth diapause-destined larvae surviving cold storage.

Significant Findings:

1) With only one year of data, it is difficult to determine the critical chilling period needed to break diapause for each harvest date. It was observed that emergence took longer with shorter cold storage durations (Figures 1) and cool storage durations less than 1 month (Figure 2).
2) For the most part, larvae either died or remained in diapause when maintained on a 12:12 L:D photoperiod at 68F (Figure 3). Very few moths emerged under these conditions. We did notice a peak of emergence in the 5th harvest, most likely due to the presence of a 3rd generation of codling moth.
3) The proportion of larvae entering diapause for the first harvest was very low, and few survived cold and cool storage. Approximately half of the larvae in the second harvest (August 15) were diapause destined since only half survived cold storage for more than 2 months. By the 3rd harvest most of the larvae were diapause destined and easily survived cold storage for more than 2 months.
4) Although cold storage did kill a majority of the larvae in the first and second harvest within the first 8 weeks of storage, we still observed cocooning or moth emergence after more than 14 weeks of cold storage. After the 3rd harvest, cold storage had a less pronounced effect on mortality. Many of the moths emerged following more than 3 months of cold storage.

Results and Discussion:
We did notice trends in harvest date on diapause, survival after cold storage, and the effects of cool storage on moth emergence. As the number of days at 34°F storage increased there was a decrease in the span and duration of moth emergence (Figure 1). As the number of days at 50°F cool storage increased there was an overall decrease in the span and number of days to emergence up to 35 days of storage, after which, there was little change in number of days to emergence (Figure 2).

There was an overall decline in the number of cocooned larvae as harvest progressed through the season (Figure 3). For harvests 1-4 the number of dead and diapaused exceeded to total number of emerged moths (Figure 3). Harvest 5 had more moths emerged than died in the strips, but the number of moths was still less than the number of diapausing larvae (Figure 3). Harvest 6 was the only harvest in which the number of moths exceeded the number of dead or diapausing cocooned larvae (Figure 3). This was most likely due to a significant third flight on codling moths in September.

Harvest 1 (Figure 4) had only 2 moths emerge (sample numbers 10405 and 10409) which correlate to 6 wks @ 34°F and 4 or 8 wks @ 10°C, respectively. This represented a 0.67% emergence of the total number on insects in the harvest.

Harvest 2 (Figure 5) had a number of moths emerge over a span of cold and cool storage regimes. However, only 1 moth emerged for any given sample, and represented only 3.7% of the total number of insects in the harvest.

Harvest 3 (Figure 6) had single moths emerge from a range of cold and cool storage treatments, with emergence from samples held at 34°F for over 16 wk. Total moth emergence was only 8% of the total number of insects in the harvest.

Harvest 4 (Figure 7) had samples with multiple moths emerging from individual treatments, with as many as 4 in one sample (40110), which was not held at 34°F, but spent 9 wk at 50°F. Longer durations of cold storage resulted in less moths emerging from samples. The total number of moths emerging from this harvest was 13.8 of the total number of insects in the harvest.

Harvest 5 (Figure 8) had the highest number and proportion of moths emerging than any other harvest. There were numerous samples having more than 2 moths emerging with a ratio of moths emerging representing 33.3% of the total number of insects in the sample. Moths emerged from the longest duration of cold storage (16 wk) and the shortest duration of cool storage (0 wk).

Harvest 6 (Figure 9) had 52% of the total insects emerge as moths, with 4 samples having 2 moths and 1 sample with 3 moths.

Harvest 7 (Figure 10) had 60% of the total insects emerge as moths. However, there were very few insects in the total harvest (10 total).

These results indicate that a third flight of codling moth holds the greatest risk of codling moth surviving in the fruit than in cooler years when the 3rd flight is either curtailed or eliminated. Contrary to popular belief, larvae in early harvested apples, harvests between August and mid-September, do not hold a greater risk of codling moth survival under tropical conditions (12:12 L:D, 68°F).

Laboratory Colony Research:
We have completed infesting and treating the first series of laboratory colony studies. These conditions involved oviposition under 12:12 L:D, 68°F conditions. The samples are currently in the 6 month holding conditions to monitor for moth emergence (12:12 L:D, 68°F). We will begin the second series of oviposition at 9:15 L:D, 68°F in January 2009.

2008 Harvest Results:
We made 7 harvests of codling moth infested fruits in the Yakima and Douglas counties in the fall of 2008. To date, we have only had 1 moth emerge from any of the harvests. This moth was from harvest 4 and received only 5 wk at 50°F cool treatment before being placed in the emergence room (12:12 L:D, 68°F). The data from this harvest will not be complete until November 2009.

Figure 1. Effects of cold storage at 34°F on length to emergence of codling moth.

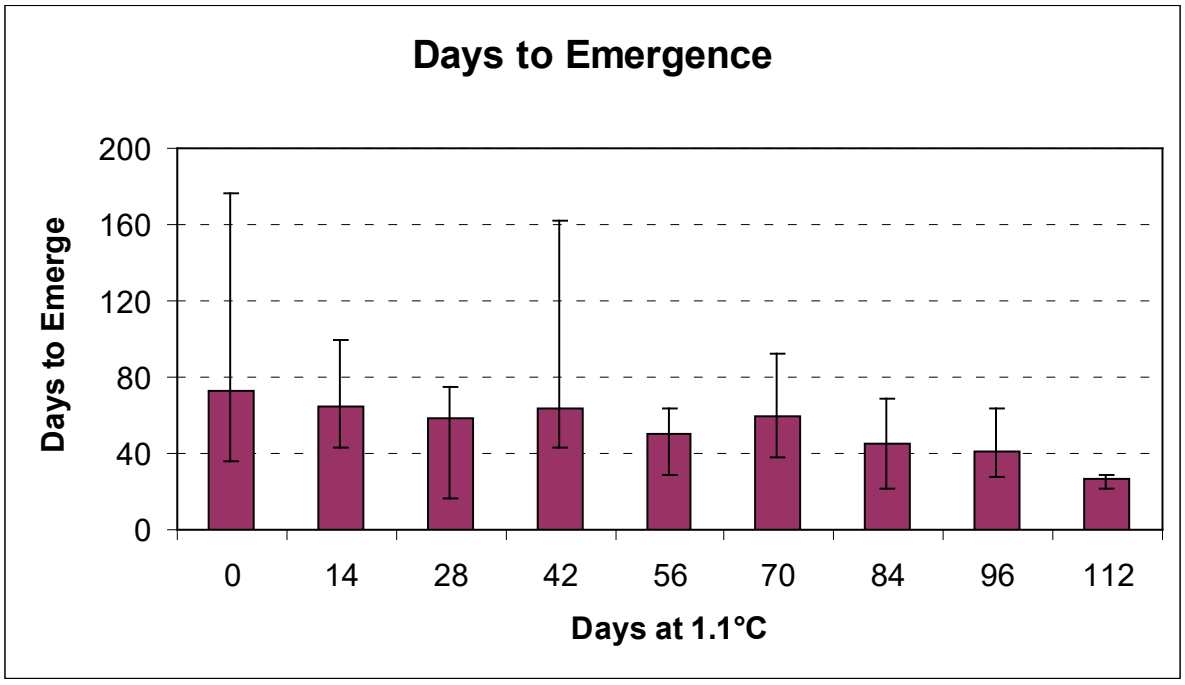

Figure 2. Effects of cool storage at 50°F on length of emergence of codling moth.

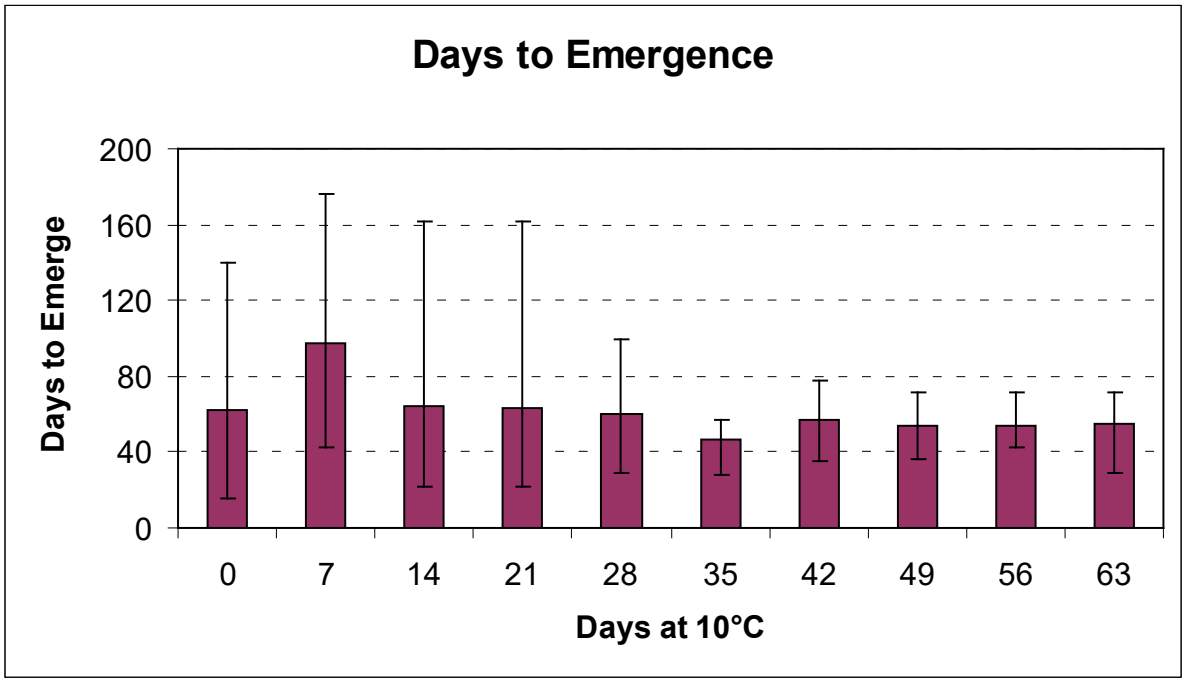

Figure 3. Total number of individual codling moths entering into cocoons (# spun) during weekly evaluations of strips. Total number moths emerging during weekly evaluatons (# Emer). Total number codling moths dead (# Dead) or still in diapause (# Diap) at the final 6 month evaluation.

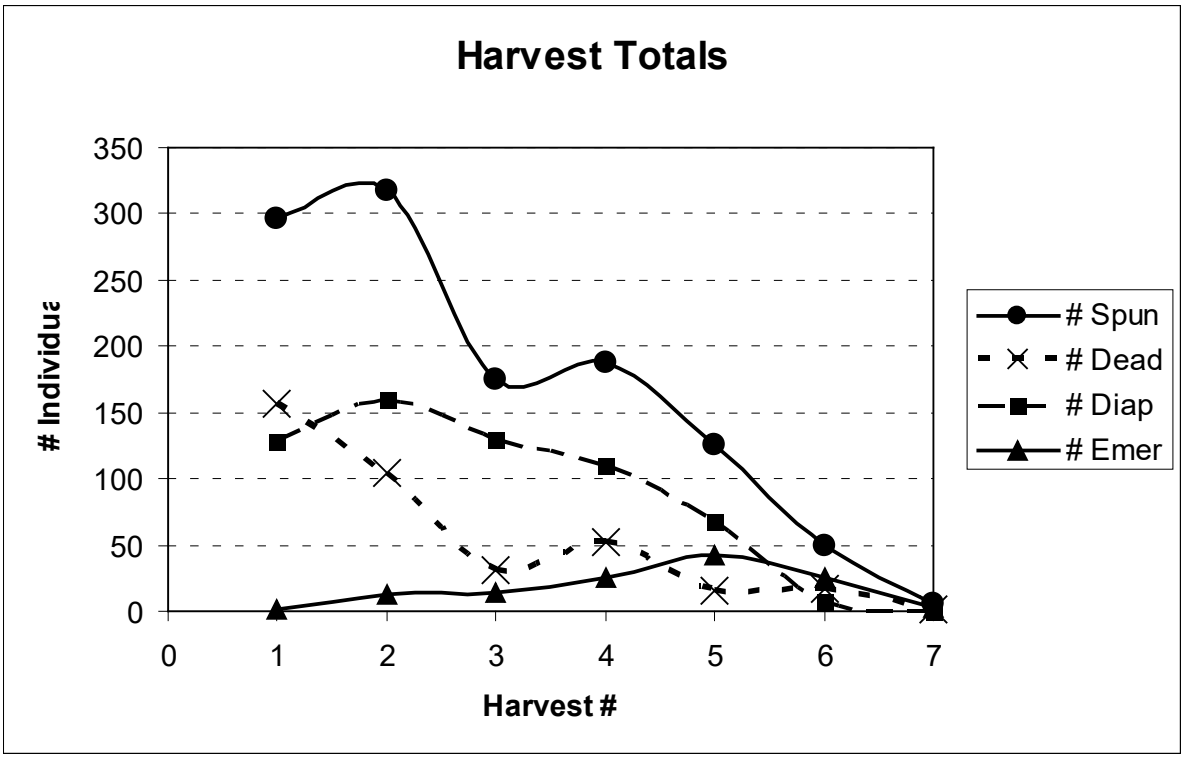

Table 1. A key to the samples numbers.

Number	Condition	Duration	# weeks
1st 2 digits	Harvest Number	1 through 7 at 2 wk intervals	
3rd digit	Weeks at 34F	1 through 9 at 2 wk intervals	0-16 wk
3rd & 4th digit	Weeks at 50F	1 through 10 at 1 wk intervals	0-9 wk

Figure 4. Results from harvest 1 (August 1, 2007).

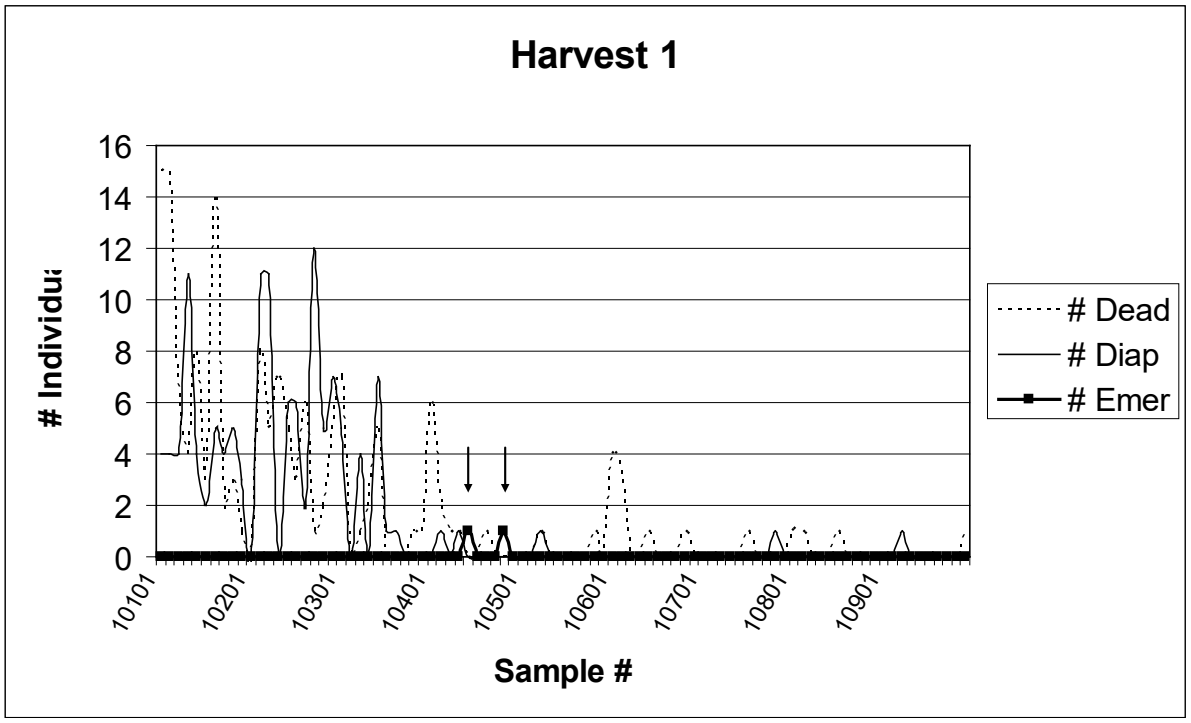

Figure 5. Results from Harvest 2 (August 15, 2007).

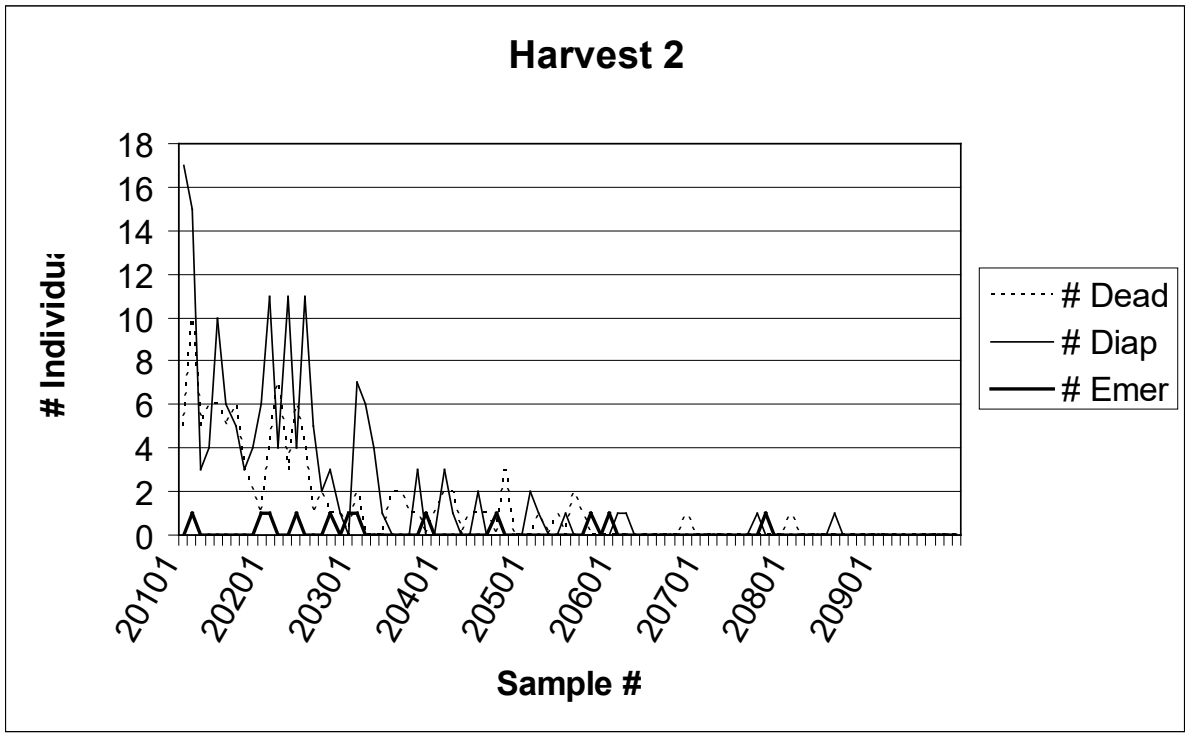

Figure 6. Results Harvest 3 (August 29, 2007).

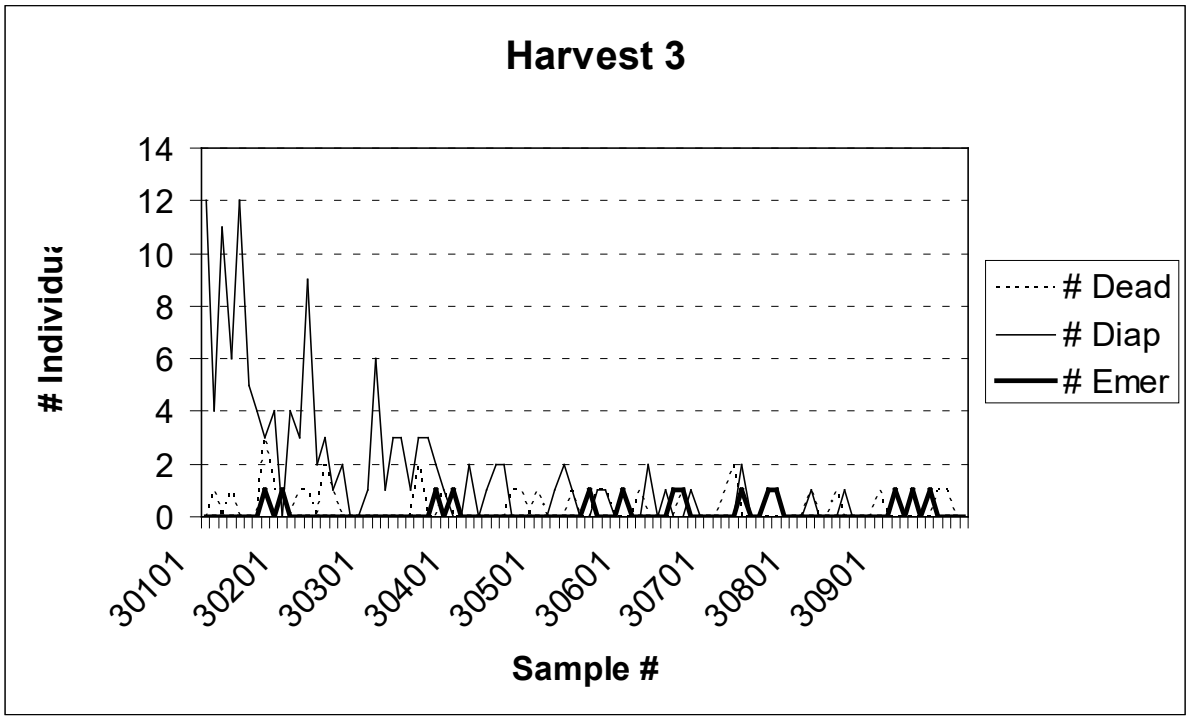

Figure 7. Results of Harvest 4 (September 12, 2007).

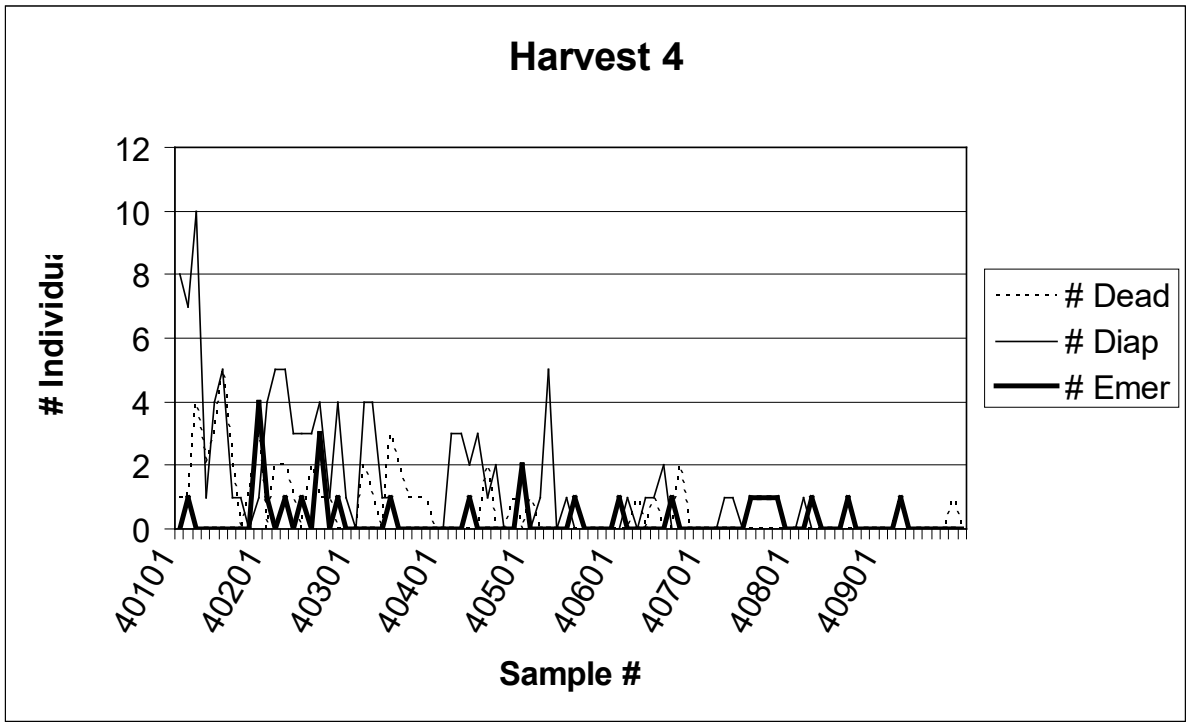

Figure 8. Results of Harvest 5 (September 26, 2007).

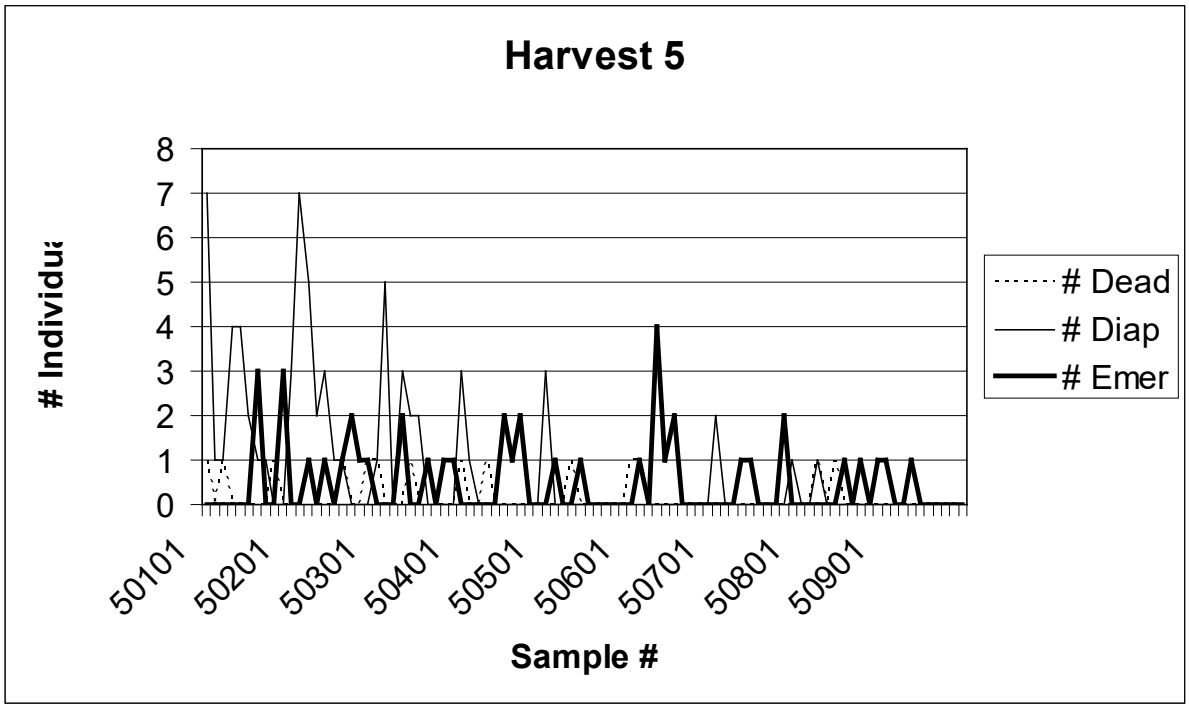

Figure 9. Results of Harvest 6 (October 10, 2007).

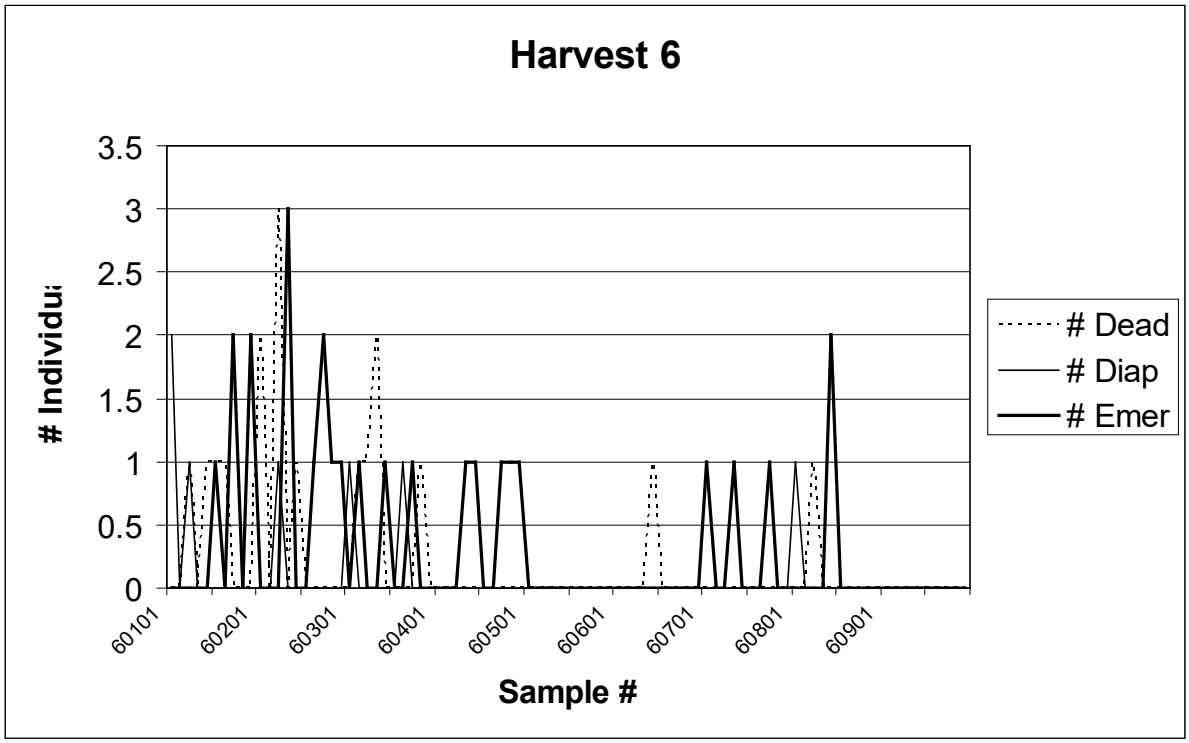

Figure 10. Results from Harvest 7 (October 24, 2007).

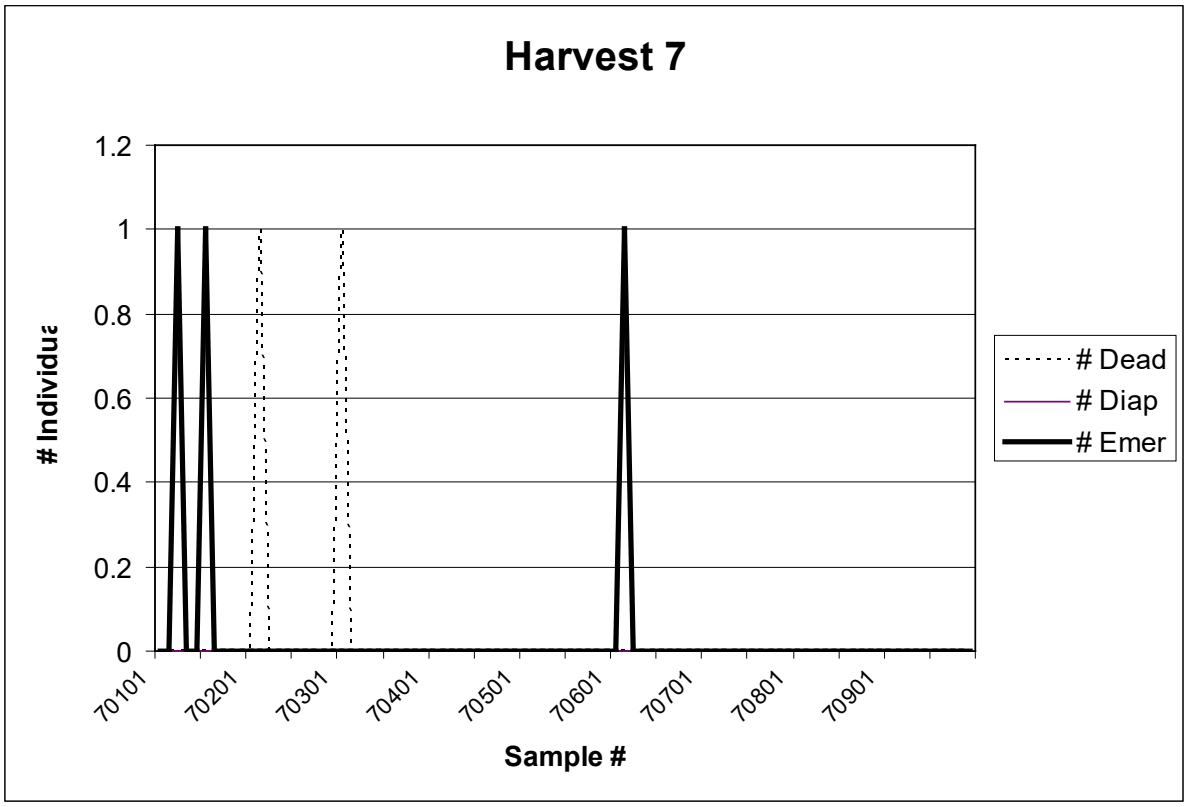

Table 2. Percentage of codling moth in diapause, dead or emerged from each harvest.

Harvest	Date	% Diapause	% Dead	% Emerged
1	August 1, 2007	43.4	52.9	0.06
2	August 15, 2007	50.8	32.8	3.8
3	August 29, 2007	74.3	17.7	8.0
4	September 12, 2008	58.5	27.7	13.8
5	September 26, 2007	54.0	12.7	33.3
6	October 10, 2007	14.0	34.0	52.0
7*	October 24, 2007	0.0	40.0	60.0

* Only a total of 5 codling moth in harvest.

Executive Summary

Project Title: Fate of codling moth in apples after harvest

Codling moth in apples destined for foreign ports in tropical climates poses a threat to maintaining these export markets. The primary threat being that we do not have accurate numbers on the probability of these moths to survive commercial handling procedures and then to survive to emergence as moths under tropical conditions. Data from one year of the study indicates that most of the larvae either die or remain in diapause when held at tropical conditions (12:12 L:D, 68°F). When chilling temperatures are added, it does reduce the time for moth emergence up to 1 month of temperatures at 50°F, but make little difference thereafter.

USDA-APHIS-PERL used emergence durations of 6 weeks. Our data indicate that it can take nearly 180 days for moth to emerge from diapause if they do not receive any cold or chilling treatments. This may greatly affect how the risk model is calculated. In addition, we have estimates of the percentages of larvae in the fruit entering diapause, completing diapause, and emerging as moths for one season. The data from Harvest 7 appears to be quite damaging to our case, but it is based on only 5 larvae in the total harvest. Only 3 moths emerged from the whole harvest.

It is apparent that additional data are needed to accurately determine the risk of codling moth in apples destined to tropical countries. It would be erroneous to base an entire export program on only 1 or 2 years of data where the occurrence of the 3rd flight may completely change the risk calculations.

CONTINUING PROJECT REPORT **YEAR:** 1 of 2

Project Title: Control of postharvest fruit rots in apple

PI:	Chang-Lin Xiao	**Co-PI (2):**	Bruce Campbell
Organization:	WSU-TFREC, Wenatchee	**Organization:**	USDA ARS, Albany, CA
Telephone:	509-663-8181 X229	**Telephone:**	510-559-5846
Email:	clxiao@wsu.edu	**Email:**	bcc@pw.usda.gov
Address:	1100 N Western Ave	**Address:**	800 Buchanan St.
City:	Wenatchee	**City:**	Albany
State/Zip:	WA 98801	**State/Zip:**	CA 94710

Cooperators: Selected packinghouses across central Washington State

Total Project Request: **Year 1:** $89,289 **Year 2:** $93,406

Other funding Sources: none

WTFRC Collaborative expenses:

Item	2008	2009
Stemilt RCA room rental	6,368.42	**6,368.42**
Crew labor	0	**0**
Shipping	0	**0**
Supplies	0	**0**
Travel	0	**0**
Total	6,368.42	**6,368.42**

Footnotes: The estimate of the RCA room rental cost was based on a projection of 40-bin space needed for this research.

Budget 1:
Organization: WA State University-TFREC **Contract Administrator:** ML Bricker; Kevin Larson
Telephone: 509-335-7667; 509-663-8181 x221 **Email:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2008	2009
Salaries[1]	54,331	**56,001**
Benefits	21,173	**23,505**
Wages (time slip)	5,000	**5,000**
Benefits	785	**900**
Equipment	0	**0**
Supplies[2]	6,000	**6,000**
Travel[3]	2,000	**2,000**
Miscellaneous	0	**0**
Total	89,289	**93,406**

Footnotes:

[1] Salaries are for Yong-Ki Kim (1.0 FTE postdoctoral research associate) at 44% benefit rate, and Robin Boal (Scientific Assistant; 0.35 FTE working on this project, partially state-supported tech position at 38% benefit rate).

[2] Supplies include:
(a) $4,000 for cost of fruit purchased from commercial orchards or packers for trials.
(b) $2,000 for lab supplies for fungicide-resistance related experiments (isolation media, chemicals, petri dish plates for isolation of fungi and fungicide sensitivity tests, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates)

[3] Travel to orchards and packinghouses across the state is required for sampling. We will be using a leased vehicle.

Objectives:

1. Develop preharvest fungicides and postharvest fungicides integrated programs for decay control.
2. Develop preharvest fungicides and postharvest biocontrol integrated programs for decay control.
3. Develop pre- and post-storage integrated programs for decay control.
4. Determine patterns of sensitivity or resistance of fludioxonil- and pyrimethanil-resistant *Penicillium expansum* and *Botrytis cinerea* to various pre- and postharvest fungicides and use the information for guiding fungicide use.
5. Establish an industry-coordinated program to monitor the shift in sensitivity of *P. expansum* to fludioxonil and pyrimethanil.
6. Collaborate with Bruce Campbell in evaluating natural compounds for management of fungicide resistance and decay control.

Significant findings:

- The pyrimethanil-resistant mutants of *Penicillium expansum* also were resistant to cyprodinil (Vangard), indicating the existence of cross resistance between pyrimethanil and cyprodinil. All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure), a DMI fungicide, indicating that use of DMIs in the orchard likely will not increase the populations resistant to fludioxonil or pyrimethanil.
- Fludioxonil-resistant and pyrimethanil-resistant mutants of *P. expansum* and their parental wild-type isolates were sensitive to pyraclostrobin (a strobilurin fungicide). However, pyrimethanil-resistant mutants became resistant to boscalid and Pristine (a mixture of pyraclostrobin and boscalid). The results indicate that if resistance to pyrimethanil develops in *P. expansum* populations, the resistance can extend to boscalid and Pristine, compromising the effectiveness of preharvest use of Pristine for blue mold control.
- Isolates of *Penicillium expansum* collected from decayed apple fruit that were drenched with pyrimethanil (Penbotec) were tested to monitor whether or not the sensitivity to this fungicide has changed since the introduction of this fungicide in 2004. The EC50 values of pyrimethanil for the isolates collected in 2008 were compared to the baseline EC50 values based on the isolates collected in 2005. Our results indicated that the sensitivity of these isolates to pyrimethanil and fludioxonil remained at similar levels.
- Experiments for objectives 1, 2, 3, and 6 have been set up on the 2008 crops in the fall of 2008. At the time of writing this report, these experiments are still in progress. The fruit are still in cold storage for either future treatments or decay development. Most experiments will have results in spring 2009.

Methods:

Various pre- and postharvest integrated programs were evaluated for control of storage diseases before packing as well as decay after packing. Red Delicious and Fuji were used for the tests. Pristine was applied one week before harvest. Fruit are currently stored in CA. In spring 2009, part of the fruit will be removed from CA at 5 and 7 months after harvest. Fruit will be inoculated with *P. expansum*. For each preharvest fungicide treatment, part of inoculated fruit will be treated with each of the three postharvest fungicides (TBZ, Penbotec and Scholar) or the biocontrol agent BioSave after inoculation. Nontreated fruit will be used as controls. This experiment is to determine residual activity of preharvest fungicides alone or in combination with postharvest fungicides and biocontrol agents in controlling blue mold infection at packing. All fruit will then be stored in cold storage for 4-8 weeks and then for 7 days at 68°F, at which time decay development will be evaluated.

Experiments were conducted to evaluate various postharvest fungicide drench treatments in combination with online treatments at packing for control of decay. This experiment is to simulate commercial operations in which fruit are treated with fungicides (for example, drench) prior to storage and then treated again on the packing line at packing. Commercially harvested fruit without use of preharvest fungicides were used for this study. Fruit were either not drenched or drenched with one of the apple-postharvest fungicides. Fruit are currently stored in CA. In the spring of 2009, part of the fruit will be removed from CA at 5 and 7 months after drenching. Fruit will be inoculated with *P. expansum*. For each fungicide-drench treatment, part of inoculated fruit will be treated with either the biocontrol agent BioSave or one of the two other postharvest fungicides after inoculation. Nontreated fruit will be used as controls. This experiment is to determine residual activity of pre-storage fungicide-drench treatments alone or in combination with on-line fungicide or biocontrol treatments in controlling blue mold from infection of wounds that occur at packing. All fruit will then be stored in cold storage for 8 weeks and then for 7 days at 68ºF at which time decay development will be evaluated.

Fungicide resistance patterns in pyrimethanil-resistant mutants, particularly cross resistance and multi-drug resistance to commonly used pre- and postharvest fungicides, were determined.

A program to monitor the potential shift in sensitivity of *Penicillium expansum* to Scholar and Penbotec was established in this study. Blue mold-decayed fruit were collected from grower lots that had been treated with Penbotec or Scholar from packinghouses and isolations of *P. expansum* from decayed fruit were attempted. Isolates of *P. expansum* were tested for sensitivity to fludioxonil and pyrimethanil and EC50 of the fungicides against selected isolates was determined and compared with the baseline sensitivity data.

Identified natural compounds from Bruce Campbell's lab were tested for activity against fungicide-resistant strains of *P. expansum* in an agar medium and apple fruit.

Results & Discussion:

Patterns of cross resistance or multi-drug resistance in pyrimethanil-resistant and fludioxonil-resistant mutants of Penicillium expansum and Botrytis cinerea.

All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure), a DMI fungicide (Table 1), indicating that the use of DMIs in the orchard likely will not increase the populations resistant to fludioxonil or pyrimethanil. Sensitivity of wild-type isolates and fungicide-resistant mutants to thiophanate-methyl (Topsin M) exhibited the same pattern of sensitivity to TBZ, indicating cross resistance between TBZ and Topsin M in fungicide-resistant mutants, including multi-drug resistance phenotypes. The four pyrimethanil-resistant mutants also were resistant to cyprodinil (Vangard), indicating the existence of cross resistance between pyrimethanil and cyprodinil (Table 1).

Fludioxonil-resistant and pyrimethanil-resistant mutants and their parental wild-type isolates of *P. expansum* were sensitive to pyraclostrobin (a strobilurin fungicide) (Table 2). Based on both mycelial growth and spore germination assays, pyrimethanil-resistant mutants became resistant to boscalid and Pristine (a mixture of pyraclostrobin and boscalid). The results indicate that if resistance to pyrimethanil develops in *P. expansum* populations, the resistance can extend to boscalid and Pristine, compromising the effectiveness of preharvest use of Pristine for blue mold control.

We also generated 18 fludioxonil-resistant mutants and more than 40 pyrimethanil-resistant mutants of *Botrytis cinerea*. Selected mutants are being used for further tests for patterns of resistance to other fungicides commonly used in apple orchards.

Table 1. In vitro sensitivity of mycelial growth of fludioxonil- and pyrimethanil-resistant mutants and their parental wild-type isolates of *Penicillium expansum* to triflumizole, thiophanate-methyl and cyprodinil.

Isolate	Phenotype	EC_{50} (mg/L) and phenotype Triflumizole (DMI)		thiophanate-methyl (benzimidazole)		Cyprodinil (anilinopyrimidine)	
3354	TBZ^{S} Flu^{S} Pyr^{S}	0.121	S	3.211	S	0.353	S
3294	TBZ^{HR} Flu^{S} Pyr^{S}	0.175	S	> 200	HR	0.371	S
4277	TBZ^{S} Flu^{HR} Pyr^{S}	0.139	S	1.820	S	0.362	S
4284	TBZ^{S} Flu^{HR} Pyr^{S}	0.143	S	4.033	S	0.276	S
4262	TBZ^{HR} Flu^{HR} Pyr^{S}	0.238	S	> 200	HR	0.187	S
4272	TBZ^{HR} Flu^{HR} Pyr^{S}	0.252	S	> 200	HR	0.139	S
4256	TBZ^{LR} Flu^{LR} Pyr^{R}	0.290	S	18.990	LR	9.280	R
4258	TBZ^{LR} Flu^{LR} Pyr^{R}	0.330	S	16.940	LR	22.269	R
4252	TBZ^{HR} Flu^{LR} Pyr^{R}	0.290	S	> 200	HR	6.814	R
4253	TBZ^{HR} Flu^{LR} Pyr^{R}	0.293	S	> 200	HR	7.450	R

TBZ = thiabendazole, Flu = fludioxonil, Pyr = pyrimethanil, S = sensitive, R = resistant, LR = lowly resistant, HR = highly resistant.

Table 2. In vitro sensitivity of fludioxonil- and pyrimethanil-resistant mutants and their parental wild-type isolates of *Penicillium expansum* to pyraclostrobin, boscalid and Pristine®

Isolate	Phenotype	EC_{50} (mg/L) and phenotype Pyraclostrobin (QoI)		Boscalid (carboxamide) mycelial growth		germination		Pristine	
3354	TBZ^{S} Flu^{S} Pyr^{S}	0.235	S	0.500	S	0.214	S	0.066	S
3294	TBZ^{HR} Flu^{S} Pyr^{S}	0.087	S	1.096	S	0.105	S	0.058	S
4277	TBZ^{S} Flu^{HR} Pyr^{S}	0.125	S	1.028	S	0.508	S	0.061	S
4284	TBZ^{S} Flu^{HR} Pyr^{S}	0.127	S	1.330	S	0.266	S	0.054	S
4262	TBZ^{HR} Flu^{HR} Pyr^{S}	0.184	S	7.314	R	0.101	S	0.044	S
4272	TBZ^{HR} Flu^{HR} Pyr^{S}	0.260	S	4.864	R	0.202	S	0.058	S
4256	TBZ^{LR} Flu^{LR} Pyr^{R}	0.254	S	4.004	R	1.607	R	0.353	R
4258	TBZ^{LR} Flu^{LR} Pyr^{R}	0.344	S	8.295	R	2.439	R	0.757	R
4252	TBZ^{HR} Flu^{LR} Pyr^{R}	0.212	S	5.039	R	1.394	R	0.319	R
4253	TBZ^{HR} Flu^{LR} Pyr^{R}	0.284	S	6.323	R	1.679	R	0.331	R

TBZ = thiabendazole, Flu = fludioxonil, Pyr = pyrimethanil, S = sensitive, R = resistant, LR = lowly resistant, HR = highly resistant.

Monitoring the shift in sensitivity of P. expansum to fludioxonil and pyrimethanil.

Decayed apple fruit were sampled from grower lots that had been drenched with Penbotec. Of the 19 isolates collected and identified, 17 were identified as *P. expansum*, and all but one of these isolates were identified as TBZ resistant.

The EC50 values of pyrimethanil for the isolates collected in 2008 ranged from 0.898 to 1.529 mg/L, with an average of 1.233 mg/L (Fig. 1). The baseline EC50 values of pyrimethanil based on the 120 isolates collected in 2005 ranged from 0.519 to 2.054, with a mean of 1.340 mg/L. The results indicated that the sensitivity of these isolates to pyrimethanil remained at a similar level as the baseline population.

For the 17 isolates tested in 2008, the EC50 values of fludioxonil for fungal mycelial growth ranged from 0.015 to 0.025 mg/L with an average of 0.021 mg/L (Fig. 1). In comparison, the baseline EC50 values of fludioxonil based on the 120 isolates collected in 2005 ranged from 0.011 to 0.068 with an average of 0.020 mg/L. It appears that the sensitivity of these isolates to fludioxonil remained at a similar level as the baseline population.

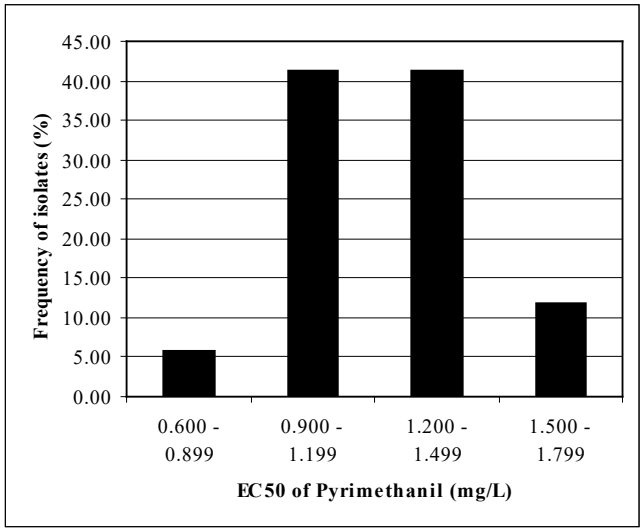

Fig. 1. Distribution of EC50 values of pyrimethanil for *Penicillium expansum* isolates collected in 2008.

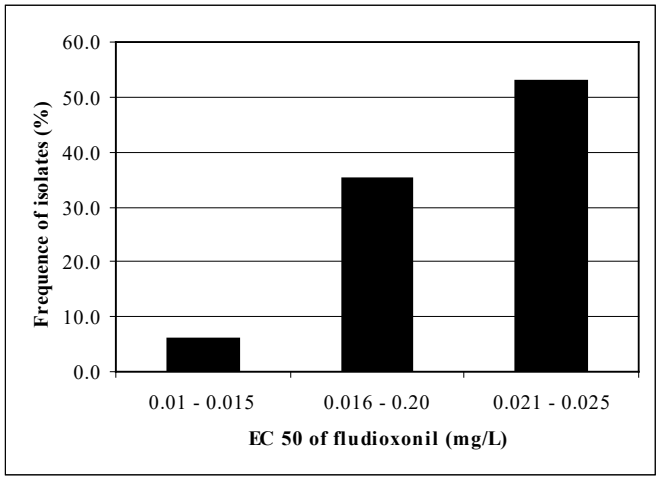

Fig. 2. Distribution of EC50 values of fludioxonil for *Penicillium expansum* isolates collected in 2008.

Preharvest Pristine in combination with postharvest biocontrol agent or fungicide for blue mold control.

Pristine was applied to Fuji apples 7 days before harvest. Fruit are currently stored in CA. Fruit will be removed from CA 5 months after harvest, washed and brushed through a research packing line, wounded and inoculated with *P. expansum*, and treated with either BioSave or a fungicide. Inoculated fruit will be stored at 32°F in air for 8 weeks and one additional week at room temperature. Decay will be assessed after cold storage as well as after one additional week at room temperature.

The experiment ends in the spring of 2009. Results will be forthcoming.

Pre-storage fungicide drench in combination with postharvest biocontrol agent or fungicide for blue mold control.

Two trials (one on Fuji and one on Red Delicious) were set up in 2008. Fruit from commercial orchards were drenched with either Scholar or Penbotec and are currently stored in CA. The fruit will be removed from CA 5 and 7 months after harvest, washed and brushed through a research packing line, wounded and inoculated with *P. expansum*, and treated with either BioSave or a fungicide. Decay will be evaluated after a certain period in storage.

These trials end in the late spring of 2009. Results will be forthcoming.

Residual effects of Scholar and Penbotec on control of blue mold in Fuji apple fruit.

In a previous study, we documented that residues on/in Red Delicious apple fruit that were drenched with Scholar or Penbotec prior to storage remained stable in storage conditions and can largely protect the fruit from infection by blue mold. In this study, we evaluated whether the residual activity also occurs on Fuji apple fruit. We set up an experiment on the 2008 crops of Fuji. The experiment will end in the late spring of 2009. Results will be forthcoming.

Evaluate natural compounds for controlling fludioxonil-resistant Penicillium expansum.

This research was done in collaboration with Bruce Campbell. In our previous study, we evaluated 2,5-DHBA 18 mM and 2,5-Dbald 1 mM as a chemosensitizing agent to overcome fludioxonil resistance of *Penicillium expansum* on apple fruit. We found that when used in combination with fludioxonil, these two compounds did not improve control of blue mold caused by a fludioxonil-resistant strain. To further explore the potential of using natural compounds to overcome fludioxonil resistance, Campbell lab did additional lab tests and found that octylgallate could be a promising chemosensitizing agent to overcome fludioxonil resistance in *P. expansum*. On the 2008 crop, we set up a trial to evaluate octylgallate in combination with Scholar (fludioxonil) for control of blue mold caused by a fludioxonil-resistant strain. The fruit are currently in storage for decay development. Results will be forthcoming.

CONTINUING PROJECT REPORT **YEAR**: 2008 (2 of 3)
WTFRC Project Number: CP-07-701

Project Title: Augmenting fungal control in apples with natural compounds

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Total project funding request: **Year 1:** $32,421 **Year 2:** $33,555 **Year 3:** $34,730

Other funding Sources

Agency Name: None
Amount requested/awarded:
Notes:

Budget 1
Organization Name: WRRC, USDA-ARS **Contract Administrator:** Gwyn Watson
Telephone: 510.559.6029 **Email address:** gwyn.watson@ars.usda.gov

Item	2008	2009	
Salaries	$21,880	$23,924	
Benefits			
Wages			
Benefits			
Equipment	$4,491	$4,449	
Supplies	$5,987	$6,357	
Travel	$1,197		
Miscellaneous			
Total	$33,555	**$34,730**	

Footnotes:

OBJECTIVES: The goal of the research outlined in this proposal is to find new, safe natural compounds that effectively improve activity of conventional fungicides for pre/post-harvest treatment of apple. We are trying to identify molecular targets of these compounds using genomic tools and determine effective methods for delivery of discovered compounds. This research will greatly improve the ability to suppress decay of apples, a priority identified by the WTFRC. Our specific objectives are:

1. Augment antifungal activity of natural compounds based on structure-activity relationships of analogs of identified antifungal natural compounds (Year 1 and 2).

2. Identify the most efficient molecular targets [*e.g.*, mitochondrial superoxide dismutase (Mn-SOD)] for newly discovered compounds using functional genomics approaches (Year 1 and 2).

3. Determine an effective method for delivery of newly discovered natural compounds, leading to a target-specific strategy for a safe and economic approach to fungal pathogen control in the field or during processing and storage (Year 3).

SIGNIFICANT FINDINGS (Year 2):

- We investigated the mechanism of two fludioxonil resistant strains of *Penicillium expansum*, *i.e.*, FR2 and FR3, provided by Dr. Xiao. Our study indicates that fludioxonil resistance of the FR2 and FR3 strains is a result of UV-induced mutation of cellular redox homeostasis.
- Increased sensitivity of FR3 to oxidizing agents, compared to its parental strain (W2), indicated the oxidative stress response system of FR3, such as the mitogen-activated protein kinase (MAPK) pathway, had become defective as a result of UV-treatment.
- Alternatively, the other resistant strain, FR2, showed higher tolerance to oxidizing agents compared to its parental strain (W1), suggesting fludioxonil resistance of FR2 was based upon gain-of-function, possibly increased antioxidation activity.
- To overcome the resistance in both strains, redox-active natural phenolics were successfully used as chemosensitizing agents that targeted various elements of the oxidative stress-response pathway. Co-application of certain natural phenolic compounds with fludioxonil overcame fludioxonil-resistance in two mutant strains, FR2 and FR3, of *P. expansum*.
- Natural phenolics were also found that served as chemosensitizing agents for overcoming resistance to strobilurin.
- In conclusion, our data proved the effectiveness of targeting cellular oxidative stress response systems for control of fungi. Certain natural compounds are effective synergists to commercial fungicides and can be used for improving control of apple pathogens. Use of such compounds for fungal control reduces environmental and health risks associated with commercial fungicides, lowers costs and reduces development of resistance to fungicides.

METHODS

Microorganisms. *Penicillium expansum* FR2 and FR3, fludioxonil resistant mutants (Li and Xiao, 2008) and their parental strains, W1 and W2, respectively, were grown at 28°C (82.4°F) on potato dextrose agar (PDA). *Aspergillus fumigatus* AF293, wild type, and *A. fumigatus* MAPK deletion mutants *sakA*Δ and *mpkC*Δ (Xue *et al*. 2004; Reyes *et al*. 2006) were grown at 37°C (98.6°F) on PDA.

***Chemicals*.** Test chemosensitizing agents: thymol [5-methyl-2-(isopropyl)phenol], 2,3-dihydroxybenzaldehyde, gallic acid and ester analogs (methyl-, ethyl- and octyl- gallates); antifungal agents: fludioxonil, kresoxim-methyl, antimycin A; and oxidizing agents: menadione, hydrogen

peroxide (H_2O_2), diamide, were purchased from Sigma Co. (St. Louis, MO). Each compound was dissolved in dimethylsulfoxide (DMSO; absolute DMSO amount: < 2% in media) except H_2O_2 and diamide, which were dissolved in water, for incorporation into media.

***Antifungal bioassays*.** Sensitivities of filamentous fungi to the compounds were based on percent radial growth of treated (T) compared to control (C), receiving only DMSO, colonies and/or based on the Vincent equation [% inhibition of growth = 100 (C-T)/C, C: diameter of fungi on control plate; T: diameter of fungi on the test plate] (Vincent 1947), if necessary. Fungi (5 x 10^3 spores) were diluted in phosphate buffered saline and spotted on the center of PDA plates (triplicates) with or without antifungal compounds. Growth was observed for 3 to 7 days. Effectiveness of chemosensitization by thymol (0.2 to 0.6 mM), octylgallate (0.05 to 0.2 mM) or 2,3-dihydroxybenzaldehyde (2,3-D; 0.05 to 0.3 mM) was assessed by incorporating each compound into growth media with fludioxonil or strobilurin (kresoxim-methyl; 0.02, 0.04, 0.06 mM). Radial growth was recorded as described above. Oxidizing agents, menadione (0.001 to 0.512 mM), hydrogen peroxide (0.5 to 5 mM) or diamide (0.5 to 5 mM) were incorporated into media at respective levels and fungal sensitivities were measured by fungal radial growth, as described above. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of a compound where no fungal growth was observed.

RESULTS AND DISCUSSION

Characteristics of* P. expansum *fludioxonil resistant mutants. UV-induced generation of *P. expansum* FR2 and FR3, fludioxonil-resistant mutants, was previously reported elsewhere (Li and Xiao, 2008). Further characterization of these mutants performed in this study is summarized as follows:

(A) Reduced growth rate of FR2 and FR3 on normal medium: FR2 and FR3 mutants showed reduced radial growth on PDA (w/o fludioxonil) compared to their respective parental strains, *i.e*., W1 and W2, respectively (FR2: 11-17% reduction; FR3: 25-30% reduction). Results indicated that FR2 and FR3 possessed inherently lower hyphal-growth activity.

(B) Fludioxonil resistance of FR3: When grown on fludioxonil-containing medium, FR3 showed higher resistance to fludioxonil than W2. FR3 showed only a 3-14% growth reduction as compared to a 17-100% growth reduction of W2 on media containing 0.02-1.0 mM fludioxonil compared to W2 controls. Interestingly, the growth rate of FR3 exposed to fludioxonil always exceeded that of FR3 controls [*i.e.*, 19-35% higher radial growth with fludioxonil (0.02-1 mM) than without fludioxonil].

(C) Fludioxonil resistance of FR2: FR2 also showed higher fludioxonil resistance than W1, with FR2 showing a 21-38% growth reduction compared to a 66-100% reduction of W1 under fludioxonil treatments (0.02-1 mM) compared to growth of W1 controls. However, unlike FR3, the growth rate of FR2 exposed to fludioxonil was always lower than that of untreated FR2 [4-25% lower growth exposed to fludioxonil (0.02-1 mM) compared to control FR2]. Sporulation of fludioxonil treated FR2 was very poor, whereas that of fludioxonil treated FR3 was normal. Hence, though both strains exhibited resistance to fludioxonil, different mechanisms were probably involved between the strains.

Differential responses of* P. expansum *mutants to oxidizing agents. We hypothesized that the mechanism(s) of fludioxonil resistance in *P. expansum* FR2/FR3 mutants resulted from a mutation in the HK-MAPK signaling system. Some fungi having mutations in certain MAPK genes can escape toxicity of fludioxonil. We observed in FR2/FR3 the characteristics of fungi having a mutation in the HK signaling system, showing reduced hyphal growth while resistant to fludioxonil (Hagiwara *et al*. 2007). Since the HK-MAPK pathway is the key signaling system for fungal defense against environmental stresses, such as oxidative stress, we reasoned that the FR2/FR3 mutants should be hypersensitive to exogenously applied oxidizing agents.

Menadione, a redox cycling quinone, is a source of toxic superoxide radicals (Castro *et al*. 2007; Fernandes *et al*. 2007). FR3 was approximately twice as sensitive to menadione than the parental W2 strain (MICs for menadione: 0.256 mM < W2 < 0.512 mM *vs.* 0.128 mM < FR3 < 0.256 mM; **Fig. 1**).

FR3 was also more sensitive to other oxidizing agents, H_2O_2 (3 mM) or diamide (3.5 mM; thiol-oxidizing agent) than W2 (**Fig. 1**).

Interestingly, the response of the FR2 mutant to the oxidizing agents was directly opposite to that of the FR3 mutant. Unlike FR3, FR2 was almost twice as tolerant to menadione than its parental strain, W1 (MICs for menadione: 0.128 mM < W1 < 0.256 mM *vs.* 0.256 mM < FR2 < 0.512 mM; **Fig. 1**) and was also relatively tolerant to H_2O_2 (at 4 mM), where W1 showed no growth (**Fig. 1a**), or diamide (at 2.5 mM) (FR2: 29% less growth than FR2 w/o diamide; W1: 42% less growth than W1 w/o diamide; **Fig. 1**).

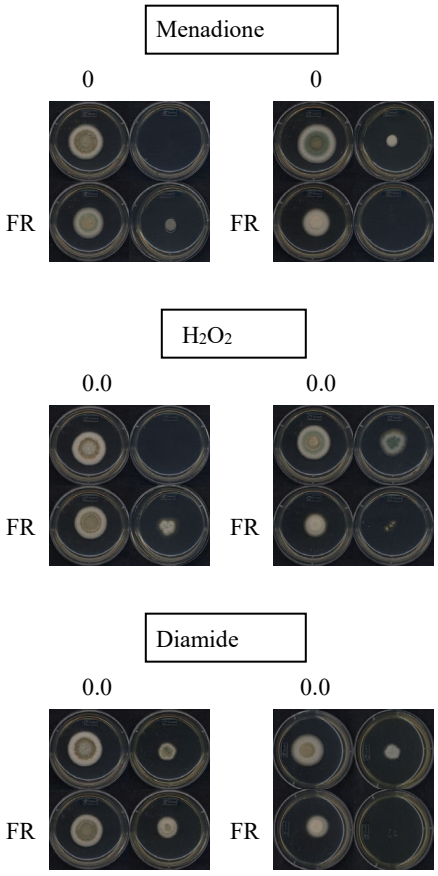

Figure 1. Differential responses of *Penicillium expansum* parental strains, W1 and W2, and respective fludioxonil-resistant mutants, FR2 and FR3, to oxidizing agents, menadione, hydrogen peroxide (H_2O_2), or diamide.

The hypersensitivity of the FR3 mutant to oxidizing agents, in addition to characteristic reduced hyphal-growth, indicates the HK-MAPK oxidative stress response pathway was defective in this strain. Since fungicidal activity of fludioxonil is exerted through a normal/functional MAPK system, mutation of the HK-MAPK pathway may explain why the FR3 strain was able to escape the mode of action of fludioxonil (Kojima *et al*. 2004).

On the other hand, FR2 appeared to represent a gain-of-function phenotype. This strain showed increased tolerance to the oxidizing agents. This increased tolerance suggests an increased antioxidative capacity that possibly could ameliorate cellular redox fluctuations under fludioxonil treatment. However, the energy expenditure for heightened antioxidative activity, under conditions without stress, may explain the lower hyphal growth of FR2. We previously observed that overexpression of the antioxidation gene *sodA*, encoding mitochondrial superoxide dismutase (Mn-

SOD) of *A. flavus*, in the *S. cerevisiae* wild type strain resulted in reduced growth on normal growth medium (*i.e*., w/o oxidative stress) (Kim *et al*. 2006). Collectively, our results indicate that disruption of normal cellular redox homeostasis, either through up- or down-regulation of antioxidation activity, can be at least one mechanism of fludioxonil resistance in *P. expansum*.

Chemosensitization of fludioxonil resistant strains using redox-active natural compounds. Chemosensitization using redox-active natural compounds, such as thymol or 2,3-dihydroxybenzaldehyde (2,3-D), was found to be effective in overcoming fungal resistance to conventional antifungal drugs (Kim *et al*. 2008a,b). In that study, fungi having a mutation in their oxidative stress response system (*e.g*., MAPK gene deletion) were more sensitive to thymol or 2,3-D than nonmutant strains (Kim *et al*. 2008a,b). We reasoned that we could further disrupt cellular redox-homeostasis of the FR2/FR3 strains using redox-active compounds. Chemosensitization could produce an additional stress on these strains to render them vulnerable to fludioxonil. The redox-active compounds could impair cellular components in addition to depleting activity of antioxidant enzymes, such as Mn-SOD as a result of oxidative stress. We used the previously proven chemosensitizing agents, thymol and 2,3-D, and included octylgallate (3,4,5-trihydroxybenzoic acid octyl ester) as redox-active chemosensitizing agents to fludioxonil for the FR2/FR3 study.

We examined the chemosensitizing activity of octylgallate, thymol and 2,3-D in co-applications with fludioxonil. Growth of FR2, FR3 and their parental strains was almost completely inhibited when fludioxonil (0.02 or 0.06 mM) was co-applied with 0.15 mM of octylgallate (**Fig. 2**). Thymol or 2,3-D also exhibited some chemosensitizing activity to fludioxonil in the *P. expansum* strains. However, this activity for 2,3-D was negligible in the mutant strains (**Table 1;** at end of this report).

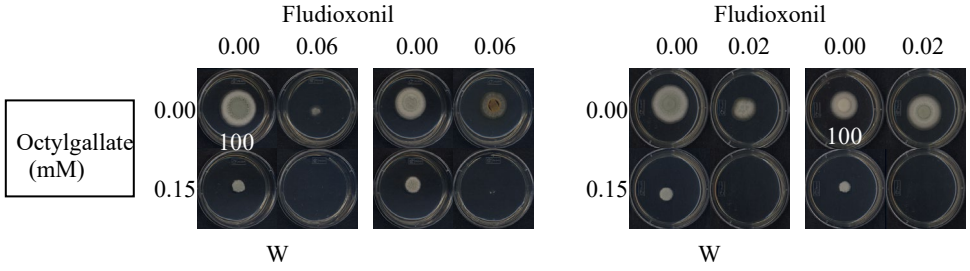

Figure 2. Representative bioassay showing growth of FR2, FR3 (fludioxonil-resistant mutants of *P. expansum*) and their respective parental strains, W1 and W2, in co-applications of fludioxonil (0.02 or 0.06 mM) and octylgallate (0.15 mM). (See also Table 1). Note: Radial growth rates of each strain w/o any treatment was considered as 100% growth (control), and the relative growth rate in each treatment was determined accordingly (SD < 5%).

We previously observed an antifungal synergism with thymol and 2,3-D when co-applied in growth media (Kim *et al*. 2008b). A similar synergistic effect was found in our current study. When thymol (as low as 0.2 mM) and octylgallate (as low as 0.1 mM) were co-applied there was complete growth inhibition of W1/FR2 and W2/FR3 (data not shown). This synergism suggests that thymol and octylgallate affect a common cellular target, the oxidative stress response system. Therefore, the chemosensitizing effect of these compounds to fludioxonil probably results from generating the same disruption of the oxidative stress response system of the fungus.

Chemosensitization of* P. expansum *to a mitochondrial respiration inhibitor. The responses of FR2 and FR3 to the oxidizing agents (**Fig. 1**) showed the redox homeostasis systems of these mutants were abnormal. We decided to further investigate the responses of these mutants to another fungicide, strobilurin (kresoxim-methyl). The responses of these mutants to strobilurin treatments

were not substantially different from those of the respective parental strains (**Table 1**). Hence, there was no indication the mutant strains were resistant to this fungicide. The mode of action of strobilurin is different from that of fludioxonil. Strobilurin inhibits complex III of the mitochondrial respiratory chain, resulting in a disruption of energy production. Coinciding with this disruption is an abnormal release of electrons that additionally damages cellular components by oxidative stress. Mn-SOD plays an important role in protecting cells from such oxidative damage. We were interested to see if co-applying redox reactive chemosensitizing agents, targeting Mn-SOD, could augment the fungicidal effects of strobilurin. As shown in **Table 1**, octylgallate (as low as 0.05 mM) in combination with strobilurin (0.02 mM), resulted in complete growth inhibition of both parental and mutant strains of *P. expansum*. This chemosensitization also occurred with co-application of 2,3-D or thymol (**Table 1**).

Summary: Certain safe natural phenolic compounds have the potential to serve as potent chemosensitizing agents to enhance activity of conventional antifungal drugs or commercial fungicides. We demonstrated how a number of phenolic compounds greatly improved effectiveness of fludioxonil and activated a process for overcoming fludioxonil-resistance (**Fig. 3**). These compounds also greatly enhanced the activity of strobilurin. Our results indicate this enhanced activity is from disruption of cellular redox homeostasis by targeting the antioxidative stress response systems (*e.g*., Mn-SOD) with redox-active natural compounds. Chemosensitization by safe, natural compounds can lower effective dosages of conventional antifungal drugs and fungicides. It can also be used to overcome resistance to these agents. Consequently, the lower dosage requirement could reduce environmental impact, health risks and costs.

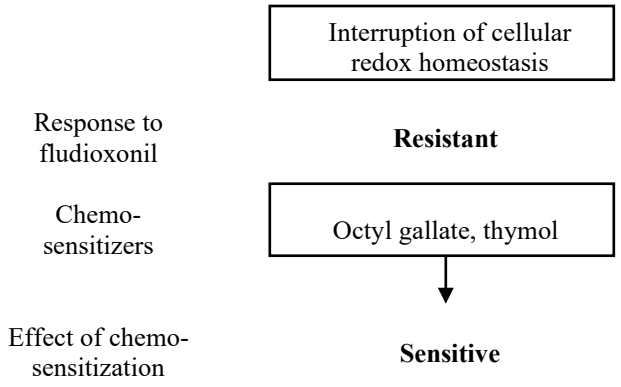

Figure 3. Diagram showing chemosensitizing effects of safe, natural compounds, which enhance antifungal activities of and/or overcome fungal resistance to conventional fungicides such as fludioxonil.

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Table 1. Percent growth of strains of *Penicillium expansum*, fludioxonil-resistant mutants (FR2 and FR3) and respective parental strains (W1 and W2), to fungicides fludioxonil (Flud) and strobilurin (kresoxim-methyl) (Kre-Me) and redox-active natural phenolics, alone and in combination (co-applied). The phenolics include octylgallate (OcGal), 2,3-dihydroxybenzaldehyde (2,3-D) and thymol (Thy). Numbers in parentheses are concentrations (mM) of each compound used, and percent numbers (%) are relative growth rate (radial growth) of fungi compared to "no treatment" controls of each strain (=100%) (SD < 5%). *P. expansum* strains were grown at 28°C (82.4°F) on potato dextrose agar. Growth was observed for 3 to 7 days.

	Fungicide alone	Chemosensi-tizer alone	Co-applied		Fungicide alone	Chemosensi-tizer alone	Co-applied
W1	Flud (0.06) 27%	OcGal (0.15) 34%	0%	**W2**	Flud (0.02) 64%	OcGal (0.15) 36%	0%
	Flud (0.06) 14%	2,3-D (0.20) 71%	~0% (few colonies)		Flud (0.04) 78%	2,3-D (0.30) 75%	0%
	Flud (0.04) 44%	Thy (0.60) 50%	0%		Flud (0.04) 26%	Thy (0.20) 58%	0%
	Kre-Me (0.02) 54%	OcGal (0.05) 69%	0%		Kre-Me (0.02) 52%	OcGal (0.05) 73%	0%
	Kre-Me (0.02) 50%	2,3-D (0.15) 81%	0%		Kre-Me (0.02) 54%	2,3-D (0.20) 79%	0%
	Kre-Me (0.02) 44%	Thy (0.60) 40%	0%		Kre-Me (0.02) 47%	Thy (0.40) 50%	~0% (few colonies)
FR2	Flud (0.06) 80%	OcGal (0.15) 52%	~0% (few colonies)	**FR3**	Flud (0.02) 129%	OcGal (0.15) 38%	0%

Flud (0.06) 78%	2,3-D (0.20) 70%	48%		Flud (0.04) 119%	2,3-D (0.30) 85%	96%
Flud (0.06) 86%	Thy (0.60) 36%	~0% (few colonies)		Flud (0.04) 135%	Thy (0.20) 83%	~0% (few colonies)
Kre-Me (0.02) 59%	OcGal (0.05) 77%	0%		Kre-Me (0.02) 67%	OcGal (0.05) 79%	0%
Kre-Me (0.02) 59%	2,3-D (0.15) 77%	0%		Kre-Me (0.02) 65%	2,3-D (0.20) 82%	0%
Kre-Me (0.06) 41%	Thy (0.40) 41%	~0% (few colonies)		Kre-Me (0.02) 61%	Thy (0.20) 83%	~0% (few colonies)

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Integrated biological control of woolly apple aphid

PI: Elizabeth H. Beers
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Cooperators: Jay Brunner, WSU-TFREC; Vince Jones WSU-TFREC; Tom Unruh, USDA-ARS; Steve Cockfield, Okanogan Valley IPM.

Total project funding request: Year 1: $38,238 **Year 2**: **$33,962** **Year 3**: $35,320

Other funding Sources

Agency Name: Washington State Commission on Pesticide Registration
Amount requested/: $14,792 (for 2009)
Notes: To be presented at the January 2009 meeting. Funded in 2008 ($12,948)

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Kevin Larson; ML Bricker
Telephone: 509.663.8181**;** 335.7667 **Email address:** kevin_larson@wsu.edu; / mdesros@wsu.edu

Item	2008	2009	2010
Salaries	11,007	**11,448**	11,906
Benefits	941	**954**	992
Wages	19,432	**15,560**	16,182
Benefits	1,858	**2,178**	2,265
Equipment	0	**0**	0
Supplies	2,000	**1,000**	1,040
Travel	3,000	**2,822**	2,935
Miscellaneous		**0**	
Total	$38,238	**$33,962**	$35,320

Objectives

1. Identify and quantify the predators and parasitoids of woolly apple aphid in eastern Washington (years 1 and 2).
2. Determine the relative impact of the predators and parasitoids (years 2 and 3).
3. Determine the effect of orchard pesticides on the key natural enemies (years 2 and 3).

Significant Findings

- Woolly apple aphid (WAA) populations started to appear on the aerial parts of the apple plants in May, with two peak population periods (July/Aug and mid-Sept/mid-October).
- *Aphelinus mali* was the only parasitoid species observed attacking WAA.
- Syrphids were the most frequently encountered predators (62.7%), followed by lacewings (23.6%) and coccinellids (8.9%). Other predators (4.8%) included nabids, *Deraeocoris*, spiders, and earwigs.
- Rimon used during the 2nd generation of codling moth caused higher WAA populations than the check; populations in the Delegate and Altacor treatments were also slightly, but not statistically, higher.
- There was some indication that Rimon suppressed predatory mites, but not enough to cause a spider mite outbreak in this late-season test.

Methods

Objective 1. Woolly apple aphid (WAA) colonies were sampled from April through October, 2008 in order to obtain qualitative and quantitative information on the natural enemy complex of this pest. Sampling occurred in 21 orchards throughout eastern Washington State, with approximately equal numbers of organic and conventional orchards. Orchards were selected on the basis of selective insecticide programs and a history of previous WAA infestations. Samples consisted of 50 shoot WAA colonies (when available) per orchard. We first inspected the shoots for motile adult predators, and then cut a 10-cm section of shoot that contained the WAA colonies and placed it in a self-sealing plastic bag. The samples were brought to the laboratory and inspected for predators and parasitoids under the scope. The coccinellid and lacewing larvae found in our samples were reared to adults for species identification (data not available). In the laboratory we also calculated the percentage parasitism of WAA by the parasitoid using a subsample of 15 aphid colonies.

During each orchard visit, we also counted the number of WAA colonies in a 5 minute search to estimate population size and growth.

Objective 3. This test was conducted in a 25-acre block in a commercial orchard (Arrowhead Ranch), in Bridgeport, WA. The block consisted of twelve-year-old 'Cameo' apples planted 7 by 16 feet. The block was irrigated with drip irrigation. The plots were 13 rows × 26 trees, or about 0.87 acres in size. There were five treatments with four replications.

The treatments consisted of a third and fourth cover sprays during the second generation codling moth. Four of the treatments were registered insecticides having codling moth activity, and the fifth was an untreated check, permissible because of the low codling moth pressure in this block. Applications were made with an airblast sprayer calibrated to deliver 100 gpa. The third cover was applied 23-26 July, and the fourth cover, 12-14 August.

Woolly apple aphid populations and their natural enemies were evaluated prior to third cover, and biweekly through mid-October. Two types of evaluations were made. The first was a timed count of colonies to look at overall population trends. During the timed counts, the green apple aphids, syrphids, chrysopids and coccinellids were also counted. The second evaluation was a detailed examination of the stage distribution of the aphids and natural enemies found in the colonies. Woolly

apple aphids were classed as adult, immature, with wing buds or alate. Mummies caused by the parasitoid *A. mali* were recorded, as well as syrphid eggs (hatched or unhatched) and larvae.

Phytophagous and predatory mites were also sampled at weekly intervals. Fifty leaves/plot were collected and kept cool during transportation and storage. Mites were brushed from the leaves with a mite brushing machine (Leedom, Mi-Wuk Village, CA) and collected on a revolving glass plate coated with a sticky substance. The composite sample on the plate was counted using a stereoscopic microscope. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), *Tetranychus urticae* Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor [the eggs of TSM and MCD could not be distinguished, and were recorded as a group]; western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); a stigmaeid predatory mite, *Zetzellia mali* Ewing, and motile stages of apple rust mite (ARM), *Aculus schlechtendali* (Nalepa).

Cumulative mite days (CMD) were calculated for tetranychid, predatory, and rust mites, giving an estimate of population densities integrated over the course of the test. CMDs are the sums of the average density of mites on two dates multiplied by the number of intervening days:

$$CMD = \Sigma 0.5(P_a+P_b)D_{a-b}$$

where P_a is the population density (mean mites/leaf at time a), P_b is the population density at time b, and D_{a-b} is the number of days between time a and time b.

Data were analyzed using the Statistical Analysis System (SAS 1988). PROC GLM was used to conduct an analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test.

Results and Discussion

Objective 1. Woolly apple aphid (WAA) populations started to appear in the field around May. This time coincides with a moderate increase in average temperature. This is also the time crawler movement from edaphic colonies begins, establishing aerial colonies in the tree.

The majority of woolly apple aphid populations appeared to have one of two peaks: one in July/August, and one in late September/mid-October. One orchard had a very extended peak spanning this entire period.

The only parasitoid of WAA observed was *Aphelinus mali*. There was considerable fluctuation of the percentage of parasitism throughout the season, with no discernable pattern; fluctuations could be due to either a decline of the host or application of non-selective pesticides.

The predators observed attacking WAA colonies were syrphids, lacewings, coccinellids, predatory bugs and spiders. Overall, syrphids (62.7%) were the most abundant predator observed in our samples (Fig. 2). It should be noted that our sampling system of collecting only predators that are associated with the WAA colonies may favor less mobile predators such as the larvae of syrphids, lacewing and coccinellids and that our sampling took place during the day; nocturnal predators such as earwigs and spiders may be underrepresented by this sampling method.

Specimens reared to the adult stage have been prepared for identification, which should be complete by the spring of 2008.

Eastern Washington has an important complex of WAA natural enemies including both a parasitoid and generalist aphid predators. These natural enemies, integrated with other management tools, have the potential to maintain WAA populations at low densities. Research in Objectives 2 and 3 will help determine both the impact of the natural enemy complex and how to protect it from nontarget effects.

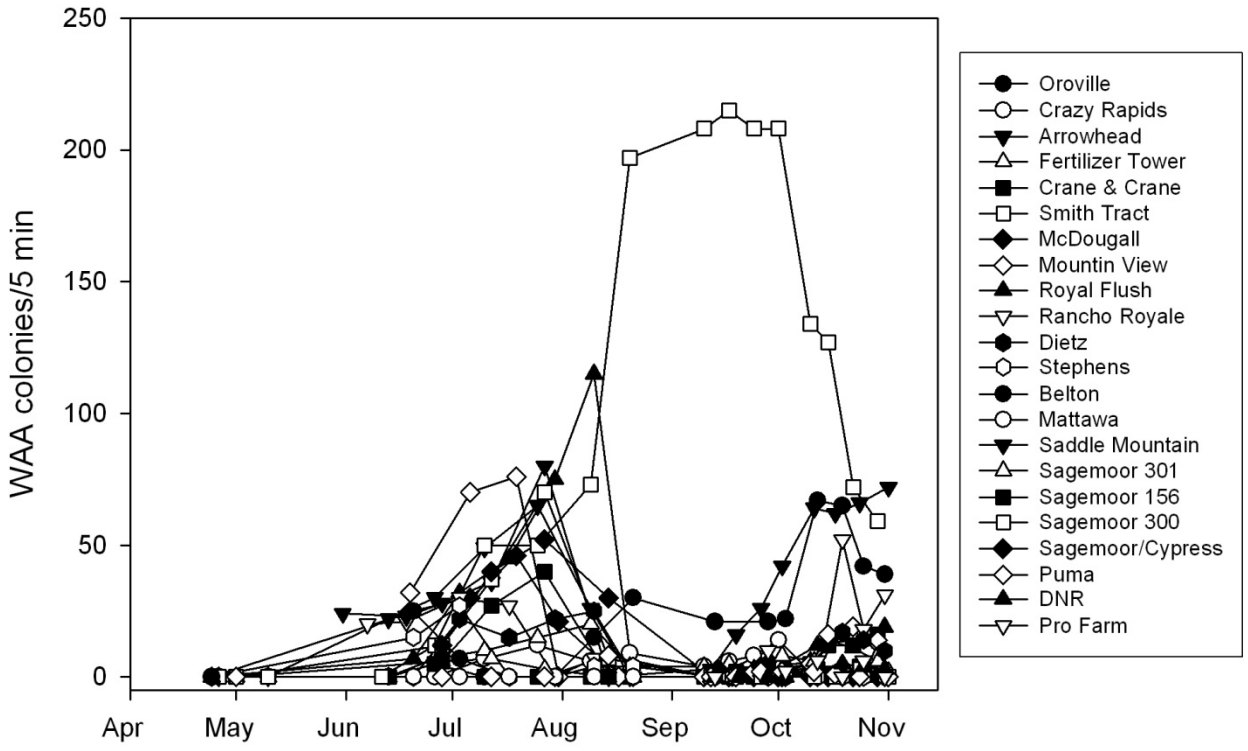

Fig. 1. Woolly apple aphid colonies/5 min search, 21 orchards in central Washington, 2008

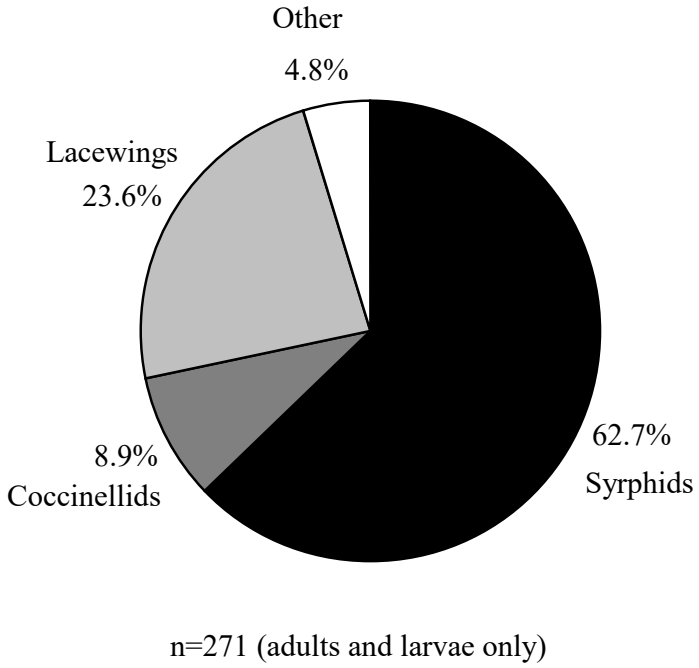

Fig. 2. Composition of the predator complex in 21 orchards in central Washington, 2008.

Objective 3. Woolly apple aphid densities were moderate in late July when the test was initiated (Fig. 3). This type of population level often increases dramatically during the cooler weather in September and October. However, the number of colonies declined dramatically in late August due to a spell of unseasonably hot weather on 15-17 August (100+ °F). Populations slowly rebounded as the weather moderated, but never attained their former levels. Woolly apple aphid colony densities were significantly higher than the check in the Rimon treatment from late August through early October. This trend was reflected in the seasonal sums, where Rimon had the highest overall populations (Fig. 4). The Delegate and Altacor treatments had similar colony numbers, and although numerically higher than the check, no statistical differences among treatment means were detected on either individual count dates or in seasonal sums. The Intrepid treatment was most similar to the check, and never statistically different from it.

The tetranychid populations in this block consisted entirely of European red mite, and the predatory mites consisted entirely of *G. occidentalis*. European red mite populations were moderate during the course of the test, never exceeding seven mites/leaf. The population persisted through mid-September, which is somewhat unusual in central Washington. No significant differences were found among treatments in tetranychid mite densities (Fig. 5). For the predatory mites, differences occurred on several count dates. The densities in the Intrepid treatment were significantly lower than the check in late August (Fig. 6), but rebounded in early September, resulting in an overall high CMD at the end of the season. Predatory mite densities were lower in the Rimon treatment on several dates, resulting in a CMD that was significantly lower than the highest treatment (Intrepid), but not different than the check. Apple rust mites were low throughout the course of the test, with no meaningful treatment differences (data not shown).

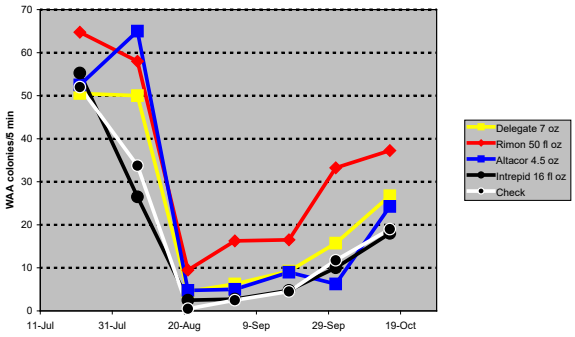

Fig. 3. Timed counts of colonies/5 min/plot on individual dates and post-treatment sums, Arrowhead, 2008.

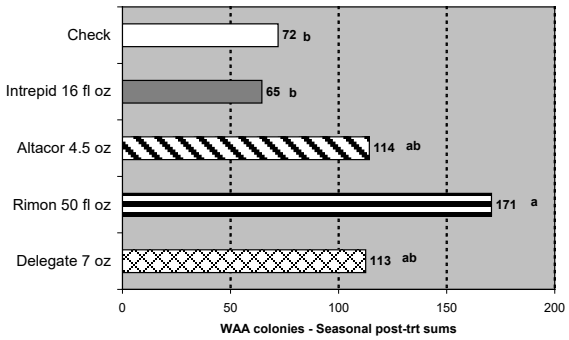

Fig. 4. Post-treatment sums of colonies/5 min counts, Arrowhead, 2008

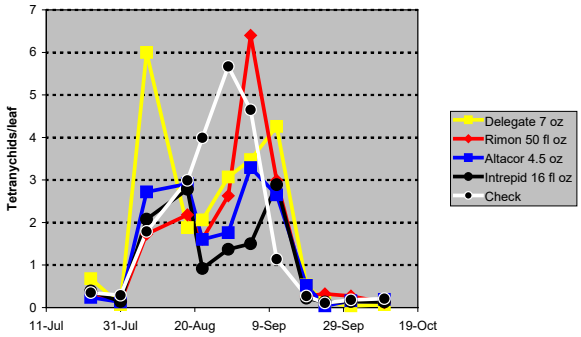

Fig. 5. Tetranychid mite densities over time, Arrowhead, 2008.

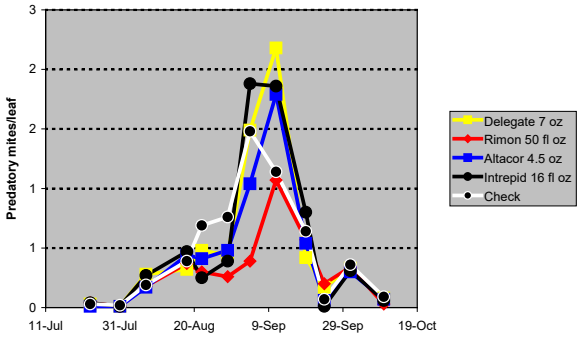

Fig. 6. Predatory mite densities over time, Arrowhead, 2008.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3

Project Title: Interaction of dispersal and management of CM and OBLR

PI:	Vince Jones	**Co-PI(2):**	Jay Brunner
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Total project funding request: **Year 1:** $60,112 **Year 2:** $62,452 **Year 3: $65,213**

Other funding Sources: None

Budget 1:
Organization: WSU-TFREC **Contract Administrator:** ML Bricker; Kevin Larson
Telephone: 509-335-7667; 509-663-8181 x221 **Email:** mdesros@wsu.edu; kevin_Larson@wsu.edu

Item	Year 1: 2007	Year 2: 2008	Year 3: 2009
Salaries[1]	$28,332	$28,332	**$29,151**
Benefits	$10,200	$11,049	**$11,660**
Wages	$12,000	$12,480	**$12,979**
Benefits	$1,380	$1,959	**$2,336**
Equipment	$0	$0	**$0**
Supplies[2]	$6,000	$6,300	**$6,615**
Travel[3]	$2,200	$2,332	**$2,472**
Total	$60,112	$62,452	**$65,213**

Footnotes:
[1] Tawnee Melton 0.75 FTE
[2] Supplies include general lab supplies and ELISA-specific supplies, field supplies including traps, lures, markers, cell phone charges
[3]Travel costs are within-state travel to field sites and vehicle costs

Objectives:

1. Evaluate methods to age-grade CM caught in traps throughout the season. This will be used to evaluate times of peak reproductive potential during which control measures should be optimized.
2. Determine the effect of flight of specified distances on the reproductive ability of CM and OBLR males and females using laboratory assays.
3. Evaluate the effects of different cover sprays on dispersal of CM using protein markers and investigate the effects of border sprays of kaolin on movement patterns. We will also compare the age/mating classes by sex of CM individuals that have flown long distances to the unmarked individuals caught in the same traps.

Significant Findings:

We have collected and processed nearly 3,500 moths to help in the development of an age-grading system for codling moth. Data analysis is not yet complete.

Our baseline testing of OBLR using the flight mills showed that the averge longest single flight of males (5,916') was ≈6.7 fold further than females (880'). Male average total flight duration was 2 fold greater than females, but females flew 1.7 fold more frequently. Maximum male flight distance was 14.5 miles, versus 4.5 miles for females.

Baseline testing of CM showed no significant differences in flight duration, distance, or number of flights between the sexes. Maximum flight distance recorded was 12.1 miles.

Our test of cover sprays on dispersal of CM showed no significant differences between the Assail and Guthion treatments, but both blocks showed highest trap catch at the ends of the blocks.

Our kaolin studies showed that a three tree border can reduce CM migration significantly, even in the face of high population pressure. We will concentrate heavily on this aspect next year.

Significant Progress:

Objective 1. We monitored for CM at four different locations this year, but had low trap catches at two of the locations. We caught 2,396 moths at TFREC and 1,073 at the Wenatchee Valley College (WVC) orchard. A key part of the study this year (besides confirming the age grading system) was to evaluate the difference in age classes caught between pheromone traps and the Trécé DA Combo lure, season-long. We found that the relative frequency of different age classes were not significantly different between the two lure types at the TFREC orchard, but were different at WVC (biased towards older individuals). The differences at the WVC orchard seemed to be the result of very low capture of young individuals compared to TFREC and may be the result of the mating disruption being used there versus the non-MD situation at TFREC. In MD situations, we have previously found that males take longer to find females; similarly, we would expect it to be harder for them to find a pheromone lure, meaning that they would be older when they were caught in the traps.

Plan for next year. We need to perform a much more comprehensive analysis of the current year's data sets as well as last year's data sets to evaluate our age grading classification and integrate this into our models to determine how age structure might affect our management recommendations.

Objective 2. We have completed and tested 24 digital flight mills that can be used to evaluate dispersal capabilities of CM and OBLR and the effect of dispersal on reproduction. We are able to run 24 insects simultaneously and the data are recorded by a computer. Using this set up, we can determine how far and fast they fly, whether they stop and rest, the number of flights, and the duration of flight. Our initial intent with the flight mills was to understand how flight affects the reproduction of migrating individuals which we will still pursue, however, we realized that the flight mills would be a good way to evaluate pesticide effects on flight and we are pursuing both experiments.

The flight mills use magnetic levitation and teflon bearings to reduce friction to a minimum. A foot long arm is attached to the bearing and moths are attached to the arm by gluing a small insect pin to

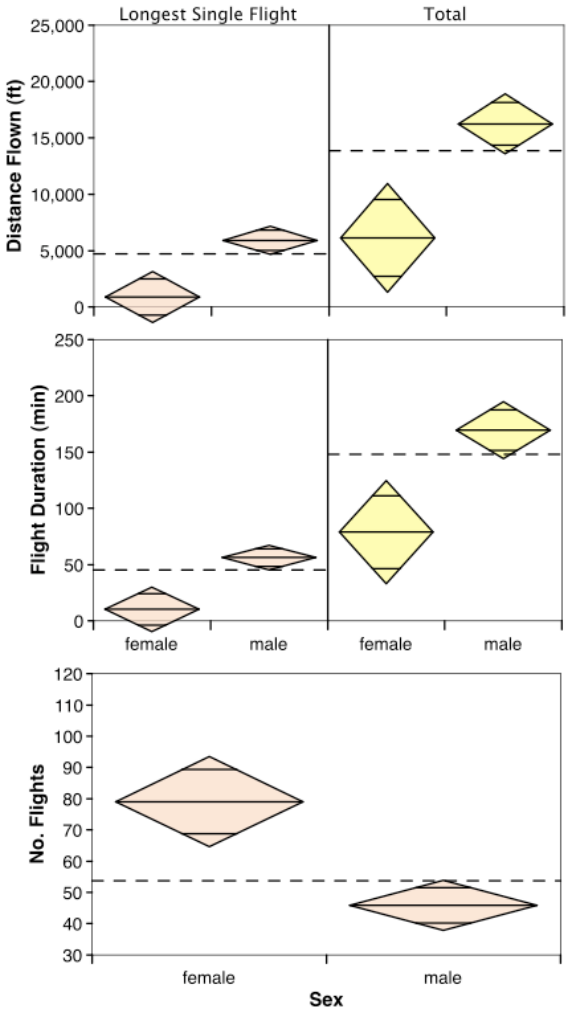
the back of the moth on the thorax (to prevent interference with the wing motion). The pin is stuck into a cork, and then pushed on the end of the arm. Moths fly in circles, and a small magnet attached near the bearing is detected by a sensor on every revolution and recorded for each moth. After flight, the moths are easily separated from the insect pins so that they can be used in our reproductive experiments.

Obviously, testing moths on the flight mills is extremely artificial and is not indicative of normal behavior in the field. Instead, flight mill data gives us good information on the physiological capabilities of the moths. For example, if we record the average longest single flight distance for CM females at 7,710 feet, that shows what the *potential* flight distance is on average, but doesn't indicate that would occur in the field where volatiles, environmental conditions, and host plant density would all potentially affect behavior. The flight mills are important in understanding how far moths can potentially move unassisted, what the effects of pesticides are on movement, and how movement affects reproduction. Being able to evaluate the moths in the laboratory means that we have a much less expensive way to test things than using field experiments where multiple factors affect results and require large numbers of replicates to quantify field effects.

To date, we have performed the preliminary studies needed so that we can design our experiments on pesticide and flight effects on reproduction of migrating individuals. Our preliminary studies examined the effect of sex and diet (honey water vs plain water). Moths were reared to the pupal stage, then sexed and separated for emergence. We used two-day-old males or females that had been held at the 16:8 (L:D) at 25°C, and provided half the group water and the other half a 20% honey-water solution. Moths were placed on the flight mills late in the afternoon and recorded for a 14-hour period over the natural dusk - dawn cycle.

Analysis: We examined five different parameters to determine flight characteristics based on diet and sex. These included the average longest single flight distance and duration, the average total flight distance and duration, and the average number of flights. We eliminated moths that did not fly for more than 5 minutes during the 14-hour period, because such moths were likely improperly mounted so that the glue interfered with normal flight. We tested for normality before statistical analysis and applied a logarithmic data transform before using ANOVA to test for significant differences in any of the parameters; all data is displayed graphically as mean diamonds.

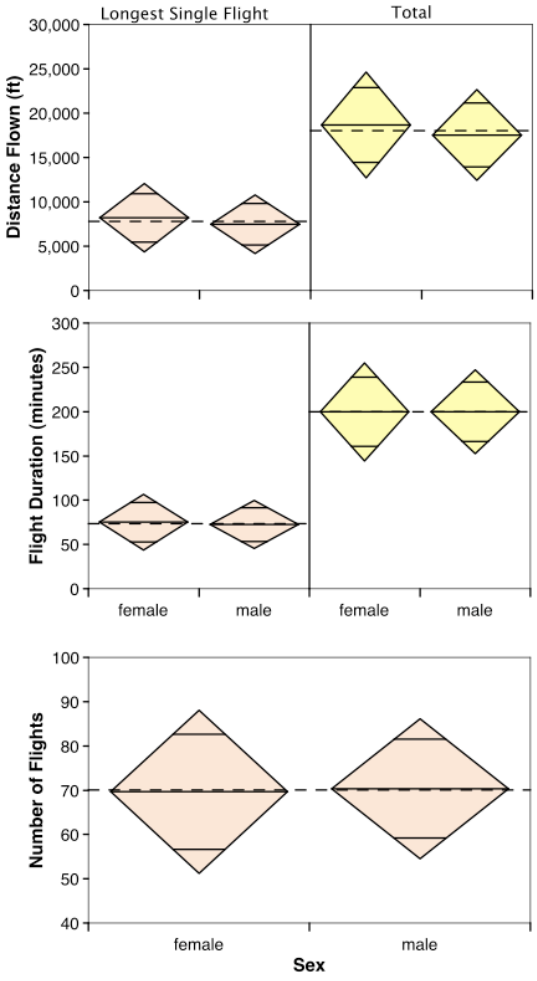
Fig. 1. Mean diamond plots for OBLR flight distance, duration, and number of flights. Dotted lines on plots indicate the overall mean for a comparison, the line in the middle of the diamond is the mean, the upper and lower lines are the 95% confidence intervals. If the 95% confidence intervals do not overlap, the treatments are significantly different.



Results: Evaluation of the data for both moth species revealed a highly skewed distribution; that is, a relatively high proportion of the moths flew a short distance, but about 25% of the population flew an extraordinarily long distance.

The data for OBLR showed that there is a highly significant difference between the sexes in terms of flight ability, but that diet was only marginally significant. Males flew further and for a longer duration both for the longest single flight (1.1 vs. 0.16 mile) and in total (3.1 vs 1.2 miles) (Fig. 1). Females had nearly twice the number of flights as males (79 vs. 46). While the data show no significant differences between diets, the trend is for individuals fed honey to fly further and longer. It is possible the differences would have become significant if more females were flown (a total of 42 females and 137 males were flown). Overall, it is clear that males fly nearly 4 times further for the longest single flight and 2.5 times further over the total flight period; the differences in the total flight period are less than expected by flight duration alone because females fly nearly twice as often as males.

Fig. 2. Mean diamond plots for CM flight distance, duration, and number of flights. Dotted lines on plots indicate the overall mean for a comparison, the line in the middle of the diamond is the mean, the upper and lower lines are the 95% confidence intervals.



With CM, we found no significant differences between the sexes in any of the parameters, nor were the parameters affected significantly by the diet (Fig. 2). On average, the longest single flight was ≈1.5 miles and averaged 73 minutes, while the average total distance flown was ≈3.4 miles and 198 minutes (Fig. 2). In terms of the number of flights, on average, moths started and stopped 70 times throughout the test period. The maximum distance flown was 12.2 miles and the longest single flight lasted nearly 12.5 hours.

Plan for next year. We are currently collecting data to determine pesticide effects on dispersal, starting with the comparison of Assail and Guthion on OBLR and CM flight. We will then begin with the studies on effect of flight of various distances on reproduction.

Objective 3. Methods - Cover Spray Effects: We set up two different plots to evaluate the effect of Assail versus Guthion cover sprays on the dispersal of codling moth using protein markers. The first plot was set up in Manson during the first generation. Unfortunately, codling moth populations at the site were extremely low and even though we had 120 traps out, only 14 moths were caught. The second plot was set up during the second generation in Quincy, and we were able to capture 1,651 moths, of which roughly 10% (171) were positively marked.

The second plot consisted of two blocks: one was 19.5 acres (≈ 1487’ long x 560’ wide) and treated with Guthion and the second was 12.9 acres (≈794’ long x 687’ wide) and treated with Assail. Treated areas in each block were 350’ long by 112’ wide and situated on one end of the block, thus the furthest distance outside the marked area that could be recorded in each block was 1375’ in the Guthion block and 575’ in the Assail block - after those distances, there was a wide road (85’-105’)

between the blocks and adjacent orchards. We set up four transects in each plot away from the marker treated areas with equal distances for the full length of the Assail block; in the longer Guthion block, we added a lower density of traps out to the end of the block. For comparison of dispersal in the two plots, we initially only looked at the moth captures in the transects out to the 575' in the Guthion block, then compared the dispersal in the full range of traps with the understanding that any trap capture beyond 608' in the Guthion block would *a priori* have longer dispersal distances on average.

We used the Trécé DA Combo lures so that we would obtain at least a small number of female moths and to have consistency in trap catch as both blocks were under mating disruption. Each moth captured was sexed and then dissected to determine moth age and mating status.

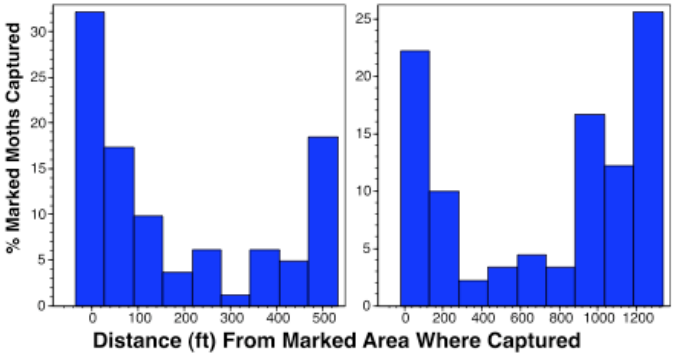
Results - Cover Spray Effects: As usual, there was a strong bias towards males (91% males) in the DA Combo lures with no significant difference in sex ratio between the two treatments. Because of the low percentage of females captured, unless mentioned, all analyses are restricted to male moths. Analysis of the age distributions of marked moths captured in each block also showed no significant differences between the blocks and averaged 11% young, 54% middle aged, and 35% old. This ratio is similar to that of unmarked moths caught in the plots.

In terms of the average distance moved, there were no significant differences resulting from either pesticide ($p = 0.3$) or age ($p = 0.08$) when the Guthion plot data were restricted to the same trapping grid size as found in the Assail plot. The differences in average distance caught for the two pesticides were 263.7' for Assail and 187' for Guthion, which are exactly the opposite of what we found 2 years ago. Part of the difference is that in our current year's experiment, the Assail plot had a reduced size, which reduced long trap catches. Examination of the Guthion plot actually showed that 63% of the marked moths trapped in the Guthion plot were at distances >524'.

Probably the most interesting factor in the data was the differences in trap capture within the plot (Fig. 3). When evaluating the data for moth capture in both treatments, individuals dispersing from the edge of the plot tend to be found at high levels near the marking area and at the furthest edge of the block. Moth behavior is apparently strongly affected by the wider drive rows found at the end of the block. A possible explanation might be the trap density (and hence competition between traps) is effectively lower at the ends where traps are not found outside the plot, but our larval distribution studies performed a few years ago for improving the Taiwan sampling program showed the same trends of heaviest populations being found on the borders in roughly 80% of the orchards sampled.

Plans for Next Year - Cover Spray Effects. The problems of getting a large enough population of codling moth and large paired blocks that are >1400' long make it difficult to detect differences caused by pesticide on dispersal distances. This is further complicated by the weather which may both degrade the marker residue or which may be windy enough to restrict movement of the moths. To address some of these problems, we will use our flight mills to assay moths exposed to lower rates of pesticides for a 24-hour period before being placed on the flight mill. These assays should tell us if there are physiological changes in the moths that alter their flight capacity and assess the effects of age and mating status. Doing this should allow us to check several pesticides with much less effort and variability than using the field assays.

Fig. 3. Percentage of marked moths captured at different distances from the marked area. In both plots, moth captures rose sharply at the end of the plot where a wide drive row was present.



Methods - Kaolin Effects on Movement: We set up two different trials to evaluate the

ability of a three tree row barrier to reduce CM movement from high population to low population areas. In the first trial, a large abandoned orchard was removed adjacent to a commercial block. Although the grower applied a Surround treatment to the border, it was only applied once at a low rate and washed off completely within a small amount of time. We sampled 795 trees at the orchard, but in all plots, damage was less than 0.05%, so no analysis was possible.

The second trial was run in a small planting at WSU-TFREC with extraordinary levels of codling moth pressure, coming from surrounding infested blocks as well as present within the block. Early in the first generation, we started treating the bottom half of the plot with insecticides and left the top part of the plot untreated. A three-row barrier of Surround was applied twice to the center of the plot and to one edge (Fig. 4). We sampled every tree in the block by evaluating 60 half-fruit per tree for CM damage at the end of the first and second generations. There were no sprays applied during the second generation. We compared the damage found on either side of the Surround barrier, looking at both overall damage in each subplot, and looking at the differences within a plot in the three tree rows adjacent to the Surround barrier and then the three rows furthest from the surround barrier.

Results: The heavy insecticide treatments of the lower four plots in the first generation resulted in very low damage levels in those plots compared to the top four plots that were untreated. Overall, damage was highest on the north (right) border of the block and decreased in each successive plot to the south (left). There were no significant differences between the upper and lower three rows, except in the second plot from the left in the top row.

In the second generation, the average damage in the top plots increased significantly (3-4 fold) over the levels in the first generation, but the lower block damage levels did not increase significantly from the first generation. The greatest increase was in the lower left plot which was not protected by the surround barrier, but that still remained very low.

The data show that the Surround barrier is very good at reducing migration between plots. The three plots on the bottom right were unaffected by the high populations on the topside of the Surround border. The bottom left plot that was not protected by the barrier is the only question. It appears that the TFREC blocks to the north of our test block were the source of pressure and the kaolin border stopped migration from those blocks and then funneled the moths down the tree rows in the upper plots. However, it is still a question why moths that laid eggs on the top left block did not move down into the unprotected block below in greater numbers.

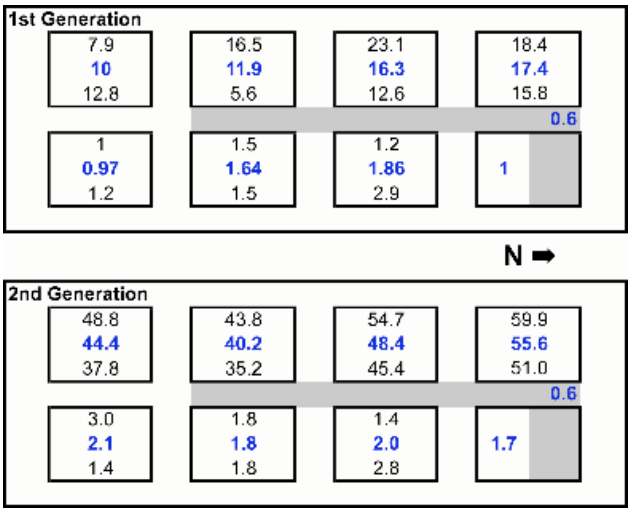


Fig. 4. Layout of kaolin experiment at WSU-TFREC. Each square represents a plot; gray areas were treated twice with Surround in the first generation. The three numbers represent the average % damage for the plot (center number) and the three rows furthest and closest to the Surround border. The one number in the lower right plot is the average for the plot; not enough trees were present to tabulate.

Plans for next year: We plan on setting up multiple plots with kaolin borders to evaluate movement. We hope to spray four different orchard blocks and use similar methods to those described above, but on a much larger scale.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3

Project Title: Defining natural enemy biology and phenology to improve IPM

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State/Zip:	WA 98951	**State/Zip**:	Canada V0H 1Z0

Cooperators: Jay Brunner, WSU-TFREC; Qing-He Zhang, Sterling International, Inc., Spokane

Total Project Request: Year 1: $132,478 **Year 2: $137,978** **Year 3:** $135,378

Other funding Sources

Agency Name: USDA-CSREES SCRI Grant $2.244M

Budget 1:
Organization: WSU-TFREC **Contract Administrator:** ML. Bricker, Kevin Larson
Telephone: 509-335-7667, 509-663-8181 x221 **Email:** mdesros@wsu.edu, kevin_larson@wsu.edu

Item	Year 1	Year 2	Year 3
Salaries[1]	43,604	**45,176**	47,162
Benefits[2]	7,203	**8,004**	7,792
Wages	8,000	**8,320**	8,653
Benefits	1,256	**1,498**	1,359
Equipment	0	**0**	0
Supplies	3,500	**3,640**	3,786
Travel[3]	3,500	**3,640**	3,786
Total	67,063	**70,278**	72,538

Footnotes:
[1]*Half-time project manager (Nik Wiman); 0.33FTE Associate in Research (Callie Baker).*
[2]*Wiman (7%); Baker (34%).*
[3]Within state travel for surveys.

Budget 2:

Organization: USDA-ARS **Contract Administrator:** Janet Tsukahira
Telephone: 510-559-6019 **Email:** jtsukahira@pw.ars.suda.gov

Item	Year 1	Year 2	Year 3
Salaries*	38,782	**37,231**	35,493
Benefits	11,635	**11,169**	10,647
Wages	0	**0**	0
Benefits	0	**0**	0
Equipment	1,450	**0**	0
Supplies	500	**500**	500
Travel	0	**0**	0
Miscellaneous	1,200	**1,200**	0
Total	53,567	**50,100**	46,640

Footnotes:

* Salaries are for a term appointment with 13, 12, and 11 months in 1st, 2nd, & 3rd years, respectively

Budget 3:

Organization: Agriculture & Agri-Food Canada **Contract Administrator:** Karen St. Martin, Goewin Demmon
Telephone: 250-494-7711 **Email:** stmartink@agr.gc.ca, demmong@agr.gc.ca

Item	Year 1	Year 2	Year 3
Salaries		0	0
Benefits		0	0
Wages[1]		**10,500**	11,000
Benefits		**2,100**	2,200
Equipment		**0**	0
Supplies[2]		**3,500**	1,500
Travel[3]		**1,000**	1,000
Miscellaneous		**500**	500
Total		**17,600**	16,200

Footnotes:

[1] Summer student wages with 20% benefits plus inflation in year 3.
[2] Supplies in year 1 include cost for synthesizing several grams of *Ascogaster* pheromone for group.
3 Travel costs are for local travel within Okanogan Valley.

Objectives:

1. Characterize the phenology of key natural enemies using banding studies in the late fall and orchard sampling from early spring to late fall.
2. Evaluate various semiochemicals as a method of monitoring natural enemy abundance / phenology and impacts of control treatments.
3. Use video monitoring to identify predator species attacking codling moth and develop a polyclonal antibody for expanded predator gut content analysis of codling moth.
4. Further investigate the life history of tachinid parasitoids of leafrollers and their potential for enhancing management of leafrollers.
5. Integrate the information on natural enemy phenology and abundance into the WSU-Decision Aid System to help users gauge the impact of pesticide sprays at different times of the season.

Significant Findings:

We were able to leverage WTFRC funding including this grant to obtain a $2.244M Specialty Crops Research Initiative grant to improve BC in apples, pears, and walnuts. Overall, we matched roughly $500K in WTFRC funding with enough funds from WSU, UC Berkeley, and Oregon State University get an overall commitment of $4.488 M. That grant will allow expansion of this project and incorporation of sub-lethal effects of the new insecticides on natural enemies, include economic and sociological analysis to optimize IPM strategies.

Beating trays severely underestimate natural enemy activity in the orchards. At the same orchards where we collected 21 total *C. nigricornis* on beating trays, our attractant traps caught thousands.

Early season *Deraeocoris* populations may not be predictable by air temperatures and may require bark temperatures (or estimates using solar radiation sensors), but we are starting to work on preliminary phenology models.

We tested 15 different semiochemicals or combinations in the orchard for activity and nine of them for longevity. We have highly active lures for *C. nigricornis*, and several other lacewing species and potentially less active attractants or combinations for syrphids, *Deraeocoris*, and *Orius*.

Video monitoring equipment was purchased and debugged late in the season. We will have more data next year on that objective.

We are still testing polyclonal antibodies to detect CM in predator gut contents. However, a highly efficient LAMP protocol was developed and should be in use this coming year.

Tachind flies that emerged from LR hosts exposed to a sublethal dose of either Esteem or Intrepid (at $\approx LD_{10}$ for LR) were less fecund and produced 60 and 20%, respectively, of the progeny reared from untreated larvae.

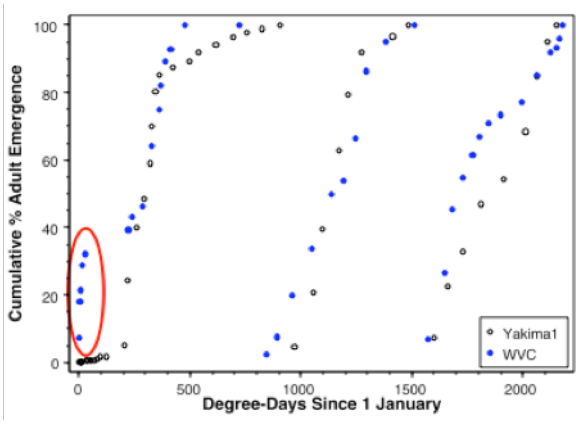
The most common alternate tachinid host collected was the western spruce budworm; however, it is not an overwintering host. We are still identifying some of the specimens collected this year.

Significant Progress:

Objective 1. We have phenology data from two studies: (1) from overwintering bands placed in the orchard then brought to an outdoor shaded shelter by early December; and (2) beating tray samples collected in eight orchards in Wenatchee and Yakima from early spring into the fall. The band data have not yet been analyzed; only data from the beating tray samples are currently available. The diversity of natural enemies varied between orchards, but the tendency was for equal diversity and greater abundance of predators in the Yakima samples.

Results. Data are being worked into phenology models for the more abundant species. Preliminary analysis of the *Deraeocoris brevis* sample suggests that we may need to record not just air temperatures, but also bark temperatures. When we compare data taken from WVC and a Yakima orchard, we find initially that there is a discrepancy between the two for emergence of overwintering adults (Fig. 1): i.e., there was a very rapid increase on a DD scale for the WVC site compared to the Yakima site. The WVC data showed that only 3 DD accumulated between 12 March and 9 April, so that when plotted on a DD basis emergence appeared to occur very rapidly. This was likely a function of the air temperature profile versus actual temperatures beneath the bark where *D. brevis* overwinter. Warmer temperatures found on the bark would have accelerated emergence compared to what our air temperature data showed. Once the air temperatures started to warm, the curves for the overwintered adults and the two summer generations were quite similar between the two sites.

Fig. 1. Cumulative % adult emergence of *D. brevis* at two orchards. Circled area shows area where differences in bark and air temperature affect the model.



Our data from the beating trays also show their limitations and why we need to move beyond that sampling method. For example, our beat tray samples in the WSU-Sunrise, WVC, and WMO orchards together produced only 21 *Chrysopa nigricornis* adults all season long. Using attractant traps in the same orchards resulted in capture of thousands of *C. nigricornis*. Our trap data clearly indicate that understanding of the importance of various species and their abundances is severely handicapped by limitations of the sampling methods that are the current standards in the industry.

Plan for next year: We will set up weather stations at two of the sites with air temperature probes and probes placed in bark of the apple trees. The sites will be near AWN sites, so that we can correlate bark temperature with air temperature and solar radiation from the AWN solar radiation sensors.

We will again sample eight orchards (four in Yakima area, four in NC Washington), starting in early spring and continuing through the fall. Band samples collected this fall will be evaluated for natural enemy emergence this coming spring and we will analyze the spring 2008 emergence data.

Objective 2A. *Determine release rates of semiochemicals*. Release rates were evaluated in three trials in the spring, summer, and fall. In order to regulate release rates, we used heat-sealed polyethylene bags of different thicknesses with a small square of felt inside to absorb the chemical. We evaluated six different compounds with two different dispensers in the spring (3 June to 1 July), eight different compounds with four different dispensers in midsummer (25 July to 22 Aug), in the fall filled in the gaps in our data on release rates from five different compounds and four different bag thicknesses (3 Oct to 31 Oct). We used four replicates of each lure and placed them in the field at TFREC and brought them back to the lab and weighed them at weekly intervals or less, depending on the time of the season and lure. Weight loss over time was fit to equations to estimate the lure longevity.

We also had two additional lure release rate trials that evaluated trap catch of the various natural enemies using the polyethylene bags of 1.5, 2, 4, and 6 mils thick for seven different compounds and 1.5 and 2 mils for two other compounds. The final trial was performed in the fall and only evaluated trap catch of squalene using a range of different amounts and multiple membranes to lower the release rate. *At this point, we have all the data for the predators, but have not yet been able to analyze the parasitoids or tachinids collected, so the reported spectrum of activity is to be considered a minimum.*

Results: Lure release rates for a given dispenser could be ranked from highest to lowest during summer>spring>fall. Our polyethylene bags proved to be good at regulating the release rate of five of the attractants out to at least 30 days during the midsummer, two were regulated enough to obtain 12-18 day longevity, one for a week, and one for no more than 5 days (Table 1).

In the dose-response trial, the only predators captured in large enough numbers to analyze were *Chrysopa nigricornis, Chrysoperla plorabunda*, and syrphid flies. Several of the compounds attracted *C. nigricornis* at various levels, but all were eclipsed by squalene, which captured 7,119 over the ten weeks of the trial (it appears to be a species specific lure). In a separate plot that had high aphid numbers, we caught 8,911 *C. nigricornis* in a 6-week period with only 10 squalene traps (average of 20.7 lacewings per trap per day). Peak trap captures occurred when a 2 mil bag was used, only the trap catch for the 6 mil bag was significantly lower than the 2 mil bag. For *C. plorabunda*, 2-phenyl ethanol and benzaldehyde both captured low numbers; with peak catch occurring in the 1.5 and 4-6 mil bags, respectively. Syrphid flies were captured best by geraniol and 2-phenyl ethanol in the thinner bags.

Because of the large numbers of *C. nigricornis* caught in squalene traps (sometimes >150 in a few days), we ran another trial in the fall (28 Aug to 2 Oct) to see if we could reduce trap catch by changing the amount of squalene in the lure or by using multiple membranes (double bagging) across which the material would have to diffuse. We found that by changing the lure loading we could manipulate trap captures more predictably than by using multiple membranes. We also sexed all the *C. nigricornis* caught in this trial and found that squalene attracts primarily males (2,477 males and 97 females) and that females were never caught unless at least four males were present on the trap.

Plan for next year: We are currently evaluating release rates of different dispensers in laboratory fume hoods to determine if we can extend the life of some of the different attractants. Next year we will test another suite of lures and will reevaluate some of the lures that ran out too early in our tests which may have affected the trap capture this year (*e.g.*, cis-3-hexen-1-ol and octyl aldehyde).

Objective 2B. *Evaluate field effectiveness and spectrum of activity of the different attractants.* We

Table 1. Attractants tested during summer 2008 and potential target natural enemies from our results.

Chemical	Moxee	WVC	WSU-Sunrise	Quincy	Quincy B	Potential Targets
3 hexenyl acetate	x	x	x	x	x	**CN**
benzaldehyde	x	x	x	x	x	Tachinids, Syrphids, **CP**, *Orius*
cis-3-hexen-1-o1	x	x	x	x	x	Syrphids, *Orius*
iridodial (IR)	x	x	x	x		**CN, CO**
methyl salicylate (MS)	x	x	x	x		*CN, CP, CC,* syrphids, **D**, *Orius*
squalene (SQ)				x	x	***CN***
MS + IR	x	x	x	x		**CN, CP, CO**
SQ + MS			x	x		**CN, CP, CO**
SQ + IR			x	x		**CN, CP, CO**
SQ + MS + IR				x		**CN, CP, CO**
water (control)	x	x	x	x		**D**, *Anthocoris, Orius*
caryophyllene					x	?
geraniol					x	Syrphids
octyl aldehyde					x	?
2-phenyl ethanol					x	Syrphids, **CP**

CN = *C. nigricornis*, **CO** = *C. oculata*, **CC** = *C. coloradensis*, **CP** = *C. plorabunda*, **D** = *Deraeocoris brevis*

had plots in four different locations this year (in addition to the trials above). The different attractants evaluated at each orchard were slightly different, in part because of the different sizes of orchards available (Table 1). We tested iridodial (a lacewing pheromone from Sterling International, Inc.) alone and in combination with methyl salicylate and a three way combination of iridodial + methyl salicylate + squalene. In the combination lures, we tended to get a mixture of species, primarily *Chrysopa oculata* when iridodial was added to the combination. Some of the differences in species combination are undoubtedly a result of local species differences, but in all locations where we used a squalene or squalene in combination with another lure resulted in large captures of *C. nigricornis*.

Fig. 2. Chickadee feeding on CM larvae (top). Wooden block cleaned out by bird feeding (bottom).

None of the lures in any locations caught significant numbers of ladybird beetles, but we did have some lures that increased trap captures for *Deraeocoris*, *Anthocoris*, syrphid flies, and tachinid flies, but these seemed to be location specific.

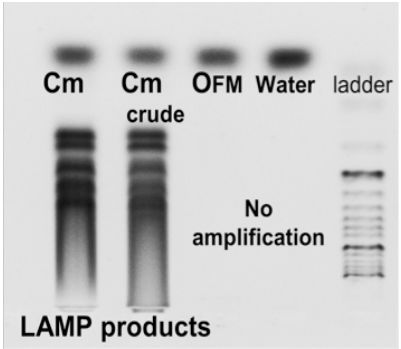
Plan for next year: We will expand our studies to new chemicals to attempt to find a lure that works well for ladybird beetles, and try some different combination lures to attempt to increase trap catch for *Deraeocoris*, *Anthocoris*, syrphid flies, and tachinid flies. We will also try different types of traps to see if we can enhance the capture of some species using visual cues, such as yellow colored traps.

Objective 3A. *Video monitoring*: A CM cocoon monitoring system was designed using a 4-camera digital video recorder designed for police and public bus surveillance. The DVR and the cameras run directly on 12 v DC, allowing monitoring at sites without power. Each camera was modified for close-up observation by taping a lens from an inexpensive field loop to the front of the camera. Two cameras had built-in infrared lighting and two cameras were equipped with a custom 12 v LED that uses less electricity. Two such DVR's and cameras (8 total) were tested in the last week of August and early September. Virtually no predation by insects was recorded over this short period, but bird predation was observed. Figure 2 shows one example of a predation event, in this case a chickadee that has a CM larva in its beak. Within just a couple of minutes that bird completely removed and consumed the five CM cocooned in the wooden block.

Objective 3B. *Gut content analysis methods to replace PCR:* Two approaches are being pursued: 1) development of a polyclonal antibody based on cocooned larval hemolymph proteins (storage or diapause proteins) and 2) development of the loop-mediated isothermal DNA amplification method known as LAMP. At the time of writing this report, we are still seeking a DNA sequence coding for a hemolymph protein suitable for the antibody production. We hope by spring, or even by the reporting session, such a protein will be identified.

We have successfully designed a LAMP amplification system based on the internal transcribed spacer gene (ITS2) of codling moth. LAMP requires several long primers to amplify the DNA like PCR, but it is cheaper, requires less equipment and labor, and, once optimized, is more resistant to contamination than PCR and allows use of crude extracts. Figure 3 provides an example of our LAMP amplifications of CM both clean and crude DNA extractions, seen as a ladder like smear on the gel.

Fig. 3. LAMP assay showing selectivity and effects of crude vs. refined extracts.



Plan for next year: Video monitoring will be performed season-long and quantified more. Our work on the polyclonal antibody

will continue along with field collections that can be run when the antibodies are available. We will also optimize the system for fluorescent visualization in unopened tubes and apply the LAMP procedure to samples collected and stored.

Objective 4A. *Determining the impact of IGR's used for leafroller control on sterility of tachinid adults. Nemorilla pyste* reared from hosts that have been exposed to sublethal doses of Esteem (pyriproxyfen) and Intrepid (methoxyfenozide) are affected reproductively. Sterility is more frequent among flies that are reared from treated hosts, and flies that are able to reproduce do so at reduced rates. Flies reared from untreated larvae that were offered six hosts daily over the course of adult life produced an average of 140 eggs and 32 progeny per female fly. Flies reared from hosts treated with Esteem produced an average of 93 eggs and 19 progeny per female, and flies reared from hosts treated with Intrepid produced just 63 eggs and 6 progeny per female. Exposure to Esteem in the host also affected the sex ratio of progeny; there were 1.7 male progeny for every female produced, while flies reared from Intrepid-treated and untreated hosts maintained a 1:1 sex ratio in their offspring. Clearly, *Nemorilla* is highly sensitive to IGR exposure through the host, and exposure is detrimental to the rate of population increase in these parasitoids. The sublethal levels of the compounds that were used to treat the hosts in the lab are minute compared to field rates, and by the time the adult stage is reached, the flies have had an average of 20 days post-exposure to metabolize any of the compound that they were exposed to as larvae in the host.

Objective 4B. *Evaluating alternate hosts for overwintering of the two tachinid species, and their probable locations.* Field collections have been made in a variety of habitat types that are relatively common in and around fruit growing areas. These include orchard borders, riparian habitat associated with irrigation or natural water, and woodland areas (xeric steppe has been excluded based on lack of host species). A number of tachinid hosts have been collected from these habitats. Adult flies have also been collected in these habitats from sweep net samples and volatile traps. Most hosts and many flies still need identification. Of the hosts of identified importance to both *Nemorilla pyste* and *Nilea erecta* in central Washington, the most common is *Choristoneura occidentalis,* the western spruce budworm. *C. occidentalis* is abundant on evergreen trees and is physically and behaviorally similar to the orchard host OBLR, and like OBLR overwinters as small larvae, so it is not an overwintering host. Populations of OBLR, PLR, and other Tortricidae with similar biology are common on native deciduous trees including willows, cottonwoods, alder, dogwood and oceanspray.

Objective 4C*. Evaluating the longevity of tachinids under field conditions.* On the first day of life, male and female *Nemorilla pyste* were placed singly in plastic vials (150 ml) with screened openings, which were housed in LPD traps and hung in trees. Vials were checked daily for fly survival. In general, survival was very poor in the vials; less than 50% of flies survived past day 2 and all flies died by the 5th day. Flies contained in similarly sized plastic cups in the lab, but also provided with water, did not reach 50% mortality until day 4, and 100% mortality did not occur until day 13. Flies contained in the lab and provided with carbohydrate (honey-water) did not reach the 50% mortality threshold until day 16, and 100% mortality was not reached until day 51. While field mortality estimates should be much lower than lab estimates, it seems that the current method of evaluating field mortality of the flies is not providing reasonable data. This is probably because; a) flies were too confined to efficiently thermoregulate, b) fly survival is apparently highly dependent on access to fluids, and longevity is enhanced by access to sugary fluids.

Plan for next year: Research next year in Objective 4A will concentrate on the exposure of adult flies to residues and evaluating their reproductive response. In Objective 4B, we will make more collections and identifications of possible overwintering hosts. In Objective 4C, we will use larger enclosures that allow greater range of movement for the flies to more effectively regulate their temperature, as well as provide some fluids.

Objective 5. This objective is scheduled to occur in the last year of the grant as more details on natural enemy phenology become available.

CONTINUING PROJECT REPORT **YEAR**: 1 OF 2

Project Title: Management of codling moth and leafrollers in apple orchards

PI: Jay F. Brunner
Organization: WSU-TFREC
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Email: jfb@wsu.edu
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State/Zip: WA 98801

Cooperators: Mike Doerr, WSU-TFREC; Steve Gacrzynski, USDA-ARS-Yakima; John Dunley, WSU-TFREC; Ashfaq Sial (graduate student), WSU-TFREC.

Total Project Request: **Year 1***:* $30,881 **Year 2: $30,212**

Other funding Sources

Agency Name: Washington Commission on Pesticide Registration
Amount requested or awarded: $28,324
Notes: Funding of WSCPR contingent on funding from WTFRC.
Agency Name: Private Chemical Companies
Amount requested or awarded: ≈ $35,000
Notes: Funding by private companies is variable and not predictable. The amount shown is based on experience over the last three years. Funds go towards leafroller colony rearing, field surveys, and temporary labor and travel.

The financial information provided in addition to sponsor support simply communicates research program support costs vs. specific project cost-share commitment.

WTFRC Collaborative expenses: NONE

Budget 1:
Organization: WSU-TFREC **Contract Administrator:** ML Bricker; Kevin Larson
Telephone: 509-335-7667; 663-8181 X221 **Email:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2008	2009
Salaries [1]	18,170	**18,879**
Benefits [1]	7,240	**7,563**
Wages [2]	3,000	**1,500**
Benefits	471	**270**
Equipment	0	**0**
Supplies [4]	1,000	**1,000**
Travel [3]	1,000	**1,000**
Miscellaneous	0	**0**
Total	30,881	**30,212**

Footnotes:
[1] Kathleen Pierre (3 months - Associate in Research) and Mike Doerr (2 months - Administrative Professional).
[2] Temporary or hourly workers.
[3] Pays for a vehicle used part-time on this project plus fuel and maintenance costs.
[4] Leafroller diet components, plastic Petri dishes, glassware.

Objectives:

1. Develop baseline toxicity bioassays for codling moth and leafroller of new insecticides under development.
2. Select populations of leafrollers (in the laboratory) to determine their inherent potential to develop resistance to selected insecticides.
3. Develop molecular markers to use as a tool for early detection of resistance development in leafrollers and codling moth.
4. Survey codling moth and leafroller populations using discriminating concentrations for key insecticides.
5. Characterize cross-resistance in leafrollers between old and new insecticides.
6. Evaluate new insecticides for control of codling moth and leafrollers in field tests.

Significant findings

1. Field-aged bioassays and field trials confirmed previous laboratory results showing that Altacor (chlorantraniliprole) has activity against codling moth eggs, primarily when laid on residues. The same effects were not observed for Belt, a new insecticide in the same class, which at least partially explains why this product does not provide control of codling moth in field tests.
2. Laboratory selection for resistance in obliquebanded leafroller to Altacor (chlorantraniliprole) and Delegate (spinetoram), to insecticides registered for use in 2008, showed that after five or four generations, respectively, resistance to Altacor were seven times that of the susceptible laboratory colony while resistance to Delegate were only 3.5 times that of the laboratory colony.
3. Every population of obliquebanded leafroller collected in the field (6) showed significant levels of resistance to Altacor (chlorantraniliprole) relative to the laboratory colony. Resistance ratios ranged from two to five. There was some suggestion from the data that resistance to Altacor was correlated to resistance to the organophosphate insecticide azinphosmethyl (Guthion).
4. The field collected obliquebanded leafroller populations showed the same level of resistance or enhanced susceptibility to Delegate (spinetoram) as they did to Success (spinosad). These data demonstrate that resistance to Success will be conferred on Delegate as was expected.
5. Data from 2008 show obliquebanded leafroller populations resistance to Proclaim (emamectin benzoate) for the first time since it was registered in 2006. Previous data had not shown any sign of resistance in field collected populations of leafrollers.
6. An international effort to characterize baseline resistance in codling moth to Altacor did not reveal any concerns for resistance, though there was considerable variation in the response of different populations to discriminating concentrations.
7. Ashfaq Sial, the graduate student working on this project, has demonstrated his abilities in molecular work by isolating a gene that confers resistance to organophosphate insecticides, Ace-1 gene, and cloning and sequencing it. While this is not novel information is shows a step in methods development that can be applied to new insecticides.
8. Field trials with Delegate and Altacor confirmed earlier studies, which showed them to be highly effective leafroller control products.

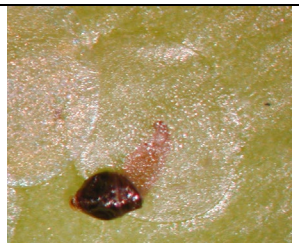
Methods:

Methods used in this project were outlined in last year's new project proposal and have not changed significantly enough to warrant their repetition here. If there are specific questions with regard to methods, consult the 2007 new proposal or contact the PI for more information.

Results and Discussion:

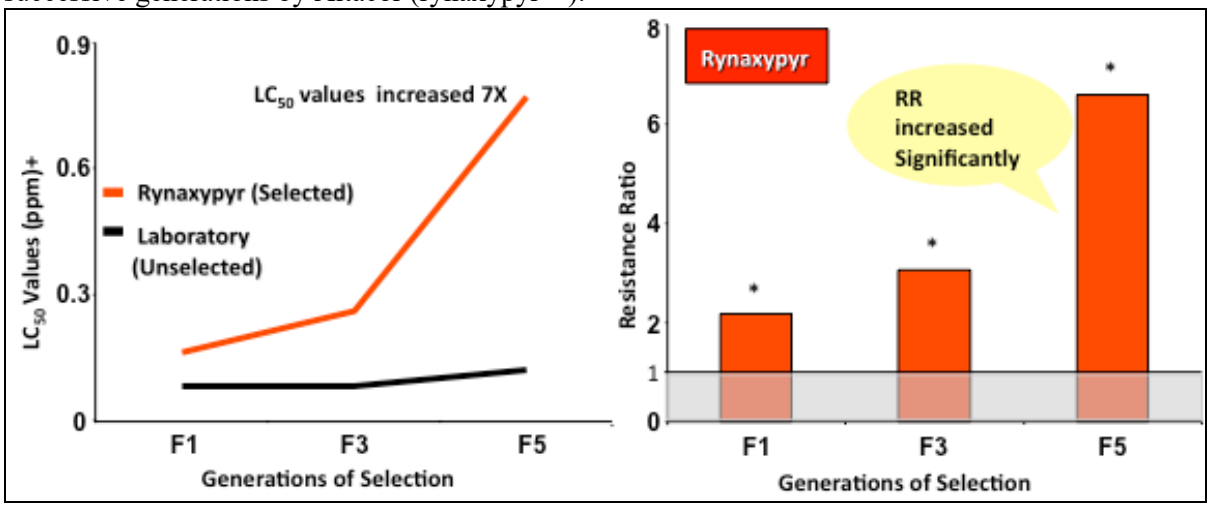
Baseline Bioassays: Laboratory bioassays helped characterize the effect of Altacor on codling moth eggs. The combined ovicidal, ovo-larvicidal and true larvicidal activity of this product helps explain its potency against this key pest (Fig. 1). Another new insecticide, Belt, showed poor ovicidal activity against codling moth at least partially explaining why it does not provide robust control of this pest in the field.

Figure 1. Ovicidal, ovo-larvicidal, and larvicidal affect of Altacor on codling moth.

		
True ovicidal activity. The larva died within the egg.	Ovi-larvicidal activity. The larva died in the process of exiting the chorion.	True larvicidal activity. The larva exited the egg, fed briefly, and died in close proximity to the egg.

Selecting for Resistance: One way to determine the risk of resistance development is to select populations in the laboratory over successive generations and determine if and at what rate tolerance to a chemical develops. We have selected 2,000 obliquebanded leafroller neonates with an LC_{70} concentration each generation. The LC_{70} value increased as tolerance was expressed more and more. Selection with Altacor resulted in a significant increase in the LC_{50}, resistance ratio of more than 2, while after five generations the LC_{50} value had increased almost seven fold relative to the unselected laboratory colony (Fig. 2). In addition heritability (h^2) had declined in the Altacor selected population indicating that much of the heterogeneity in the population has been selected against.

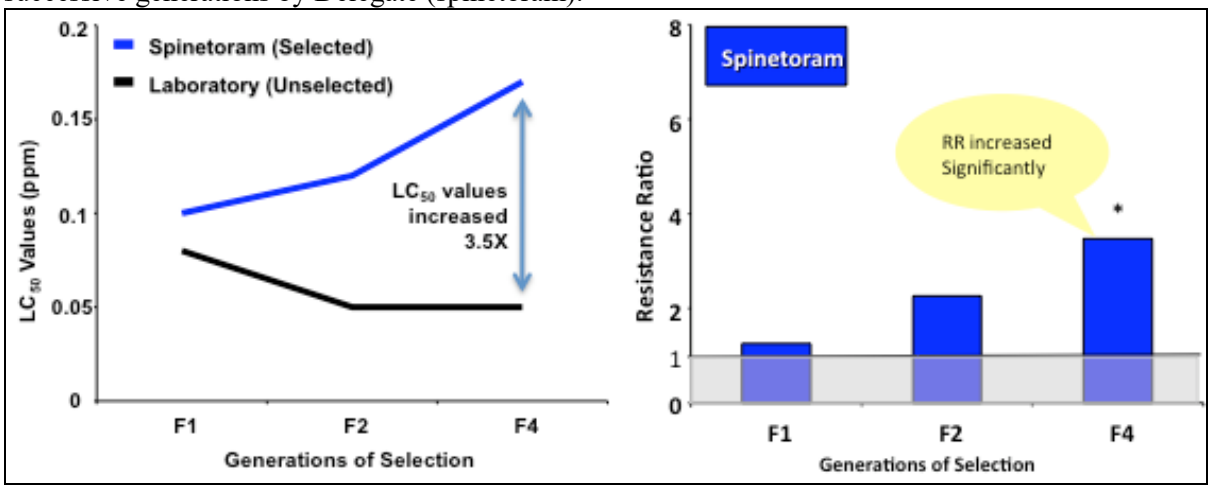
Figure 2. Increase in LC_{50} values and resistance ratios for obliquebanded leafroller selected on successive generations by Altacor (rynaxypyr™).



After four generations of selection with Delegate the LC_{50} value had increased only about 3.5 times (Fig. 3) but this represented a significant resistance ratio. However, the heritability (h^2) had not declined in the Delegate selected population indicating that there was more heterogeneity in the population yet to be selected against. These data demonstrate the risk of these two new insecticides to

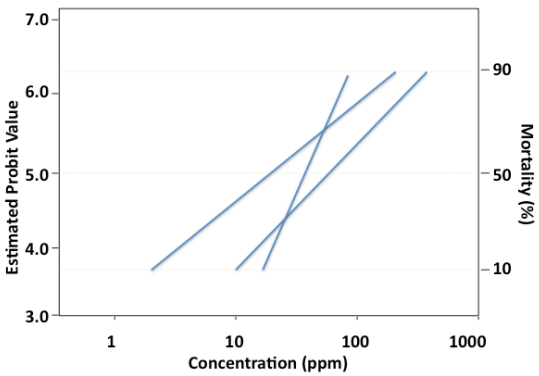
resistance development and underscores the need to follow sound resistance management strategies to at least slow the development of resistance in the field. *The selected populations form the basis for exploring the biochemical basis for resistance and eventually the molecular expression of that resistance and the potential hope that markers can be developed.*

Figure 3. Increase in LC_{50} values and resistance ratios for obliquebanded leafroller selected on successive generations by Delegate (spinetoram).



Surveys of Field Populations: A survey of codling moth populations from across the state revealed no concerns with tolerance to Delegate or Altacor. Delegate data were from topically treated adults using technical spinetoram dissolved in acetone. The demonstration that this method can provide reliable and repeatable dose-response lines (Fig. 4) will allow us to use moths captured in pheromone traps to assess more codling moth populations than is possible if bioassays are restricted to neonates. These data provide baselines for future comparisons where resistance is considered a potential problem.

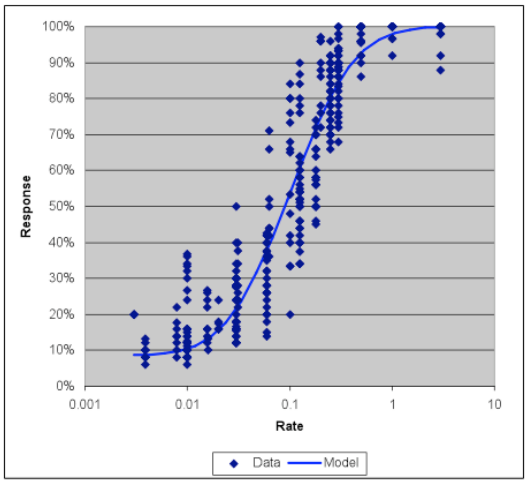
Figure 4. Dose-response lines for codling moth adults captured in pheromone traps and treated topically with technical spinetoram.



Baseline data were also generated against codling moth and obliquebanded leafroller for a new chemical of the same class as Altacor. This product appears very promising as another control tool for fruit pests.

We have participated in an international project looking at susceptibility of codling moth to Altacor. This has been a very good collaborative experience and data thus far shows no major difference in tolerance between field and laboratory (susceptible) populations (Fig. 5).

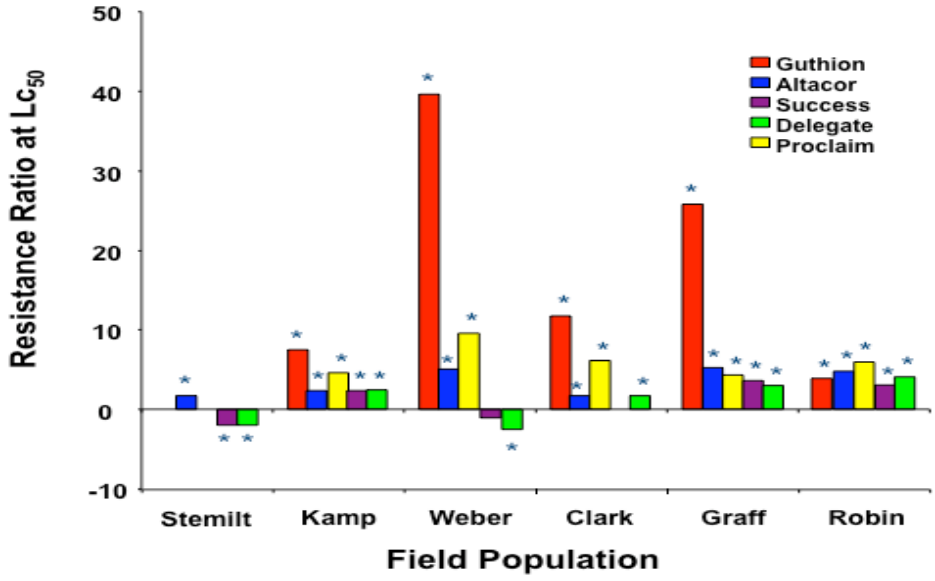
Figure 5. Pooled US data of percent mortality of codling moth neonates exposed to different concentrations of Altacor.



Field populations of obliquebanded leafroller were collected from six different orchards in 2008. Populations were reared in the laboratory and tested

using a diet incorporation bioassay to determine their susceptibility to Guthion, Altacor, Delegate, Success and Proclaim. Results of these bioassays are shown in Fig. 6. There were not enough individuals reared to test each insecticide for each population, LC_{50} values were determined for Altacor, Delegate and Success with each population. Every population of obliquebanded leafroller collected in the field (6) showed significant levels of resistance to Altacor (chlorantraniliprole) relative to the laboratory colony. Resistance ratios ranged from two to five. These preliminary data did not seem to suggest any correlated cross-resistance to the organophosphate insecticide Guthion (azinphosmethyl). The obliquebanded leafroller populations showed the same level of resistance or enhanced *susceptibility* to Delegate (spinetoram) as they did to Success (spinosad). These data demonstrate that resistance to Success will be conferred on Delegate as was expected. Resistance to Proclaim (emamectin benzoate) was documented for the first time since its registration in 2006. Previous data had not shown any sign of resistance in field-collected populations of leafrollers.

Figure 6. Resistance ratios of five insecticides from six obliquebanded leafroller populations, 2008.



* above bar denotes significant resistance ratio.

These field-collected populations will continue to be reared in the laboratory and used in further selection experiments to determine if the mechanism of resistance noted from the selected laboratory population is the same as that expressed in the field populations. It is possible that two different mechanisms are functioning in these populations.

Mechanisms of Resistance and Development of Molecular Markers: Now that resistant populations, field-collected and laboratory selected, are available the next phase of this research will focus first on testing resistant strains with synergists, PBO and DEF, that will begin to characterize if certain enzymes, esterases, glutathione-*S*-transferases, mixed function oxidases, are involved in degradation of the insecticide. If one or more biochemical targets are identified likely then characterization of the genes involved will be attempted using standardized approaches. This work will be conducted in cooperation with Dr. Garzynski.

Efficacy Evaluations: Ten field trials were conducted in 2008 to evaluate new insecticides for efficacy, timing and rates against codling moth and obliquebanded leafroller. Thirteen different active ingredients were tested in these trials. Key studies showed that Altacor has ovicidal activity,

which provides flexibility in its use pattern in apple and the opportunity to coincidentally control codling moth eggs and leafroller larvae early in the season. Most of these efforts are supported through gift grants from private chemical companies. Data from these trials form the basis for recommendations in WSU Extension Bulletin EB-0419 "Crop Protection Guide for Tree Fruits in Washington".

Obliquebanded leafroller larvae were controlled by a blossom thinning application of lime sulfur. This was a hand-gun application so preliminary information would need to be validated using standard airblast equipment.

Trt	Insecticide	Rate (form/acre)	Live Larvae 12 DAT
1	Rex Lime Sulfur 28%	10 gallons	2.3b
2	Untreated		26.8a

Means in the same column followed by the same letter are not significantly different (*P*=0.05, Tukey Kramer HSD).

In two large plot field trials (unreplicated) Altacor and Delegate provide excellent control of obliquebanded leafroller. We also conducted replicated small plot trials in 2008 against overwintered and summer obliquebanded leafroller that showed very good results.

Obliquebanded leafroller larval control at petal fall.

Trt		Rate	Total number OBLR/40 shoots					
				7 DAT				14 DAT
No	Insecticide	(form./acre)	Pretrt	Larvae	Pupae	Dead	Feeding	Larvae
Apple Trial								
1	Altacor 35WG	3 oz	4.00	0.06	0.02	0.88	6.54	0.00
2	Proclaim 5SG	4 oz	4.30	0.46	0.00	0.60	5.10	0.32

Trt		Rate	Total number OBLR/40 shoots				
				10 DAT			
No	Insecticide	(form./acre)	Pretrt	Larvae	Pupae	Dead	Feeding
Cherry Trial							
1	Altacor 35WG	3 oz	3.00	0.00	0.00	0.30	4.50
2	Delegate 25WG	7 oz	3.30	0.00	0.00	0.10	2.70

DAT - Days After Treatment