Apple Crop Protection Research Review

CONTINUING PROJECT REPORT **YEAR**: Year 1 of 3
WTFRC Project Number: CP-10-103

Project Title: Chlorochroa ligata pheromone and development of management strategies

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Cooperators: Mike Doerr, WSU-TFREC, Wenatchee, WA; Clive Kaiser, Oregon State University, Milton-Freewater, OR; Kent Daane, UC Berkeley, Berkeley, CA.

Total Project Request: **Year 1**: 26,526 **Year 2: $25,649** ***Year 2 w/BMSB: $45,949***

Other funding sources

Agency Name: **California Pistachio Commission**
Amt. requested: $8,000
Notes: Work to be done in CA

Agency Name: **Blue Mt. Horticulture Association**
Amt. awarded: $5,000
Notes: Work to be done in eastern OR

Agency Name: **SCRI**
Amt. requested: $125,000 (requested)
Notes: Collaborative national project

Agency Name: **SCBGP**
Amt. requested: $50,000 (requested - tentative)
Notes: Collaborative project with OR

WTFRC Collaborative expenses: None

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

Item	2010	2011	2011-BMSB	2011-total
Salaries (Doerr; 0.08333 FTE)	0	**5,248**	**0**	**5,248**
Benefits (Doerr)	0	**1,684**	**0**	**1,684**
Wages	7,000	**6,400**	**4,000**	**10,400**
Benefits (15%)	1,036	**960**	**600**	**1,560**
Equipment	0	**0**	**0**	**0**
Supplies (traps and lures)	5,000	**0**	**5,000**	**5,000**
Travel	500	**1,000**	**500**	**1,500**
Miscellaneous	0	**0**	**0**	**0**
Total	13,536	**15,322**	**10,100**	**25,422**

Footnotes: Supplies are for construction of traps.

Budget 2
Organization Name: UC Riverside **Contract Administrator:** Mayela Castillo
Telephone: 951-827-4816 **Email address:** mayela.Castillo@ucr.edu

Item	2010	2011
Salaries (4 weeks post-doc)	6,240	**4,160**
Benefits (4 weeks post-doc)	1,750	**1,167**
Wages	0	**0**
Benefits	0	**0**
Equipment	0	**0**
Supplies	5,000	**5,000**
Travel	0	**0**
Miscellaneous	0	**0**
Total	12,990	**10,327**

Footnotes: Supplies are for production of pheromone.

Budget 3
Organization Name: OSU **Contract Administrator:** Cynthia Cox
Telephone: 541-737-4066 **Email address:** Cynthia.cox@oregonstate.edu

Item	2010	2011	2011BMSB
Salaries	0	0	**0**
Benefits	0	0	**0**
Wages	0	0	**4,000**
Benefits (14.8%)	0	0	**600**
Equipment	0	0	**0**
Supplies	0	0	**0**
Travel	0	0	**500**
Miscellaneous	0	0	**0**
Total	0	0	**5,100**

Budget 4
Organization Name: WSU, Skamania County Extension **Contract Administrator:** Joan Root
Telephone: 509-335-2885 **Email address:** rootj@wsu.edu

Item	2010	2011	2011BMSB
Salaries	0	0	**0**
Benefits	0	0	**0**
Wages	0	0	**4,000**
Benefits (14.8%)	0	0	**600**
Equipment	0	0	**0**
Supplies	0	0	**0**
Travel	0	0	**500**
Miscellaneous	0	0	**0**
Total	0	0	**5,100**

Objectives:

1. Improve and scale up the production of the pheromone of *Chlorochroa ligata*. (DONE)
2. Determine the optimized release rate for the *Chlorochroa ligata* pheromone. (DONE)
3. Develop life history and ecology information for *Chlorochroa ligata*. (years 1 and 2)
4. Determine the potential for a dual lure for *Chlorochroa ligata* and *Euschistus conspersus*. (year 2)
5. Evaluate the concept of an attract-and-kill station for stink bugs. (years 1 and 2)
6. Survey for presence of the brown marmorated stink bug (BMSB) in key regions of eastern WA.
7. ***BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug (year 2)***

Significant Findings:

- Synthesis of *C. ligata* pheromone was challenging due to two steps in the five-step process that represented bottlenecks in production of high quality pheromone. While collaborative efforts with a private company producing the pheromone were encouraging we never received any product.
- There was little difference in release rates of *C. ligata* pheromone from polyethylene lures of different thickness in the laboratory or under field conditions. Therefore all four lures were evaluated in the field for attraction.
- *C. ligata* pheromone lures made of polyethylene of different thickness placed in pyramid traps captured the same number of bugs in two different trapping studies. The slow release (evaporation) rate of the *C. ligata* pheromone may be a limiting factor in using it as a monitoring tool.
- When multiple *C. ligata* pheromone lures (3 or 10) were added to traps the number captured increased by 5 to 6 times compared to a trap with only one lure.
- *E. conspersus* pheromone release rates from polyethylene lures of different thicknesses increased as the thickness decreased. The thinnest lure's release rate was about 4 times that of the commercial lure. It should be possible to use a polyethylene lure partially covered with foil to reduce the pheromone release rate and more closely approximate that of the commercial lure.
- A commercial *E. conspersus* lure captured ten times more bugs than the *C. ligata* lures.
- Traps baited with the *E. conspersus* or *C. ligata* pheromones attracted (captured) primarily conspecifics, that is, there own species.
- Trap captures of *E. conspersus* and *C. ligata* tracked seasonal activity, indicating that each species has only one generation per season.
- Laboratory screening of insecticide residues showed that Thiodan, Lannate and two pyrethroids (Danitol and Warrior) were most effective in killing *E. conspersus* adults.
- Treating the panels of pyramid traps with insecticide did not prove to be an effective way of intoxicating stink bugs, probably because they crawled up the outside edges of panels.
- Most (70%) of the stink bug adults placed inside jugs escaped within four days. However, if a kill strip was placed in the jug or the inside was treated with an insecticide the numbers that escaped were very low.

- When trap captures of summer *E. conspersus* adults near orchard boarders was compared with fruit injury in the board row there was a good relationship (R^2=0.88), giving hope that traps could be a useful tool in indicating risk of crop damage.

Methods:

Synthesis of *C. ligata* pheromone (*year 1*). Millar's laboratory will work on methods of improving and scaling up the synthesis of the *C. ligata* pheromone to provide the quantities needed for field trials. In published work, Millar's group has shown that (methyl (*R*)-3-(*E*)-6-2,3-dihydrofarnesoate, methyl (2*E*,6*E*)-farnesoate, and methyl (*E*)-5-2,6,10-trimethyl-5,9-undecadienoate) is required for attraction in the field. Furthermore, they have shown an equal mixture of the (*R*)- and (*S*)-enantiomers (= the racemic form) is attractive. This is critically important, because the racemic form is much cheaper to produce. Working out an efficient synthesis of the pheromone will greatly increase the possibility of the pheromone being commercialized, so that it will become freely available to growers.


Fig. 1.

Optimize release rate of *C. ligata* pheromone (*year 1*). *C. ligata* pheromone was put in sealed polyethylene pouches with a cotton wick (Fig. 1), which were then placed in a fume hood in the laboratory and weighed at regular intervals to determine the evaporation (= release rate). After a range of release rates was identified candidate lures were placed in the field to determine which attract the most *C. ligata.* Traps were also baited with *E. conspersus* pheromone as a comparison with *C. ligata*. Traps baited with different release rate lures were checked every 2-3 days. Bugs trapped during each sample period were identified, counted and sexed. Lure trials were replicated at each of five locations and over time. Unbaited traps served as controls.

Traps. New pyramid traps were constructed from expanded PVC panels and painted yellow. Traps were 3 feet tall with an 18" base. A one-gallon clear plastic jar was fixed to the top to collect bugs (Fig. 2).


Fig. 2.

Life history of *C. ligata (years 1 & 2)*. Trapping began mid-May, slightly later than had been planned, but still in time to capture significant numbers of overwintering bugs. Trapping focused on the border areas of orchards. Traps were checked weekly, bugs removed, counted, sexed, and life stages recorded. By monitoring over the entire season we hoped to assess if the *C. ligata* pheromone is attractive to reproductively active and inactive forms, which will be important in developing monitoring strategies to make informed management decisions.

Dual attraction of *C. ligata* and *E. conspersus (year 2)*. This objective is planned for year 2 of the proposal.

Attract-and-kill station for stink bugs (*years 1 & 2*). We established colonies of *C. ligata* and *E. conspersus* in our greenhouse from field-collected individuals. We used transplanted mullein plant plus beans as a food source. We caged stink bugs (*E. conspersus*) in plastic cups treated with insecticides and monitored mortality at 1 h, 1d and 3d, after which mortality in the untreated controls increased beyond limits considered acceptable. Residual activity of candidate insecticides was assessed by treating cups with insecticide and placing them in the field for selected periods after which stink bugs were exposed to aged residues. Mortality was recorded 1 h, 1d and 3d after exposure. Stink bug adults were also exposed to insecticide treated panels to determine if they would become intoxicated. Stink bug adults were also exposed to insecticide residues and an insecticide tape inside the plastic jar on top of traps to determine rates of escape and mortality.

Results and Discussion:

Synthesis of *C. ligata* pheromone (*COMPLETED*). The Millar laboratory began synthesizing *C. ligata* pheromone in late winter of 2009-10. The pheromone synthesis process must have as few steps as possible to minimize costs. Each step must produce clean product with minimal byproducts. Starting materials and intermediates should be as cheap as possible. The synthesis process was challenging due to two of the five steps, which had low yield, still 20 grams of pheromone were delivered on 1 April and we began release rates studies. The initial pheromone production was sufficient to begin early trapping studies with optimized lures (Obj. 2). The Millar laboratory synthesized more pheromone and another 16 grams of *C. ligata* pheromone was provided on 1 July, which was sufficient to complete planned studies. Millar's laboratory also provided pheromone of another stink bug species, *Thyanta pallidovirens*, the red-shoulder stink bug, which was used in monitoring at some sites in the summer. We obtained commercial lures for *E. conspersus* and removed pheromone to use in the same kind of lure as we used for *C. ligata*. The Millar laboratory will provide *C. ligata* pheromone for 2011 research activities, but it is unlikely that the synthesis process can be tweaked to increase yield. This may make commercial production of the *C. ligata* pheromone expensive. We worked with a commercial company interested in producing *C. ligata* pheromone but they ran into the same barriers to efficient synthesis as experienced by the Millar laboratory.

Optimize release rate of *C. ligata* pheromone (*COMPLETED*). The release rate of *C. ligata* pheromone from polyethylene lures of different thicknesses did not differ significantly (Fig. 3). Pheromone released from a cotton wick was only about 25% higher than the thinnest polyethylene lure. The *C. ligata* pheromone is a relatively large molecule with a slow evaporative rate. In 14 days the *C. ligata* lures released between 4% and 7% of the total load under laboratory conditions. Under constant temperature conditions the fastest releasing *C. ligata* lure would last more then 150 days. Of course under higher temperatures in summer the release rate would be expected to be higher and therefore the lure would not last as long as predicted from laboratory studies.

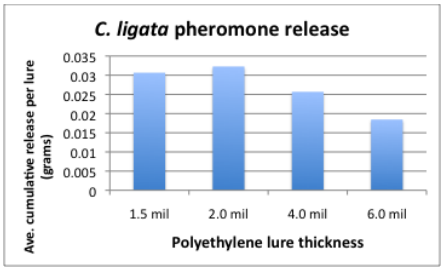

Figure 3.

C. ligata pheromone lures were field aged for 49 days and weighed every 7 days in the laboratory to determine pheromone loss. The release rate was low, similar to the laboratory and no real differences were observed between lures of different polyethylene thickness.

The polyethelene lures provided a very good release profile for the *E. conspersus* pheromone (Fig. 4), which is a smaller molecule relative to the *C. ligata* pheromone. The thinnest *E. conspersus* lure released 95% of the pheromone in 10 days with the slowest lure releasing 42% compared with the commercial lure releasing 27%. In the field the fastest releasing lure would probably last less than 10 days. More studies on the optimization of the *E. conspersus* lures under field conditions are needed.

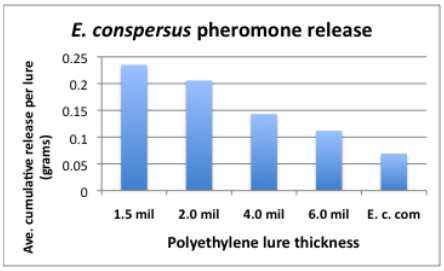

Fig. 4.

We also conducted some preliminary release rate studies with the *T. pallidovirens* pheromone using polyethylene lures. The release rate of this pheromone was even faster than that of *E. conspersus* with the fastest lure releasing 81% of the pheromone in only five days in the laboratory. We were not able to conduct field studies comparing lure release rate and capture of *T. pallidovirens* in 2010.

Four lures for *C. ligata* that were evaluated in the laboratory were also field tested by placing them in the jug atop traps (Fig. 2). Captures in *C. ligata* lure-baited traps were compared with a control trap (no lure) and a trap baited with a commercial lure for *E. conspersus*. There was no difference in the capture of *C. ligata* in traps baited with different lures (Fig. 5 - *C. ligata* data only) over a 47-day period. These data reflect the pheromone release rates observed in the laboratory where there was little or no difference in release rate was observed under constant temperature conditions.

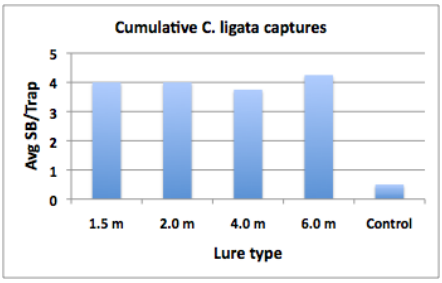

Figure 5. Average cumulative capture of *C. ligata* in polyethylene lures of different thickness.

In an attempt to enhance the attraction of *C. ligata* pheromone we conducted what would be described as a preliminary study in late summer. We compared captures in traps baited with a single lure, baited with three lures and baited with ten lures. The trap baited with three and ten lures captured between five and six times more *C. ligata* adults than the trap baited with the single lure. There is some hope that the attraction of *C. ligata* could be enhanced by using multiple lures in the same trap.

Compared to captures of *E. conspersus*, capture of *C. ligata* in lure-baited traps was low. *E. conspersus* baited traps captured more than ten times as many bugs as the *C. ligata* baited traps. It is possible that populations of *C. ligata* were much lower than *E. conspersus* in the areas that were trapped, but observations of stink bugs on native vegetation would not suggest that this was necessarily the case. These data do demonstrate that the commercial *E. conspersus* lure used in our studies was highly attractive providing a good tool for monitoring.

There was some cross over of attraction of stink bug species (see table below). Traps baited with the *E. conspersus* pheromone captured predominantly *E. conspersus* individuals. *C. ligata* baited traps captured *C. ligata* individuals but also captured *E. conpersus* and traps baited with the *T. pallidovirens* pheromone captured low levels of this species but many more *E. conspersus*. The apparent cross over attraction could have been due to the relative size of the local *E. conspersus* population and not attraction of this species to other species pheromone. These studies were only conducted at one location and late in the summer so should be repeated at more locations in 2011.

	Species captured		
Pheromone	E. conspersus	C. ligata	T. pallidovirens
Consperse	39.5	0.9	0.2
Ligata	4.7	2.6	0.4
Pallidovirens	4.5	0.4	0.4

Traps. Pyramid traps made of extruded polyethylene with a gallon plastic jug at the top provided a consistent and durable monitoring tool. The trap could be made cheaply by growers/consultants out of plywood or other composite materials. Anchoring traps with a central rod was necessary to keep them stable under high wind conditions.

Life history of *C. ligata (years 1 & 2)*. By combining data from all trapping studies we were able to see the seasonal life history of *E. conspersus* and *C. ligata*. Because we did not get traps out as early as hoped we missed the initial adult activity of both species. Both

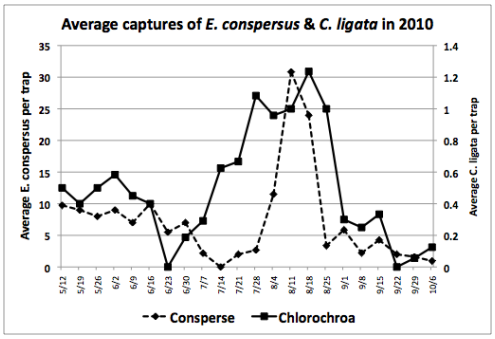

Figure 6. Average trap captures of *C. ligata* and *E. conspersus* in 2010.

species appear to have only one generation per year, confirming what has been reported in the literature and earlier studies on WA (Fig. 6). New *C. ligata* adults began appearing in early July with captures peaking in late July and early August. New *E. conspersus* adults did not appear until late July with a definite peak in mid-August. These data could have been influence by orchard border spraying, which began in mid- to late-July in most orchards.

Dual attraction of *C. ligata* and *E. conspersus (year 2)*. No activity was planned for this objective in 2010.

Attract-and-kill station for stink bugs (*years 1 & 2*). We evaluated several different insecticides against stink bugs to gain an understanding of which might be the best candidates for attract-and-kill studies. We established colonies of both *C. ligata* and *E. conspersus*, but because more *E. conspersus* were captured early in the season and they began to reproduce sooner, we used this species in all our insecticide studies. The initial screen was to expose *E. conspersus* adults to residues on plastic cups and record mortality over three days. We examine residues of the full field rate in a dilute concentration (1X – typical of a handgun application), a concentration 20% of the full rate (0.25X) and a 4X concentration (equivalent to a typical airblast sprayer application).

Insecticide residues that were most toxic in the initial laboratory screen were the carbamates Lannate (methomyl) and Carzol (formetanate hydrochloride), the chlorinated hydrocarbon, Thiodan (endosulfan) and the synthetic pyrethroids Danitol (fenpropathrin) and Warrior (lambda-cyhalothrin). Most of the newer insecticides were not effective.

While pyrethroids provided a fast 'knock down', that is the bugs appeared dead, within a short time they had recovered and more were "alive" on day 1 compared to 1h after exposure. We took some of the best insecticides from the initial screen and exposed residues in cups in the field then exposed adult *E. conspersus* to these. Thiodan residues lasted over 28 days. Warrior residues were not as effective and had a short longevity under field conditions. There was some promise of the Thiodan+Warrior combination giving a fast knock down and good mortality.


Figure 7.

We treated trap panels with insecticides, Thiodan, Warrior or a Thiodan-Warrior mix, and put the traps in a collecting tray and released 15 *E. conspersus* adults on the tray (Fig. 7). The release was repeated five times for each treatment (75 total bugs). Twenty-four hours after release the location of bugs and mortality was recorded. Many bugs left the trays and were not found. In the control 41% were accounted for after 24h. Few bugs were found dead in the trap, indicating that they were not intoxicated while climbing panels on the way to the trap. This may in part be due to their behavior of climbing primarily on the edges of the panels when moving towards the trap. A few bugs were found dead in the trays suggesting that they became intoxicated when climbing on the panels.

We next treated the inside of the jugs atop the traps with a Thiodan-Warrior mixture or a kill strip containing DVDP. Twenty or twenty-five adult *E. conspersus* were placed into the jug. This activity was repeated three times. The number of bugs alive and dead in each jug was recorded each day for six days. In the *untreated control* only 68% of bugs remained in the jug after one day, only 3% were dead. After four days only 30% remained in the untreated control jug and half of these were dead. We therefore know that given the present trap design bugs that enter the jug can escape. The good news was that after day one those bugs remaining in the jug treated with the kill strip (78%) and Thiodan-Warrior (95%) treatments were dead. If traps are to be used as an attract-and-kill device the insecticide should be placed inside the jug to kill bugs after they enter.

We monitored stink bugs at ten locations in WA during 2010, five over the entire season and five only in the second generation. Since previous research had shown that the highest level of fruit injury from stink bug feeding occurs on orchard borders we sampled ten trees on the border closest to where traps were placed. We then compared the total capture only of *E. conspersus* with the fruit injury. There was a good relationship between total captures for the entire season, and for capture only in the summer, and fruit injury (Fig. 8). While these data should be considered preliminary they show promise of using trap captures of *E. conspersus* as an indicator of the risk of an orchard for fruit injury.

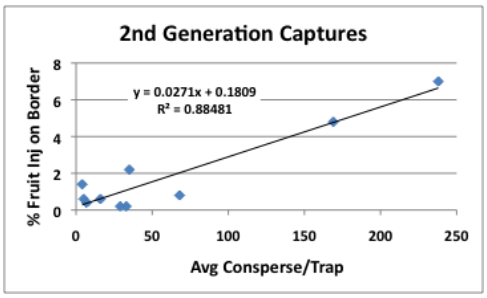

Figure 8.

Brown Marmorated Stink Bug (BMSB) – *Halyomorpha halys* (Stal): This is an exotic stink bug species first discovered in the US in the mid-1990s in Pennsylvania (Allentown). This year in the eastern US many soft fruit and apple orchards reported extremely high levels of damage (Fig. 9) and researchers in the mid-Atlantic region report high captures in traps. In 2004, it was reported from OR, and is now well established in the Portland, OR area. It has since spread north to Vancouver, south to Corvallis, east to Sandy, and has recently been found in Arlington, OR, just across the river from Roosevelt, WA.


Figure 9.

If there can be good news about a new pest it is that so far we have not identified any BMSB from our trapping for stink bugs in eastern WA, at least based on our preliminary evaluation of specimens collected. These data should not be viewed as conclusive however, because we were not using a pheromone known to be attractive to BMSB. It is reported in the literature that methyl *(E,E,Z)*- 2,4,6-decatrienoate is attractive to BMSB but it is not thought to be the actual pheromone of this species. Scientists are still working to identify the BMSB pheromone. We will strive to work with eastern scientists to obtain the best lure available and add monitoring traps for the BMSB to our research program in 2011 if the industry desires to fund this effort.

Comparative images of the BMSB (bottom row) and *E. conspersus* (top row) adults are shown to right.

Proposed additional activities – Objective 7: Additional funding requested for the BMSB is to initiate a monitoring program that will include trapping and visual observations of preferred hosts. Dr. Peter Shearer and Todd Murray have agreed to participate in this activity. They will coordinate sampling in the area of the Columbia Gorge while I will concentrate activities in the Yakima Valley and Central WA in association with sampling for *C. ligata* and *E. conspersus*. We will work with eastern researchers to evaluate release rates for the BMSB pheromone and incorporate this tool in our monitoring program as appropriate.

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

Project Title: Best practices for predator releases: lacewings, beetles, and mites

PI: Tom Unruh
Organization: USDA-ARS
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Organization: WSU-TFREC
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City/State/Zip: Wenatchee/ WA /98801

Co-PI(3): Dave Horton
Organization: USDA-ARS
Telephone: 509-454-5639
Email: david.horton@ars.usda.gov
Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato/WA/98951

Cooperators: Jim McMurtry, cooperating growers TBD

Total Project Request: **Year 1**: $79,117 **Year 2**: **$79,866** **Year 3**: $80, 680

Other funding sources

Agency Name: USDA-CSREES
Amount Requested/awarded: $2.7 million/NA
Notes: A multi-state multi-investigator project will be submitted in February 2011 to the OREI grant program. Predator release technology is part of the proposal

WTFRC collaborative expenses: None

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: (510) 559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	2010	2011	2012
Salaries[1]	29,230	29,230	29,230
Benefits	8,770	8,770	8,770
Supplies[2]	4,000	4,000	4,000
Travel			
Miscellaneous			
Total	$42,000	**$42,000**	$42,000

Footnotes: [1]Partial support for GS-6 technicians (benefits at 30%); [2]Purchase of insectary-reared green lacewings, ladybird beetles, and predatory mites.

Budget 2

Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker

Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2010	2011	2012
Salaries1	22,014	22,230	23,119
Benefits	1,851	1,884	1,960
Wages2	8,580	8,580	8,923
Benefits	172	172	178
Equipment			
Supplies3	1,500	2,000	1,500
Travel4	3,000	3,000	3,000
Total	$37,117	**$37,866**	$38,680

Footnotes: [1]Project Assistant (Graduate student, academic year); [2]Summer wages (graduate student); [3]Sampling supplies; [4]Travel to field sites; [5] WSU budget request has increased for 2011 and 2012 causing total grant to increase by $2,347 over that projected in 2010. While labor costs declined slightly from the initial request, travel and supplies increased the costs about $1150 per year.

Objectives:

1. Interview organic orchardists and managers who have recent experience in predator release and producers and distributors of predators to discover problems associated with releases and supply, and revise research details accordingly.

   Managers of organic production for Zirkle Fruit Co. and Stemilt Growers Inc. were interviewed both in person and by phone to learn more about common practices their growers used to release predatory beetles, lacewings, and mites. Additional interviews with organic managers and onsite visits to ranches that actively release predators are planned for spring 2011. Four vendors of lacewings, mites, and beetles were also interviewed by phone.

2. Develop and verify our capacity to differentiate between insectary-reared/released and naturally occurring predators using morphological or molecular traits.

   We can discriminate insectary-reared Chrysoperla rufilabris from endemic lacewings as larvae or adults. Convergent ladybeetle requires marking prior to release to differentiate them from native beetles. Field evaluations of marked beetles will commence in spring 2011. The predatory mite species collection and identification will be conducted in 2011 and 2012.

3. Make releases of lacewings and lady beetles, or predatory mites on two edges of several aphid-infested or mite-infested orchards and monitor populations of both pests and predators at release sites and non-release sites.

   In 2011 and 2012 all three predators will be released and evaluated in grower orchards.

4. Conduct field experiments to optimize stages to release, release timing, and test the use of feeding attractants or arrestants to maximize lady beetle and lacewing activity.

   Sprays of lacewing eggs were made in experimental settings to test organic adhesives. Galendromus occidentalis was released in a conventional orchard to test efficacy of one release method. Ladybeetle releases have not yet been tested. Field experiments to evaluate feeding attractants and additional predator release methods will be done in 2011and 2012.

5. Conduct laboratory experiments to compare efficacy of different insectary-reared species on the target pests.

   Feeding capacity, survival, and development of purchased Chrysoperla rufilabrus were compared to those traits for native Chrysopa nigricornis on diets of Rosy apple aphid and Woolly apple aphid. Feeding capacity at different temperatures will be examined in 2011 as this information may be critical for relating efficacy to timing of release in early spring.

SIGNIFICANT FINDINGS

- ✓ Growers manually apply lacewing eggs glued to paper, a labor-intensive approach.
- ✓ Only Beneficial Insectary Inc. produces *C. rufilabris*, all others are resellers.
- ✓ Hibernating ladybeetle collected in spring or fall and cold stored vary greatly in quality.
- ✓ Pesticide residues prevented predator mite establishment.
- ✓ Honeydew and waxes produced by Woolly apple aphid kill small green lacewing larvae.
- ✓ Purchased *C. rufilabris* develops and survives as well as the native *C. nigricornis.*
- ✓ *C. rufilabris* hatch successfully at temperatures corresponding to late March

RESULTS & DISCUSSION

Objective 1-Grower practices and needs. Organic managers for Zirkle and Stemilt outlined standard practices used on their ranches and on those of growers who pack with them. Lacewings were released as eggs glued to strips of paper, which were hung on branches by workers. Mites mixed with

corncob grit were dispersed into trees with a pollen blower or were placed into crotch of trees on infested bean plants. Ladybeetles were released in paper bags or boxes placed in orchard typically at night and after orchard irrigation. Both managers agreed that there was a need to reevaluate and optimize release methods and to evaluate efficacy of the releases.

Four insectary managers were interviewed and two (Rincon Vitova; Beneficial Insectary) were particularly helpful in providing potentially proprietary information, and in providing beetles and lacewings at cost. From these interviews, we discovered that the availability of ladybeetles is at the mercy of the weather. In wet years, the mountain overwintering sites of Convergent ladybeetle may be inaccessible due to snow pack well into early or mid summer. During wet winters, beetles for spring releases are likely to be those collected in the previous spring or summer. In warm, dry years, beetles may be collected in both fall and late winter. The time of collection and time in storage was found to affect energy stores (fat body), and may thus affect capacity to fly and produce eggs after release. Figure 1 shows differences in beetles collected in spring of 2009 and received in May of 2010 (10 months cold storage) and those collected in late June and received in August (2 months storage).

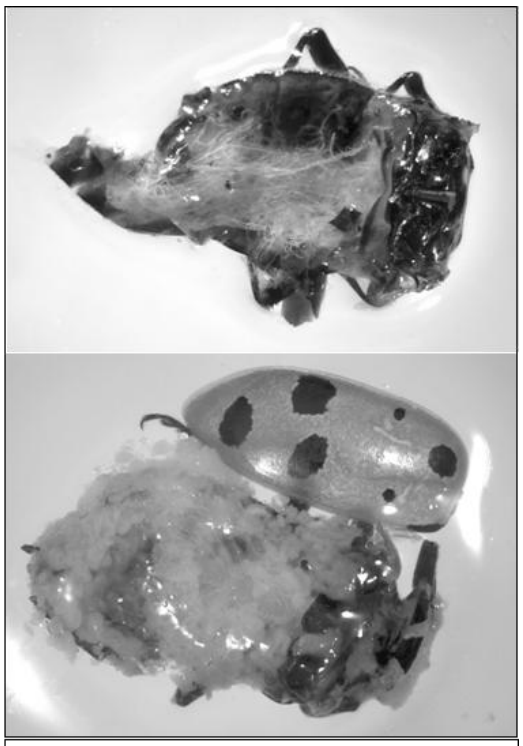

Figure 1. Convergent ladybeetle stored for 10 months (top) and for 2 months (bottom). Fat body completely obscures the strings of tracheae in beetle on bottom.

<u>*Objective 2 -Differentiating species.*</u> We are able to differentiate between native Chrysopids and *C. rufilabris* from insectaries, both as larvae and adults. We have previously determined in our cover crop work that we can mark (with protein markers) and detect the markers on ladybeetles.

<u>*Objective 3- Releases in grower orchards.*</u> This objective was intended for year 2 and 3 but preliminary releases were conducted with *Galendromus occidentalis* to test evaluation methods.

Methods: Mites were released in a mature block of 'Red Delicious' apples at a commercial orchard near Pasco, WA. Trees were planted at 7 x 15 ft row spacing. Plots consisted of a single release tree, the four trees immediately within the row and across the row in both directions from the release tree, and the trees directly adjacent to those four trees (Fig. 2). Western predatory mites, *G. occidentalis* (Typhs) from the Sterling Insectary were deployed onto the release trees on 14 July. The treatments consisted of predatory mite levels of 0, 5000, and 15000 mites per acre (0, 12, and 36 mites per tree respectively). Releases were done by transferring adult female *G. occidentalis* to a bean leaf, and attaching the leaf to the tree with a binder clip. There were six replicates per treatment.

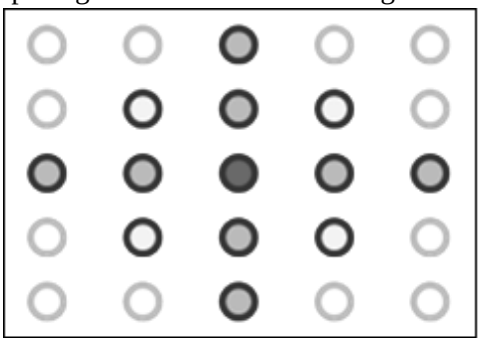

Figure 2. Center release tree, all sampled trees with dark circle.

Two sampling methods were used to assess predator and prey mite populations. The first was an *in situ* count using OptiVisors. Fifteen leaves per tree were examined in the field without detaching the leaves. Only motile stages of *Tetranychus urticae, Panonychus ulmi,* and *G. occidentalis* were counted. The second method was a standard brushed leaf sample, counted in the lab using a binocular microscope. Five leaves from each of four trees on the diagonal from the release tree (Figure 2) were collected from the field as a composite sample. This 20-leaf sample was brushed onto sticky plates using a leaf-brushing machine. All stages of *T. urticae, P. ulmi, G. occidentalis, Aculus schlechtendali,* and *Zetzellia mali* were counted. On 2 Aug, *in situ* counts and leaf brush counts were made on the 9 sample trees. This count allowed us to compare the two sampling methods.

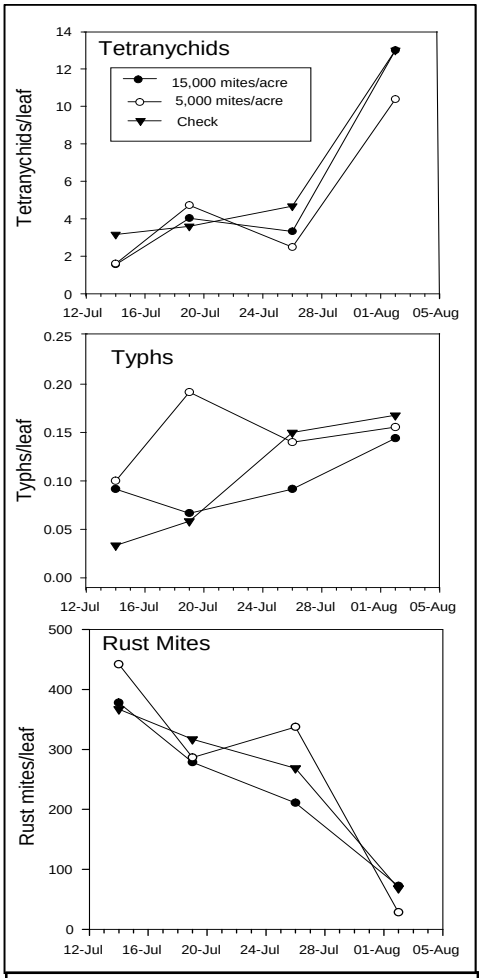

Figure 3. Summary of mite densities in samples counted by mite brushing

A bioassay was done to determine if residual pesticides on the orchard leaves affected the survival, fecundity and prey consumption of the commercially reared *G. occidentalis*. Apple leaves from both the sprayed grower orchard where predators were released and an untreated research orchard at WSU-TFREC were prepared by brushing them in the leaf-brushing machine to remove arthropods. Two cm disks were cut from the leaves and placed on water-saturated cotton in small cups. Twenty female *T. urticae* were added to each leaf disk and allowed to oviposit for 24 h. After 30 eggs had been laid on each disk, the females were removed. If egg numbers exceeded 30, the excess eggs were also removed. Egg location was marked with a felt-tip pen. One female *G. occidentalis* was loaded onto each leaf disk. The bioassay was evaluated at 24 and 48 h. *G. occidentalis* females were recorded as live, dead or missing. The number of remaining *T. urticae* eggs and *G. occidentalis* eggs were counted. *G. occidentalis* egg positions were then marked with a different color of felt-tip pen. After the 48 h evaluation, *G. occidentalis* females were removed from the disks. On the fourth day after the 48 h evaluation, the number and status of *G. occidentalis* life stages were counted.

Results: Counts of spider and rust mite were low at the time of predator release in mid-July. European red mite (ERM) was the dominant phytophagous mite species and it increased during the test (Figure 3; top panel) despite an application of Zeal on May 24. Rust mite populations were moderate initially, but declined during July (Figure 3; bottom panel. There were no statistical differences between treatment means for any mite species or group on any date. These findings provide no evidence that the released predators became established or had any effect on pest mites.

The *in situ* optiVisor mite counts (not shown) had consistently lower numbers than the leaf brush counts. *In situ* ERM counts had the best correlation with leaf brush counts, likely because their red color differentiated them from the leaf color. We had hoped the non-destructive *in-situ* samples would work but our inability to count all stages of typhs precludes this method.

Leaves from the sprayed grower block and those from the untreated block were similar in effects on mortality, prey consumption, or fecundity of the insectary-reared *G. occidentalis* (not shown). However, there was significantly poorer egg hatch and numbers of live larvae on the sprayed leaves, indicating some residues present on the leaves were sublethally toxic to the predators (Figure

4). Of the materials applied to the block both carbaryl and thiacloprid are known to have some level of toxicity to predators, although it seems unlikely that the toxic effect could have persisted for several months. The effects of other materials applied (emamectin benzoate, etoxazole, trifloxystrobin, and *Bacillus thuringiensis*) are not known. Bioassays of these materials would be helpful in determining why the predators failed to establish.

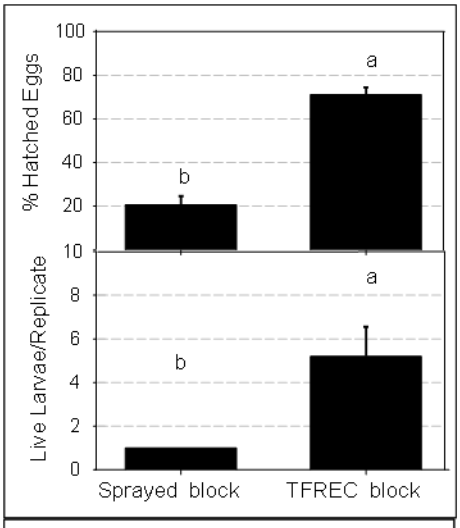

Figure 4. Differences in egg hatch and subsequent live larvae on leaves from sprayed grower block and unsprayed leaves from the WSU – TFREC

Objective 4 – Experimental studies to maximize release efficacy:

Two series of experimental field studies were conducted with lacewing eggs. First were tests of natural stickers to enhance lacewing egg adhesion when sprayed from a backpack air blast sprayer. One test included 10% and 20% egg white and water alone; egg white performed better than water as seen in Figure 5. Tests of sugar, soy and wheat flours showed little benefit (not shown).

In an experiment conducted in early November, hatch rates of lacewing eggs were observed in natural field temperatures (in ventilated white boxes). This timing was chosen because it closely mimics temperatures experienced in mid-March (Figure 6), the time of year at which releases are made. Insectary-purchased eggs were placed in an 8C incubator and a group of 200-250 eggs was placed out of doors on 7 consecutive days and hatch rates followed in relation to daily temperature. Figure 6 shows patterns for the eggs placed outside on the fir st 3 days after 1, 2, or 3 days of pre-incubation at 8C. The results show that after a delay of 3 days eggs hatch occurs synchronously with almost 50% hatch on the third day in the field. Hatch on subsequent days was more influenced by temperature patterns, with no hatch on November 9 due to low daytime temperatures. These results are positive, and show that *C. rufilabris* is likely to survive early spring temperatures.

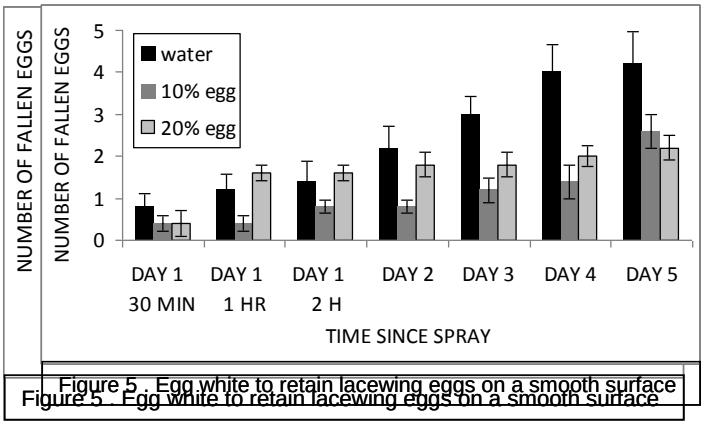

Figure 5 . Egg white to retain lacewing eggs on a smooth surface

Figure 5 . Egg white to retain lacewing eggs on a smooth surface

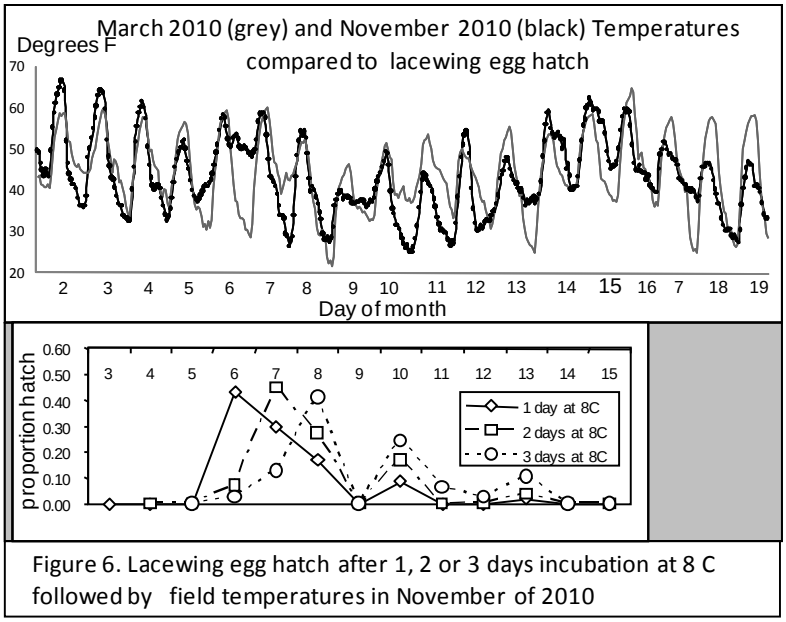

Figure 6. Lacewing egg hatch after 1, 2 or 3 days incubation at 8 C followed by field temperatures in November of 2010

Additional studies to evaluate feeding at low temperatures (objective 5) will be done in 2011; efficacy will be evaluated with predator releases in orchards in 2011 and 2012.

We assessed the effects of food availability on ladybeetle survival using beetles of optimal health (i.e., rich fat bodies; Figure 1). Beetles were confined to 9 cm Petri plates and provided one of four diets: 1) water only; 2) water and 10% sucrose; 3) water and 10% sucrose and Insect Food; and 4) water and 10% sucrose and aphids. Water and sucros e solution were provided through glass wool wicks; Insect Food was provided as a paste; pea aphid were provided on excised bell bean leaves at an intentionally low rate of 3-7 aphids/day/beetle. Aphids were provided 3 times per week while the other products were renewed weekly when Petri plates were cleaned. We hypothesized that aphid and insect food provision would support egg production and longer survival than sucrose or water. *Results*: Our hypothesis proved incorrect – over one month, no egg production occurred although mating was common; beetles fed insect food survived less well as summarized in Figure 7.

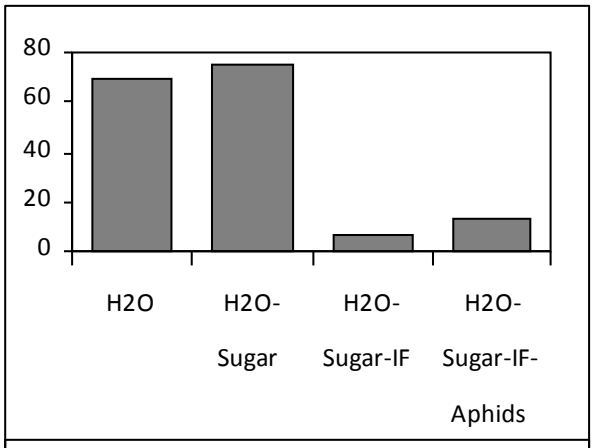

Figure 7. 32-day survival of *Hippodamia convergens* with different food sources

In a subsequent study, two cages containing 10 beetles each were provided a clean plant and water and in another set of two cages beetles were provided a plant with abundant aphids which was replaced 3-5 times/week as needed to allow beetles to feed *ad libitum*. *Results*: Beetles began laying eggs after 7 and 10 days in the two cages with aphids and produced 196 and 160 eggs in the two cages during the 2 week experiment. No eggs were produced in the aphid-free cages. These results are disappointing in one respect - they suggest a requirement for high rates of aphid consumption to stimulate oviposition, confirming similar observations in the literature.

Objective 5 – Feeding studies of insectary and native predators

We assessed whether insectary-purchased green lacewings (*Chrysoperla rufilabris*) fed and survived on a diet of two target pests, rosy apple aphid and woolly apple aphid. **Rosy apple aphid**. Our first study examined development time and survival (from egg hatch to pupation or adult emergence) of *C. rufilabris* and a resident lacewing, *Chrysopa nigricornis*. Eggs of *C. rufilabris* (purchased) and *C. nigricornis* (field-collected) were allowed to hatch in the laboratory. Newly hatched larvae were moved immediately into snap petri dishes, and fed *ad libitum* upon a diet of field-collected rosy apple aphid (plus a small section of apple leaf). We recorded survival, days to pupation, and days to emergence (at 22 ^{o}C). *Results*: The insectary-reared species developed and survived well on rosy apple aphid (Figure 8). Development of *C. rufilabris* was slightly more rapid than that shown by the native species (Figure 8; upper panel), likely due to size differences between the two species. Survival rates were very high for both species (Figure 8; lower panel).

Woolly apple aphid. Our second study explored survival of the insectary-reared lacewing (*C. rufilabris*) on a diet of field-collected woolly apple aphid. In this study, we also explored how age of lacewing larvae affected survival, due to early observations suggesting large differences in success of small and large larvae on a diet of this aphid (see below). Methods were similar to those used in the trial with rosy apple aphid, except that *C. nigricornis* was not included for comparison (we could not find *C. nigricornis* eggs in the field). *Results*: We found that newly hatched lacewings survived very poorly on a diet of woolly apple aphid (Figure 9), unlike what occurred in the previous study on a diet of rosy apple aphid (Figure 9 lower panel). We discovered that mouthparts of newly hatched lacewings regularly became stuck in the aphid's waxy honeydew as the lacewing attempted to feed

(Figure 10); over 80% of observed mortality was attributed to this honeydew factor. Conversely, large lacewing larvae (2nd and 3rd instars) were considerably more successful than newly hatched larvae, and showed excellent survival. Consumption rates of large larvae reached almost 25 aphids per day. These results suggest that releases of eggs or newly hatched larvae of lacewings may not be successful against woolly apple aphid, unless an alternative prey for hatchlings is also present in the orchard.

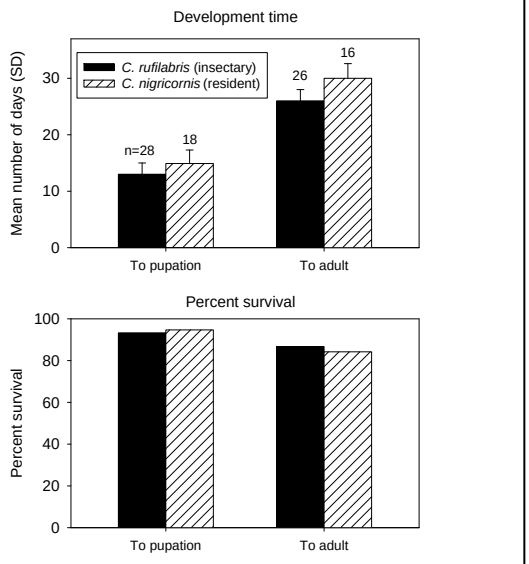

Figure 8. Upper panel: Development time (days) to pupation or adult emergence of *C. rufilabris* or *C. nigricornis* on a diet of rosy apple aphid; bottom panel: survival of both species on diet of rosy apple aphid.

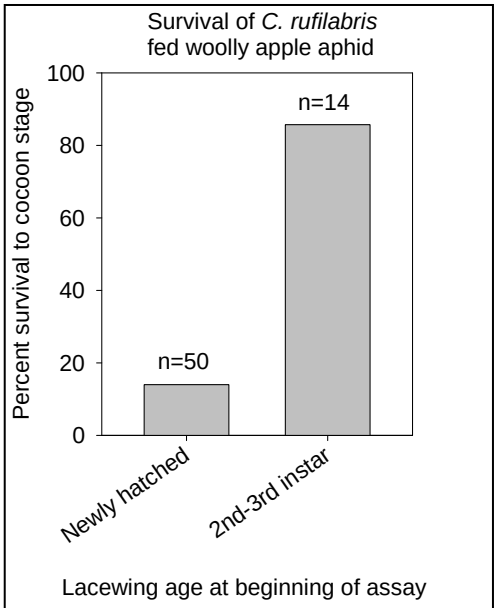

Figure 9. Survival of newly hatched or later instar *C. rufilabris* on diet of woolly apple aphid.

Figure 10. Newly hatched *C. rufilabris* with mouthparts embedded and apparently stuck in the waxy honeydew of woolly apple aphid.

CONTINUING PROJECT REPORT **Year:** 1 of 2
WTFRC Project Number: CP-10-105

Project Title: Sustainable postharvest decay control

PI: Chang-Lin Xiao
Organization: WSU-TFREC, Wenatchee
Telephone: 509-663-8181 X229
Email: clxiao@wsu.edu
Address: 1100 N Western Ave
City/State/Zip: Wenatchee/WA/98801

Cooperators: Selected packinghouses across central Washington State

Total Project Request: **Year 1:** $75,488 **Year 2: $78,681**

Other funding sources:

Agency Name: Washington State Commission on Pesticide Registration
Amt. requested/awarded: $11,247
Notes: A proposal on control of Pristine-resistant *Botrytis cinerea* was approved by WSCPR at its research review meeting held on November 30, 2010. The funding from WSCPR is contingent upon the provision of funds for the present proposal from the Washington Tree Fruit Research Commission as co-funding.

WTFRC Collaborative expenses:

Item	2010	2011
Stemilt RCA room rental	6,300	6,300
Crew labor	0	0
Shipping	0	0
Supplies	0	0
Travel	0	0
Miscellaneous	0	0
Total	6,300	**6,300**

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

Item	2010	**2011**
Salaries[1]	43,747	45,747
Benefits	17,149	18,550
Wages[2]	4,000	4,000
Benefits	592	384
Equipment	0	0
Supplies[3]	8,000	8,000
Travel[4]	2,000	2,000
Miscellaneous	0	0
Total	75,488	**78,681**

Footnotes:

[1] Salaries are for a postdoctoral research associate ($37,000/year) with 41.2% benefit rate and Robin Boal (two months at 37.8% benefit rate).

[2] Wages are for non-student timeslips.

[3] Supplies include costs of fruit purchased from commercial orchards or packers for trials and 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)

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

Objectives:

1. Manage resistance to the postharvest fungicides pyrimethanil and fludioxonil in *Penicillium expansum*.
   a. Monitor and characterize resistance to pyrimethanil and fludioxonil in *P. expansum* populations.
   b. Develop fungicide programs for controlling blue mold caused by pyrimethanil-resistant *P. expansum*.
2. Manage resistance to Pristine in *Botrytis cinerea* and *Penicillium expansum*.
   a. Establish baseline sensitivity to Pristine in *P. expansum* populations.
   b. Monitor and characterize Pristine resistance in fungal pathogen populations.
   c. Develop fungicide programs for controlling gray mold and blue mold caused by Pristine-resistant strains.
3. Evaluate non-chemical approaches for postharvest decay control.

Significant Findings:

- Resistance to pyrimethanil (Penbotec) is emerging in *Penicillium expansum* populations in some packinghouses where the fungicide as a postharvest drench has been used annually for 4-5 consecutive years. In one packinghouse, over 80% of the *P. expansum* isolates obtained from decayed fruit were resistant to pyrimethanil, resulting in the loss of blue mold control on Penbotec-drenched fruit. The results indicated that fungicide resistance practices need to be implemented in order to manage pyrimethanil resistance.

- Isolates of *P. expansum* remained sensitive to fludioxonil. TBZ-resistant strains were still present in sampled packinghouses, indicating that TBZ-resistant strains remained in *P. expansum* populations even after TBZ was not used.

- In orchards where Pristine had been used for 4-5 consecutive years, frequency of Pristine resistance in the gray mold fungus *Botrytis cinerea* ranged from 13 to 54%, whereas only approximately 2% in an orchard where Pristine had been used for only one year. The results indicated that repeated annual use of Pristine increased the population of Pristine-resistant *B. cinerea* in the orchards and that practices for managing Pristine resistance are needed.

- Boscalid only delayed conidial germination and had no fungicidal activity against *Penicillium expansum*. Pyraclostrobin and Pristine appeared to only have suppressive activity against *P. expansum*.

- Boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggesting that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline.

- All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. The results indicated that Pristine resistance does not alter the sensitivity of the isolates to DPA but there is a negative cross resistance between TBZ and DPA. The lab test results suggest that DPA may be able to control TBZ-resistant strains of *B. cinerea*, though DPA is not a fungicide.

- Experiments to evaluate preharvest fungicide programs as well as postharvest fungicides and DPA alone and their combinations for controlling gray mold caused by Pristine-resistant and/or TBZ-resistant strains of *Botrytis cinerea* have been conducted and are still ongoing in storage.

- An experiment to evaluate preharvest biocontrol agents for decay control has been conducted and is ongoing in storage. Results will be forthcoming.

Methods:

Blue mold-decayed fruit will be sampled from grower lots that had been drenched with Penbotec or Scholar from commercial fruit packinghouses. Isolates of *Penicillium* spp. were identified to species. Isolates of *P. expansum* were screened for resistance to fludioxonil, pyrimethanil, and TBZ.

Biological characteristics of pyrimethanil-resistant strains of *P. expansum*, including stability of pyrimethanil resistance, fitness parameters (mycelial growth, spore production, virulence on apple fruit, etc.), ability to compete with pyrimethanil-sensitive strains, and cross resistance to other fungicides, were determined.

Baseline sensitivities of *P. expansum* to pyraclostrobin, boscalid and Pristine were determined. Non-exposed isolates were used to establish distribution of baseline sensitivity of *P. expansum* to these fungicides.

Frequency of Pristine-resistant isolates of *B. cinerea* in apple orchards was determined. Apple fruit were collected from eight orchards 2-3 weeks before harvest. Isolation of *B. cinerea* from the calyx tissue of the fruit or from the surface of the fruit was attempted. Isolates were then tested for resistance to pyraclostrobin, boscalid and Pristine on fungicide-amended agar media.

Biological characteristics of pyraclostrobin-resistant and boscalid-resistant strains of *B. cinerea*, including resistance stability, fitness parameters (mycelial growth, spore production, virulence on apple fruit, etc.), ability to compete with fungicide-sensitive strains, and cross-resistance to other fungicides, were determined.

An experiment was conducted in a research apple orchard. Topsin, Pristine, and their mixture were applied within one week before harvest, and trees receiving no treatment served as a control. After harvest, fruit were immediately transported into the laboratory. Fruit were puncture-wounded, inoculated with different strains of the pathogen, and stored in storage for decay development.

Sensitivity to DPA, fludioxonil and pyrimethanil in Pristine-resistant isolates of *B. cinerea* was tested. To evaluate postharvest fungicides and DPA for control of Pristine-resistant *B. cinerea* on fruit, apple fruit were wounded and inoculated with Pristine-resistant or Pristine-sensitive isolate. Apples were treated with either sterile water as controls or one of the following chemical solutions: DPA, Scholar, Penbotec, DPA+ Scholar, and DPA+Penbotec. Fruit were stored in RA for decay development.

In a commercial organic Fuji orchard, Serenade MAX (*Bacillus subtilis* strain QST 713) and Sonata (*Bacillus pumilus* strain QST 2808) as preharvest sprays were evaluated for postharvest decay control.

Results & Discussion:

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

In 2010, we sampled blue mold-like decayed fruit from five packinghouses where Penbotec had been used as a postharvest drench on the 2009 crops. In total, 389 *P. expansum* isolates were obtained. Pyrimethanil-resistant strains were detected in two packinghouses where Penbotec (pyrimethanil) had been used annually as a postharvest drench since 2005. Approximately 85% of the *P. expansum* isolates obtained from packinghouse A were resistant to pyrimethanil, and 7% of the isolates from packinghouse B were resistant to pyrimethanil (Table 1). No pyrimethanil-resistant strains were detected in the other three packinghouses where Penbotec was used on 2009 crops but no or little use in the past.

All isolates were sensitive to fludioxonil. Approximately 86% and 11% of the isolates were resistant to TBZ in packinghouses A and B, respectively. TBZ-resistant strains were also present in other packinghouses, indicating that TBZ-resistant strains remained in *P. expansum* populations even after TBZ was not used.

The results clearly demonstrated that pyrimethanil resistance is emerging in those packinghouses A and B where Penbotec had been used annually in the last few years. Loss of blue mold control by Penbotec was observed in packinghouse A, suggesting that fungicide management practices are needed.

Table 1. Monitoring of resistance to postharvest fungicides in *Penicillium expansum* from apples in 2010

Source	**Drench Treatment**	**# isolates of *P. expansum***	**# isolates resistant to pyrimethanil**	**# isolates resistant to fludioxonil**	**# isolates resistant to thiabendazole**
Packinghouse A	Penbotec	177	150	0	152
Packinghouse B	Penbotec	129	9	0	14
Packinghouse C	Penbotec	26	0	0	2
Packinghouse D	Penbotec	29	0	0	16
Packinghouse E	Penbotec	28	0	0	1

Monitoring Pristine resistance in B. cinerea in apple orchards

We monitored Pristine resistance in *B. cinerea* in eight apple orchards. Pristine had been used for 4-5 years in orchards A to G and for 1-2 years in orchard H. The frequency of Pristine resistance in orchards A to G ranged from 13 to 54%, where as only approximately 2% in orchard H (Table 2). The results indicated that repeated annual use of Pristine increased the population of Pristine-resistant *B. cinerea* in the orchards and that practices for managing Pristine resistance are needed.

Table 2. Frequency of pyraclostrobin- and boscalid-resistant *B. cinerea* in 2010 from commercial Gala orchards where Pristine had been used

		Percent of isolates resistant to		
Orchard	No. of isolates	Pyraclostrobin	Boscalid	Pyraclostrobin and boscalid
A	59	56.1	45.9	45.9
B	59	56.1	54.1	54.1
C	36	60.3	52.3	52.3
D	56	18.9	17.2	17.2
E	74	24.0	13.3	13.3
F	53	20.6	16.6	16.6
G	53	60.7	49.6	49.6
H	54	1.8	1.8	1.8
Total	**444**	**37.3**	**31.4**	**31.4**

Sensitivity of P. expansum to Pristine

At 1 µg/ml of pyraclostrobin, no conidial germination was observed within 30 h of incubation at 20°C. Germination was completely inhibited at 2,000 µg/ml of pyraclostrobin for up to 7 days, but conidia were able to germinate when they were transferred to plain PDA. All of the isolates did not germinate at 5 µg/ml boscalid after 20 h of incubation at 20°C, but conidia were swollen. At 30 h of incubation, conidia were able to germinate at 100 µg/ml boscalid, indicating that boscalid only delayed conidial germination. The range of EC50 values of Pristine was from 0.009 to 0.019 µg/ml, with a mean of 0.013 µg/ml (Fig. 1). Our results indicated that boscalid only delayed conidial germination and had no fungicidal activity against *P. expansum*. Pyraclostrobin and Pristine appeared to only have suppressive activity against *P. expansum*.

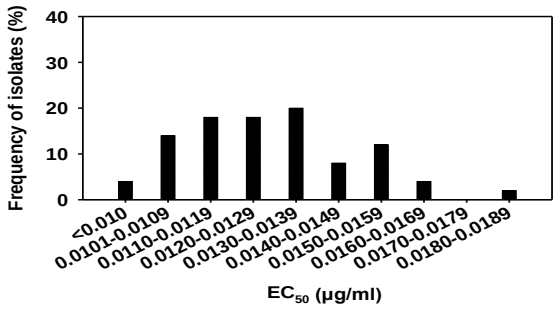

Fig. 1. Distribution of sensitivity of *Penicillium expansum* to Pristine.

Biological characteristics of Pristine-resistant strains of B. cinerea

After successive transfers on PDA and being cycled on apple fruit, most pyraclostrobin-resistant isolates remained the same levels of EC_{50} values as their initial generations with a few exceptions. All boscalid-resistant isolates retained at similar levels of resistance as the initial generations in both in vitro and in vivo at 20 and 0°C (Table 3).

There was great variability in mycelial growth, spore production, and sensitivity to osmotic stress among isolates sensitive to both pyraclostrobin and boscalid as well as among isolates resistant to pyraclostrobin, boscalid or both. When compared as groups, however, there were no significant differences in average mycelial growth, spore production, osmotic sensitivity, and conidial

germination among phenotype groups except that isolates resistant only to boscalid produced fewer conidia in vitro compared with other phenotype groups (data not shown).

Regardless of fungicide-resistance phenotypes of isolates tested in this study, all inoculated fruit developed symptoms of gray mold at both 0°C and 20°C or room temperature, indicating that there was no difference in pathogenicity between resistant and sensitive isolates (data not shown). Isolates within each fungicide-resistance phenotype group varied in virulence (lesion size on apple fruit) and sporulation at both 20°C or room temperature and 0°C. However, when they were compared as groups, no significant difference was observed in virulence on apple fruit and sporulation on the decayed tissue among the different phenotype groups.

Regardless of the phenotypes and ratios of fungicide-resistant and –sensitive isolates in the mixture, pyraclostrobin-resistant individuals were detectable in the populations, but the frequency of pyraclostrobin-resistant individuals in the populations significantly decreased after being cycled for four generations on apple fruit compared to the initial frequency (Table 4). However, no boscalid-resistant individuals in the populations were observed after being cycled for four generations on apple fruit.

The results indicated that boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggest that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline.

TABLE 3. Changes of the sensitivity of *Botrytis cinerea* isolates resistant to pyraclostrobin and boscalid after 20 (20°C) and 10 (0°C) consecutive generations on fungicide-free media and after 5 (20°C) and 3 (0°C) disease cycles on apple fruit

	EC_{50} value (µg/ml) of pyraclostrobin					EC_{50} value (µg/ml) of boscalid				
		PDA		Fruit			PDA		Fruit	
Phenotype	Initial	20°C	0°C	20°C	0°C	Initial	20°C	0°C	20°C	0°C
$Pyr^{R}Bos^{S}$	92.5	102.2	94.0	90.9	55.2*	–[y]	–	–	–	–
$Pyr^{R}Bos^{S}$	>100	>100	>100	>100	>100	–	–	–	–	–
$Pyr^{R}Bos^{S}$	>100	>100	>100	>100	>100	–	–	–	–	–
$Pyr^{R}Bos^{S}$	41.6	37.7*	31.9*	39.7	30.6*	–	–	–	–	–
$Pyr^{S}Bos^{R}$	–	–	–	–	–	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{S}Bos^{R}$	–	–	–	–	–	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	8.8	12.3	8.5	10.2	10.8
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	17.5	16.2	14.5	15.9	14.5
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	12.1	17.8	15.0	10.1	14.1
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	>20.0	>20.0	>20.0	>20.0	>20.0
$Pyr^{R}Bos^{R}$	>100	>100	>100	>100	>100	>20.0	>20.0	>20.0	>20.0	>20.0

TABLE 4. Competitions between resistant and sensitive isolates of *Botrytis cinerea* on apple fruits

Phenotype[w]	Initial frequency (%)	Final frequency (%) resistant to[x]	
		Pyraclostrobin	Boscalid
$Pyr^{R}Bos^{S}$	75	6.2*[y]	–[z]
	50	10.8*	–
	25	11.7*	–
$Pyr^{S}Bos^{R}$	75	–	0
	50	–	0
	25	–	0
$Pyr^{R}Bos^{R}$	75	7.0*	0
	50	3.4*	0
	25	3.6*	0

[w] Pyr = pyraclostrobin, Bos = boscalid, R = resistant, S = sensitive.
[x] The discrimination between resistant and sensitive was determined at 5 µg/ml of pyraclostrobin and boscalid using a mycelial growth assay and germination assay, respectively.
[y] "*" indicates that the final frequency of the resistant isolates was significantly different from that of the initial frequency according to a Chi-square test at $P = 0.05$.
[z] "–" indicates no data for this item.

Control of gray mold caused by Pristine-resistant Botrytis cinerea

A field experiment was conducted on 2010 Fuji crop. Topsin M, Pristine and their combination were applied one week before harvest. The fruit were inoculated with Pristine-sensitive or Pristine-resistant strains of *B. cinerea*. The experiment is still ongoing and results will be forthcoming.

Sensitivity to DPA, TBZ, fludioxonil and pyrimethanil in Pristine-resistant isolates of *B. cinerea* was tested. All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. The results indicated that Pristine resistance does not change the sensitivity of the isolates to DPA but there is a negative cross resistance between TBZ and DPA. All isolates remained sensitive to fludioxonil but some were resistant to pyrimethanil, likely because cyprodinil (Vangard) had been used in some of these orchards. The lab test results suggest that DPA may be able to control TBZ-resistant strains of *B. cinerea*, though DPA is not a fungicide.

An experiment was conducted to evaluate postharvest fungicides and DPA alone or their combinations for control of gray mold caused by Pristine-resistant and/or TBZ-resistant strains of *B. cinerea*. The experiment is still ongoing in storage and results will be forthcoming.

Preharvest biocontrol agents for control of postharvest fruit rots

An experiment was conducted in an organic Fuji orchard near Quincy. Biocontrol agents Sonata and Serenade were applied to the fruit 10 days and 1 day before harvest. Fruit were harvested and wounded with a finish-nail head to simulate puncture wounds. Natural inoculum was used in this study. The fruit are currently kept in cold storage for decay development and results will be available in spring 2011.

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

Project Title: Identifying fire blight resistance in M. sieversii for scion breeding

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Total Project Request: **Year 2010**: $29,000 **Year 2011**: **$25,000**

Other funding sources: None

WTFRC Collaborative Expenses: None

Budget 1
Organization Name: **USDA-ARS-NAA** **Contract Administrator: Ingrid Charlton**
Telephone: 215-233-6554 **Email address:** ingrid.charlton@ars.usda.gov

Item	2010	2011	
Salaries	4,854	4,854	
Benefits	156	156	
Wages	0	0	
Benefits	0	0	
Equipment	0	0	
Supplies	10,000	**10,000**	
Travel	0	0	
Miscellaneous	14,000	4,000	
Total	$29,000	**$19,000**	

Footnotes: Salary is for summer undergraduate student to assist Norelli in determining fire blight resistance of GMAL4593 population (Obj. 1). Supplies are to identify additional molecular markers (SNP) in *M. sieversii* (Obj. 2). **Supply funds were originally requested for only year 1 and request is now being extended to year 2** (see Significant Findings and Discussion for justification). Miscellaneous year 1 was to propagate 10 replicates of 200 *M. sieversii* accessions at commercial WA nursery (Obj. 3). Misc. year 2 is for plot establishment at Kearneysville, WV (Obj. 3).

Budget 2

Organization Name: WSU-TFREC **Contract Administrator:** M.L. Bricker & K. Larson

Telephone: 509-335-7667, 509-663-8181 x221, respectively **Email address:** **mdesros@wsu.edu**, **kevin_larson@wsu.edu**, respectively

Item	2010	2011	
Salaries	0	0	
Benefits	0	0	
Wages	0	0	
Benefits	0	0	
Equipment	0	0	
Supplies	0	0	
Travel	0	0	
Miscellaneous	0	$6,000	
Total	0	**$6,000**	

Footnotes: Miscellaneous is for plot establishment at Wenatchee, WA (Obj. 3).

OBJECTIVES:

1. Identify genetic markers for fire blight resistance in the *M. sieversii* GMAL4593 mapping population for use in scion breeding programs.
    - 2010: approximately 750 trees of *M. sieversii* GMAL4593 mapping population and controls were evaluated for fire blight resistance in the field
    - 2011: repeat field evaluation of *M. sieversii* GMAL4593 mapping population for fire blight resistance, as originally planned

2. Identify *M. sieversii*-specific molecular markers to add additional markers to the genetic map of *M. sieversii.*
    - 2010: Mapped additional markers obtained from HiDRAS project (High-quality Disease Resistant Apples for a Sustainable Agriculture) to facilitate integration of the *M. sieversii* map with other genetic maps of domesticated apple
    - 2011: Use *M. sieversii*-specific molecular markers identified in RosBREED project to fill all remaining gaps in genetic map of *M. sieversii*

3. Establish plantings of *M. sieversii* in WA and WV for the identification of additional sources of fire blight resistance in the future.
    - 2010: budwood of 200 *M. sieversii* accessions was sent to Van Well Nursery, East Wenatchee, WA for tree propagation
    - 2011: establish plantings of *M. sieversii* in Wenatchee, WA and Kearneysville, WV (fall 2011 or early spring 2012)

SIGNIFICANT FINDINGS:

Objective 1 (Identify genes for fire blight resistance in GMAL4593 mapping population)

- When a field planting of GMAL4593 mapping population was challenged with fire blight in 2010, the population segregated into resistant and susceptible individuals. This suggests we should be able to identify genes for resistance.
- Fire blight resistance in GMAL4593 mapping population was associated with specific molecular markers on chromosomes 8 and 10 of *M. sieversii.*

Objective 2 (Complete molecular map of GMAL4593)

- The total number of molecular markers in the *M. sieversii* GMAL4593 map increased from 107 (Wisniewski 2009 final report) to 164 markers.
- Worked in collaboration with RosBREED project to re-sequence *M. sieversii* parent of GMAL4593 and identify *M. sieversii*-specific molecular markers.
- To capitalize on resources being developed by RosBREED project we are requesting an additional $10,000 in supplies so that we can using *M. sieversii*-specific molecular markers to fill all remaining gaps within the *M. sieversii* genetic map.

Objective 3 (Establish new plantings of *M. sieversii* in WA and WV)

- All available field and greenhouse data on fire blight resistance of the *M. sieversii* collected by the USDA in Kazakhstan was analyzed and 200 accessions were selected for the identification of additional sources of fire blight resistance. Replicate trees of the 200 accessions are currently being propagated by a commercial nursery to establish field plants in WA and WV.

METHODS:

Objective 1: Identify a genetic locus for fire blight resistance in the *M. sieversii* GMAL4593 mapping population for use in scion breeding programs.

Because fire blight is a sporadic disease from year to year and its distribution within the orchard is usually not uniform, reliable evaluation of fire blight resistance requires artificial challenge of test plants with the fire blight bacteria in multiple trials. Vigorously growing shoots are challenged by dipping a pair of scissors in a suspension of the bacteria and then cutting the youngest leaves of the shoot tip. Resistance is determined by measuring the percent of the current season's shoot length that develops fire blight symptoms. Because economic losses from fire blight are the result of the death of young trees and woody tissue, rating cultivar resistance based upon progression of disease in shoot tissue has proven a reliable method of accessing fire blight resistance.

The GMAL4593 population consists of 190 individuals. A field planting containing 4 replicate trees of each individual was established at Kearneysville, WV in the fall 2008. These trees were challenged with fire blight in the 2010 growing season and tests will be repeated in 2011. The planting also contains the parents of the population, 'Royal Gala' and *M.* sieversii PI613981 (plant introduction number), additional susceptible control 'Jonathan', resistant controls, Geneva.41 (G.41) rootstock and wild *M.* ×*robusta* 5 (resistant parent of G.41 rootstock).

To identify genetic markers for the fire blight resistance, QTL (Quantitative Trait Loci) analysis will be performed independently for both parents in the GMAL4593 populations to identify genetic loci affecting fire blight resistance. Analysis will be conducted using MapQTL® 5 Software for simple interval mapping followed by approximate multiple QTL model analysis.

Objective 2: Identify *M. sieversii*-specific molecular markers to add additional markers to the genetic map of *M. sieversii.*

DNA for marker analysis was extracted from apple leaf tissue of all 190 individuals of GMAL4593 population and parents 'Royal Gala' and *M. sieversii* PI631981. Initially, the 2 parents and 10 individuals are screened with candidate markers to determine which markers will be informative. Informative markers are then evaluated in the entire population. Two types of markers are being used in the project, SSRs (or simple sequence repeats) and SNPs (or single nucleotide polymorphisms). SSR markers are useful in "anchoring" or integrating maps made from different mapping populations. SNP markers are more plentiful and are useful in saturating the map for greater precision. SSRs and SNPs are mapped by different methods. Both methods start with PCR amplification of plant DNA using marker-specific primers that amplify a small segment of the total DNA which contains the marker.

For SSR markers, the primers used for DNA amplification are labeled with one of three fluorescent dyes (6-FAM-blue, HEX-green, or NED-yellow). PCR products are then run on an automated laser fluorescence sequencer, ABI Prism 3730. Appropriate size standards are employed and GeneMapper software version 3.7 (ABI, Foster City, CA, USA) is used to analyze trace files, determine PCR product size and assign SSR type to individuals.

SNP markers are analyzed by a technique known as high resolution melting (HRM) using a Roche LightCycler480 instrument available at USDA-ARS-AFRS. Double stranded DNA molecules will "melt" into single strands as temperature increases. The occurrence of a SNP within the DNA will affect the temperature at which the DNA melts and precise analysis of the melting curve of each individual within the population is used to detect presence or absence of the SNP. Our average cost

in 2009 using this instrument to screen a SNP marker in the entire GMAL4593 population (192 individuals) was $109 /marker. Assuming that 50% of the markers tested will map successfully in the GMAL4593 population, the $10,000 requested for supplies in 2010 should result in 45 additional markers on the *M. sieversii* map.

The segregation of these markers within the population is then used to construct a genetic linkage map which estimates the distance between the markers within the genome. The maps are generated for each parent of the population using JoinMap software (Kyazma) version 4.0 which calculates the linkage between markers and orders the markers within each linkage group.

Objective 3: Establish plantings of *M. sieversii* in WA and WV for the identification of additional sources of fire blight resistance in the future.

Each planting in WA and WV will include the same 200 *M. sieversii* accessions and controls (See Results and Discussion) budded onto M.7 rootstock. Budwood was collected at the USDA-ARS-Plant Genetic Resources Unit (PGRU) in Geneva, NY. Trees for both plantings are being propagated by Van Well Nursery, East Wenatchee, WA. Field plantings will be established either Fall 2011 or Spring 2012. Because fire blight resistance will be determined on shoots, the tests for resistance can be conducted on young trees and the planting are expected to be of short term duration (3 to 5 years), allowing for planting at high density. Planting designs could range from a 6 foot wide, double row of trees with 3 feet between trees (trees staggered in 2 rows to create 3 feet triangles) and a 9 foot row corridor, for the shortest term, highest density planting; to a single 6 foot wide row with 6 feet between trees and 10 foot row corridor. The 6 foot wide, double row design has been successfully used for USDA-ARS-PGRU short term plants.

RESULTS & DISCUSSION:

Objective 1: Identify a genetic locus (QTL) for fire blight resistance in the *M. sieversii* GMAL4593 mapping population for use in scion breeding programs.

The GMAL4593 mapping population consists of seedling derived from a cross between 'Royal Gala' and *M. sieversii* PI631981. To successfully map a genetic locus for fire blight resistance within this population, the population needs to segregate for resistance. In other words, when challenged with the fire blight pathogen, some individuals within the population need to be consistently resistant to the disease, and others need to be consistently susceptible to the disease. Previous greenhouse evaluation of GMAL4593 for fire blight resistance by Herb Aldwinckle (Cornell University) and Phillip Forsline (USDA-ARS) in Geneva, NY found the population to be segregating for fire blight resistance.

To confirm the fire blight resistance of individuals within the GMAL4593 population and to supplement previous greenhouse data with field data, resistance was evaluated in a field planting at USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV. On May 21, 2010 approximately 2,700 vigorously growing shoots on 3-4 replicate trees of the same 190 GMAL4593 individuals were challenged with fire blight. Similar to the 2008 greenhouse trial, disease varied among individuals in the population with percent of the current season's shoot length that became diseased ranging from 5 to 100%. However, the disease rating obtained in the 2008 greenhouse and 2010 field tests were not correlated. In other words, the individuals found resistant in the greenhouse test were not the same as those identified as resistant in the field trial. Furthermore, when fire blight resistance was mapped within the GMAL4593, fire blight resistance determined in the greenhouse was associated with markers on linkage group (LG) 8, whereas resistance determined in the field was associated with markers on LG10 (Table 1).

Table 1: Association between fire blight resistance and genetic markers determined by rank sum test of Krusgal-Wallis. Significance levels: -:not significant, *:0.1, **:0.05, ***:0.01, ****:0.005 and ******:0.0005.

Linkage Group	Position (cM)	Markers	df	Greenhouse 2008		Field 2010	
				K	Significance	K	Significance
MS_LG8	**0.0**	**CH01c06**	**3**	**11.4**	***	1.4	-
MS_LG8	**12.5**	**CH01f09**	3	**13.8**	****	4.2	-
MS_LG8	21.6	GD_SNP01004	2	0.4	-	0.2	-
MS_LG10	28.7	CH03d11	1	1.3	-	0.0	-
MS_LG10	33.2	GD_SNP00869	2	0.3	-	6.9	**
MS_LG10	**57.6**	**COL**	3	7.0	*	**19.5**	******
MS_LG10	**61.5**	**Hi22a07x_202**	3	5.1	-	**20.0**	******
MS_LG10	62.0	GD100(2)	1	0.0	-	1.7	-

There are several possible explanations for these observations which should be resolved by 2011 field trials:

- Evaluation of fire blight resistance in the greenhouse was not a reliable predictor of field resistance observed in GMAL4593. Greenhouse evaluation of fire blight resistance is usually a reliable predictor of field resistance. However, if 2011 field evaluations are consistent with 2010 field evaluations, genetic markers for resistance will be developed based solely on the field evaluation of fire blight resistance.
- Resistance is controlled by the additive effect of 2 genetic loci on LG 8 and 10 whose relative impact is determined by environment. If 2011 field evaluations are more consistent with 2008 greenhouse evaluations we will develop markers based upon an additive model of 2 independent genetic loci for resistance. The current mapping analysis was based upon a model in which resistance is controlled by a single QTL. As more molecular markers are added to the map in 2011 a more complex additive model could be evaluated.
- The genetic loci controlling fire blight resistance in GMAL4593 are specific to different strains of the fire blight bacteria. 2008 greenhouse test was conducted with a native NY fire blight strain, whereas 2010 field test was conducted with a native WV strain. In 2011, GMAL4593 trees should be large enough to allow challenge with both the NY and WV strain which will allow us to directly test if loci on LG8 and LG10 are strain specific.
- The fire blight resistance of *M. sieversii* PI631981 is not highly heritable. If 2011 field evaluations are not consistent with either 2008 greenhouse or 2010 field evaluations, we will need to conclude that the fire blight resistance of GMAL4593 is not of sufficient heritability to allow the development of reliable markers for resistance.

<u>Objective 2</u>: Identify *M. sieversii*-specific molecular markers (SNPs) to add additional markers to the genetic map of *M. sieversii.*

To date we have screened 739 molecular markers in the GMAL4593 mapping population, 239 SSRs and 500 SNPs (Table 2). In order for the markers to be informative in the *M. sieversii* map they must segregate either in *M. sieversii* or in both parents. Among the SSR markers, 37% of the markers were informative for *M. sieversii* PI631981, whereas 64% were informative for 'Royal Gala'. Among the SNP markers, only 15% of markers were informative for *M. sieversii*, compared with 50% for 'Royal Gala'. The lower occurrence of SNP markers in *M. sieversii* is probably due to the broader "genetic base" or greater diversity of genes in *M. sieversii* compared to domesticated apple, making it less likely that any specific SNP markers will be present in a specific individual. Currently, there are a total of 164 molecular markers in the *M. sieversii* map. Because the current 'Royal Gala' map has a sufficient number of markers (349) we are no longer analyzing markers that are informative for only 'Royal Gala' (bottom row of Table 2)

Table 2. Summary of Marker Utilization and Distribution in GMAL4593 ('Royal Gala' X *M. sieversii* PI613981) Mapping Population.

Marker Summary	SSR	SNP
Total Screened	239	500
Segregated Both Parents	70	40
Segregated and mapped in 'Royal Gala'	31	218
Segregated and mapped in *M. sieversii*	18	36
Did not segregate	20	206
Failed Reactions	48	---
Segregated in 'Royal Gala', not mapped	52	---

To identify informative SNP markers for *M.* sieversii PI613981, we conducted "single-pass" DNA sequencing or "re-sequencing" of *M.* sieversii PI613981 in collaboration with the 'RosBREED: Enabling Marker-Assisted Breeding in Rosaceae' project. Although this type of sequencing does not cover all DNA in the genome of *M.* sieversii PI613981, it did identify many single base pair differences in the DNA of *M. sieversii* when compared to 'Golden Delicious' DNA that can now be developed into excellent location-specific molecular markers to fill the remaining gaps in the *M. sieversii* map. Funds from this WTFRC project were not used for this DNA sequencing or sequence analysis.

Because the RosBREED analysis of the data took longer than originally anticipated, during the past year we added additional SSR markers to the *M. sieversii* map. SSR markers were obtained from the European Union funded High-quality Disease Resistant Apples for a Sustainable Agriculture (HiDRAS) project. Having some of the markers from the HiDRAS project in the *M. sieversii* map will facilitate future integration of results and greater utilization of project results. However, since most of the money originally requested for molecular mapping has been spent, we now request an additional $10,000 in supplies to continue development of the *M. sieversii* map.

<u>Objective 3</u>: Establish plantings of *M. sieversii* in WA and WV for the identification of additional sources of fire blight resistance in the future.

Although many different *M. sieversii* accessions in the USDA-ARS collection have shown high levels of fire blight resistance, we do not know if these other potential sources of resistance contain the same fire blight resistance genes present in GMAL4593 or if they represent distinct, and perhaps more useful, sources of resistance. The purpose of the new *M. sieversii* plantings being established here are

to obtain reliable fire blight data for the best candidate sources of resistance. This will allow us to then use the method of association mapping to identify other sources of fire blight resistance in *M. sieversii.*

Amongst the several thousand seed of *M. sieversii* collected in Kazakhstan, a CORE collection of 110 accessions has been selected which represent a broad range of the genetic and character diversity found throughout Kazakhstan. To select accessions to be included in this trial, we first selected 37 individuals from the CORE collection that appeared resistant to fire blight and had superior fruit quality. Superior fruit quality was based upon available GRIN data for flavor, fruit weight, harvest date (favoring late), soluble solids and juice quality. Because a ratio of 1 susceptible: 1 resistant plant will be advantageous for later association mapping studies, we then selected accessions derived from sister seed (seeds collected from same mother plant in Kazakhstan) of the selected CORE accessions to result in a predicted ratio of 1 susceptible:1 resistant accession. Because we do not currently have reliable information on the fire blight resistance for most of these accessions, resistance ratio was predicted from available data. Observations had previously been made on the natural occurrence of fire blight in approximately 1,000 field grown accessions of *M. sieversii*. Accessions damaged by natural fire blight infection in the field can be considered susceptible. However, in cases where no fire blight damage occurred it is not known if these accessions are resistant to the disease or if they escaped exposure to the pathogen during favorable conditions for infection. To estimate the rate of escape, a subset of plants without field infection were propagated and challenged with fire blight bacteria in greenhouse. Approximately 60% of plants without natural fire blight infection were found to be resistant when challenged in the greenhouse. In addition to selecting accessions for a balanced resistant:susceptible ratio, additional accessions were selected based upon resistance to both fire blight and apple scab, and superior fruit quality.

Standard cultivars were included in trials so that results obtained in different locations and years can be directly compared. Resistant control cultivars ('Delicious', 'Splendour' and 'Goldrush') were included to establish the lower limit for a "resistant" rating. Two highly susceptible cultivars ('Jonathan' and 'Gala') were included to establish the high end of the disease scale when comparing tests and to ensure that a minimum disease pressure threshold is achieved in every test. Moderately resistant ('Empire' and 'Golden Delicious') and highly resistant (Geneva.41 and Robusta 5) cultivars were also included to establish the mid and low end of the disease scale when comparing tests.

Budwood of all selected accessions and control cultivars was collected at the USDA-ARS-Plant Genetic Resources Unit in Geneva, NY in late July 2010 and sent to Van Well Nursery, East Wenatchee, WA for propagation.

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

Project Title: Apple specific issues in fire blight management

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Total project request: **Year 1**: $19,300 **Year 2: $19,900**

Other funding sources: None

WTFRC Collaborative expenses: None

Budget
Organization Name: OSU Agric. Research Foundation **Contract Administrator:** Cynthia Cox
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Item	**2010**	**2011**	
Salaries FRA 3mo	10,000	10,300	
Benefits OPE 63%	6,300	6,489	
Wages			
Benefits			
Equipment			
Supplies	2,000	2,111	
Travel local	500	500	
Miscellaneous plot fee	500	500	
Total	$19,300	**$19,900**	

Footnotes: Annually**:** FRA 3 mo plus fringe, 2K M&S, 1K plot fee, 3% inflation

OJECTIVES:

1) Integration of a new material, kasugamycin, into blossom blight control programs for conventional orchards (this objective was funded from pear sources, but results are applicable to apple);

2) Evaluate fire blight suppression programs compatible with European organic certification standards;

3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

SIGNIFICANT FINDINGS:

- We continued to achieve outstanding control of fire blight of apple with Kasumin (kasugamycin) and with mixtures of Kasumin and Mycoshield (e.g., 80 ppm of each material).
- For a second season, the not-yet-registered yeast material, Blossom Protect, provided excellent control of fire blight.
- With the EU allowable organic materials, BlightBan C9-1 (*P. agglomerans*) and Serenade Max, doubling the frequency of treatment over a standard two treatment program significantly enhanced fire blight suppression.
- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), slowed expansion of fire blight in inoculated shoots of apple rootstock cultivars ELMA 26 and Nic 29.
- More dramatically, ASM drenches applied to potted 'Gala' on M26 provided nearly complete protection of the rootstock after the a high dose of the pathogen was inoculated directly into the graft union.

MATERIALS AND METHODS:

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards. Kasumin 10L (kasugamycin 10% a. i., Arysta LifeScience North America, Cary, NC) was evaluated for control of fire blight in a 'Gala' apple orchard (11-yr-old) located at the OSU Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiment was arranged in a randomized, complete block design with 4 replications and 14 treatments applied to single tree plots. Antibiotic standards, FireWall (streptomycin sulfate 17% a.i., Sipcam Advan, Research Triangle Park, NC) and FireLine (oxytetracycline hydrochloride 18% a.i., Sipcam Advan) were included as controls. In addition, a commercial formulation of the biological agent, *Pantoea agglomerans* C9-1S (BlightBan C9-1, NuFarm Americas, Burr Ridge, IL) was included as a component of some treatment combinations. Similarly, a kasugamycin-resistant selection of *P. agglomerans* C9-1S (designated C9-1^{Kr}) also was evaluated as a component of some treatment combinations. C9-1^{Kr} was obtained in the laboratory by stepwise selection on increasingly higher rates of kasugamycin over a period of several weeks. Treatments were applied t during early morning at 30% bloom (water and *P. agglomerans* treatments), 70% bloom (all treatments), and full bloom (antibiotics). Treatment suspensions were sprayed to near runoff with a backpack sprayer equipped with a hand wand (~3 liters per tree). At full bloom a motorized, 25-gallon tank sprayer equipped with a hand wand was used to fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin-sensitive strain prepared at 5 x 10^5 CFU per ml) onto each tree (2 liters per tree). Symptoms of fire blight were first observed in mid-May. Incidence of fire blight was determined by counting (and cutting) the number of blighted flower clusters (i.e. strikes) on each tree during detailed inspections made on 23-26 May and on 3-6 June. Total number of blighted flower clusters per tree (log-transformed) and incidence of blighted floral clusters (diseased clusters divided by total clusters, arcsine-square root transformed) were subjected to analysis of variance.

2) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets. Materials for organic suppression of fire blight were evaluated in a 'Golden

Delicious' apple orchard (30-yr-old) located at the OSU Botany and Plant Pathology Field Laboratory near Corvallis, OR. The experiment was arranged in a randomized, complete block design with 4 replications and 12 treatments applied to single tree plots. Materials evaluated were chosen on the expectation they would meet potentially revised 2013 National Organic Program guidelines, and were tested in various combinations and treatment timings: Bloomtime Biological (*Pantoea agglomerans* E325, Northwest Agricultural Products, Pasco, WA), Serenade Max (*Bacillus subtilis* QST 713, AgraQuest, Davis, CA) mixed with Biolink spreader-sticker (soapbark, alkylphenol ethoxylate, polysaccharide, Westbridge Agricultural Products, Vista, CA), Blossom Protect mixed with a proprietary buffering agent (*Aureobasidium pullulans* stain BCYP-B with buffer-A, Westbridge Agricultural Products, Vista, CA), and Actinovate AG (*Streptomyces lydicus* strain WYEC 108, Natural Industries, Inc., Houston, TX). The bloom thinning agents, Rex Lime Sulfur Solution (calcium polysulfide 28%, OR-CAL Inc., Junction City, OR) mixed with Crocker's Fish Oil (Crocker's Inc., Quincy, WA), were also evaluated; the timings of these materials followed a recommended protocol for bloom thinning in organic apple production. In addition, the antibiotics, FireWall (streptomycin sulfate 17% a.i., Sipcam Advan, Research Triangle Park, NC) and FireLine (oxytetracycline hydrochloride 18% a.i., Sipcam Advan), were included as controls. Treatments were applied to trees at 10% bloom (Actinovate), 30% bloom (water, lime sulfur and fish oil, Actinovate and Bloomtime Biological), 70% bloom (all treatments), full bloom (all treatments except lime sulfur and fish oil), and petal fall (Serenade Max and Blossom Protect). Treatment suspensions were sprayed to near runoff with a backpack sprayer equipped with a hand wand (3 liters per tree). Inoculation with the pathogen, measurement of fire blight, and statistical analysis were performed as described under objective 1.

3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

Rootstock only experiment. One-year-old trees of apple rootstock cvs. ELMA 26 and ELMA 9 were grown in 2 gallon pots containing growth medium, and maintained in a greenhouse at a temperature of 20-25°C (70-85°F). The experiment was arranged with six SAR-inducing treatments (including control), three timings of chemical treatment and two cultivars; each treatment combination was replicated 6 times (216 trees). In two treatments, the SAR-inducing material acibenzolar-S-methyl (ASM; Actigard 50WG, Syngenta Crop Protection) was drenched onto soil (25, or 37.5 mg a.i. per plant); in two other treatments, it was applied as applied as a truck paint (7.5 or 15 g a.i./L in 2% PentraBark), and in the 5th treatment, it was applied as foliar spray (0.25 g/L). Timings of ASM treatments occurred at 1 month prior to pathogen inoculation, at inoculation, and 1 month after inoculation. At the time of inoculation (June 10), the terminal shoot was cut with a razor blade, and pathogen cells (10^9 colony forming units per ml) were painted onto the cut surfaces. Immediately after inoculation, a plastic bag was wrapped over the cut end and left in place for one week. Length of fire blight cankers on inoculated trees was measured monthly.

Rootstock Scion experiment. Two-year-old trees of apple cv. 'Gala' on ELMA 26 were grown in a greenhouse and maintained as described above. The experiment was arranged with five ASM treatments (including control): soil drench applied once (May 21) or twice (May 21 and July 2), trunk paint applied once or twice (same dates), and non-treated. Rates of ASM were 25 or 50 mg a.i. per plant drenched onto soil, and 7.5 or 15 g a.i./L in a trunk paint with 2 % PentraBark; each treatment combination was replicated 4 times (40 trees total). Trees were inoculated on July 8; a 4-6 cm length wound was created on the rootstock by gouging the rootstock with a large nail just below the graft union. The wound was painted with a pathogen cell suspension (10^7 colony forming units per ml), and then sealed by wrapping the rootstock with parafilm for a period of 3 weeks. Disease was evaluated in early August by measuring the size of the cankered area on the rootstock, and by assigning 0-4 rating for the amount of bacterial ooze present in the vicinity of the canker.

RESULTS

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards. Trees in the study averaged 965 flower clusters. Fire blight risk, as determined by COUGARBLIGHT, was low during the bloom with light precipitation occurring on 3 days between 80% bloom and petal fall. Nonetheless, disease intensity was high with water treated trees averaging 236 blighted clusters per tree (26%). Each treatment significantly reduced ($P < 0.05$) incidence of infection and total number of infected flower clusters per tree. Kasumin and the mixtures of Kasumin and Fireline provided excellent disease control. The integrated program of *P. agglomerans* C9-1S or C9-1^{Kr} in early bloom followed by Kasumin, or Kasumin and Fireline at full bloom also provided excellent control. Within integrated *P. agglomerans*/Kasumin programs, however, treatment with the kasugamycin-resistant strain C9-1^{Kr} did not enhance control relative to treatment with the kasugamycin-sensitive parental strain, C9-1S. Integrated programs and mixtures were evaluated as resistance management strategies, with the goal of providing effective disease control combined with a reduced likelihood of kasugamycin-resistance developing in the pathogen.

		Date treatment applied*						
		17 April	19 April	23 April	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Treatment	Rate per 100 gallons water	30% bloom	70% bloom	Full bloom				
Water control	------	**X**[§]	**X**	**X**	**236**	**a**[#]	**26.0**	**a**[#]
Fireline 200 ppm	16 oz.	---	---	X	27	b	2.6	b
BlightBan C9-1	*10^8 CFU/ml*	X	X	---				
then Fireline 200 ppm	16 oz.	---	---	X	24	b	2.3	b
Kasumin 10L 100 ppm	12.8 fl. oz.	---	---	X	21	bc	2.7	b
Pantoea agglomerans C9-1^{Kr}	*10^8 CFU/ml*	X	X	---				
then Fireline 200 ppm	16 oz.	---	---	X	21	bc	2.3	b
Pantoea agglomerans C9-1^{Kr}	10^8 CFU/ml	X	X	---				
then Kasumin 10L 80 ppm	10.4 fl oz.	---	---	X				
with Fireline 80 ppm	6.4 oz.	---	---	X	19	bcd	2.1	bc
BlightBan C9-1	*10^8 CFU/ml*	X	X	---				
then Kasumin 10L 100 ppm	64 fl. oz.	---	---	X	18	bcd	2.0	bc
Kasumin 10L 80 ppm	10.8 fl. oz.	---	X	X				
with Fireline 80 ppm	6.4 oz.	---	X	X	17	bcd	1.8	bc
Kasumin 10L 100 ppm	12.8 fl. oz.	---	X	X				
with Fireline 100 ppm	8 oz.	---	X	X	16	bcd	1.7	bc
Pantoea agglomerans C9-1^{Kr}	*10^8 CFU/ml*	X	X	---				
then Kasumin 10L 100 ppm	12.8 fl. oz.	---	---	X	16	bcd	1.4	bc
Kasumin 2L 100 ppm	64 fl. oz.	---	X	X	14	cd	1.6	bc
FireWall 100 ppm	8 oz.	---	X	X	13	cd	1.4	bc
Kasumin 10L 80 ppm	10.4 fl. oz.	---	---	X				
plus Fireline 80 ppm	6.4 oz.	---	---	X	11	d	1.3	c
Kasumin 10L 100 ppm	12.8 fl. oz.	---	X	X	11	d	1.1	c

* Trees inoculated on 21 April with 5 x 10^5 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin-sensitive pathogen strain).
** Transformed log(x + 1) prior to analysis of variance; non-transformed means are shown.
*** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown.
[§] 'X' indicates material was sprayed on that specific date; '---' indicates material was not applied on that specific date.
[#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.

2) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets. Trees in the study averaged 780 flower clusters. Fire blight risk, as determined by COUGARBLIGHT, measured 'low' during the bloom period with light precipitation occurring on 3 days between 80% bloom and petal fall. Nonetheless, disease intensity was high with water-treated trees averaging 196 blighted clusters per tree (24%). Most treatment programs were designed to integrate the floral stigma-colonizing *Pantoea agglomerans* in Bloomtime Biological (which slows epiphytic build-up of pathogen populations) with materials hypothesized to provide better protection in the nectary where infection occurs (Blossom Protect, Serenade Max and FireLine). All treatment programs provided significant suppression ($P < 0.05$) of floral infection with the exceptions of Actinovate, Rex Lime Sulfur mixed with Crocker's Fish Oil (only), and the single application Bloomtime Biological followed by one application of Serenade Max. Two applications of FireLine (streptomycin sulfate) provided 95% control relative to the water-treated check. Programs finishing with Blossom Protect at the full bloom and petal fall timings also provided > 90% control.

		Bloom stage treatment applied*								
Treatment	Rate per 100 gallons water	10% bloom	30% bloom	70% bloom	Full bloom	Petal fall	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Actinovate	3 oz.	X[§]	X	X	X	---	322	a[#]	38.6	a
Water control	------	---	**X**	**X**	**X**	---	**196**	**a**	**24.2**	**b**
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---	125	ab	17.5	b
Bloomtime Biological	5.3 oz.	---	---	X	---	---				
then Serenade Max [##]	64 oz.	---	---	---	X	---	125	abc	15.5	bc
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Bloomtime Biological	5.3 oz.	---	---	---	X	---				
then Serenade Max	64 oz.	---	---	---	---	X	66	bcd	8.6	cd
Bloomtime Biological	5.3 oz.	---	X	X	---	---				
then Serenade Max	64 oz.	---	---	---	X	X	54	cd	7.7	cd
Bloomtime Biological	5.3 oz.	---	---	X	---	---				
then FireLine 200 ppm	16 oz.	---	---	---	X	---	35	de	4.4	de
Bloomtime Biological	5.3 oz.	---	X	X	---	---				
plus/then Blossom Protect	1.34 lbs.	---	---	X	X	---				
with buffer A	9.35 lbs.	---	---	X	X	---				
then Serenade Max	64 oz.	---	---	---	X	X	31	def	4.2	de
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Bloomtime Biological	5.3 oz.	---	---	---	X	---				
plus/then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs.	---	---	---	X	X				
then Serenade Max	64 oz.	---	---	---	---	X	17	ef	1.8	e
Bloomtime Biological	5.3 oz.	---	X	X	---	---				
then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs.	---	---	---	X	X	15	efg	2.1	e
Rex Lime Sulfur	2 gal.	---	X	X	---	---				
with Crocker's Fish oil	2 gal.	---	X	X	---	---				
then Blossom Protect	1.34 lbs.	---	---	---	X	X				
with buffer A	9.35 lbs	---	---	---	X	X	12	fg	1.5	e
FireWall 100 ppm	8 oz.	---	---	X	X	---	9	g	1.3	e

* Trees inoculated at full bloom with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive strain).
** Transformed $\log(x + 1)$ prior to analysis of variance; non-transformed means are shown.
*** Transformed $\arcsin(\sqrt{x})$ prior to analysis of variance; non-transformed means are shown.
[§] 'X' indicates material was sprayed on that date; '---' indicates material was not applied on that date.
[#] Within a column, means followed by same letter do not differ significantly at $P = 0.05$ according to Fisher's protected LSD.
[##] Serenade Max treatments also included Biolink spreader-sticker at a rate of 6 fl. oz. per 100 gal.

3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

a). Rootstock only experiment. Drenches and paints of the a SAR material, ASM (acibenzolar-*S* methyl) slowed expansion of fire blight in one year old, potted apple rootstock cultivars, EMLA 29 and Nic 29 apple . In non-treated trees, the expanding canker consumed nearly all of the current season growth, but generally did not expand into the woody tissue. Green shoots on SAR-treated trees had smaller cankers relative to the non-treated trees, but the effects of the SAR treatment on apple rootstocks was smaller than was observed previously in pear (see 2009 pear report). In pear, the marked effect of ASM on canker expansion occurring in woody tissue. For apple rootstocks, we attribute the smaller effect of ASM to the fact that cankers were limited to the current season shoots. Interestingly, relative to drench and spray treatments, the ASM paint treatment (15 g a.i./L) at inoculation showed the largest reduction in canker size (top right panel).

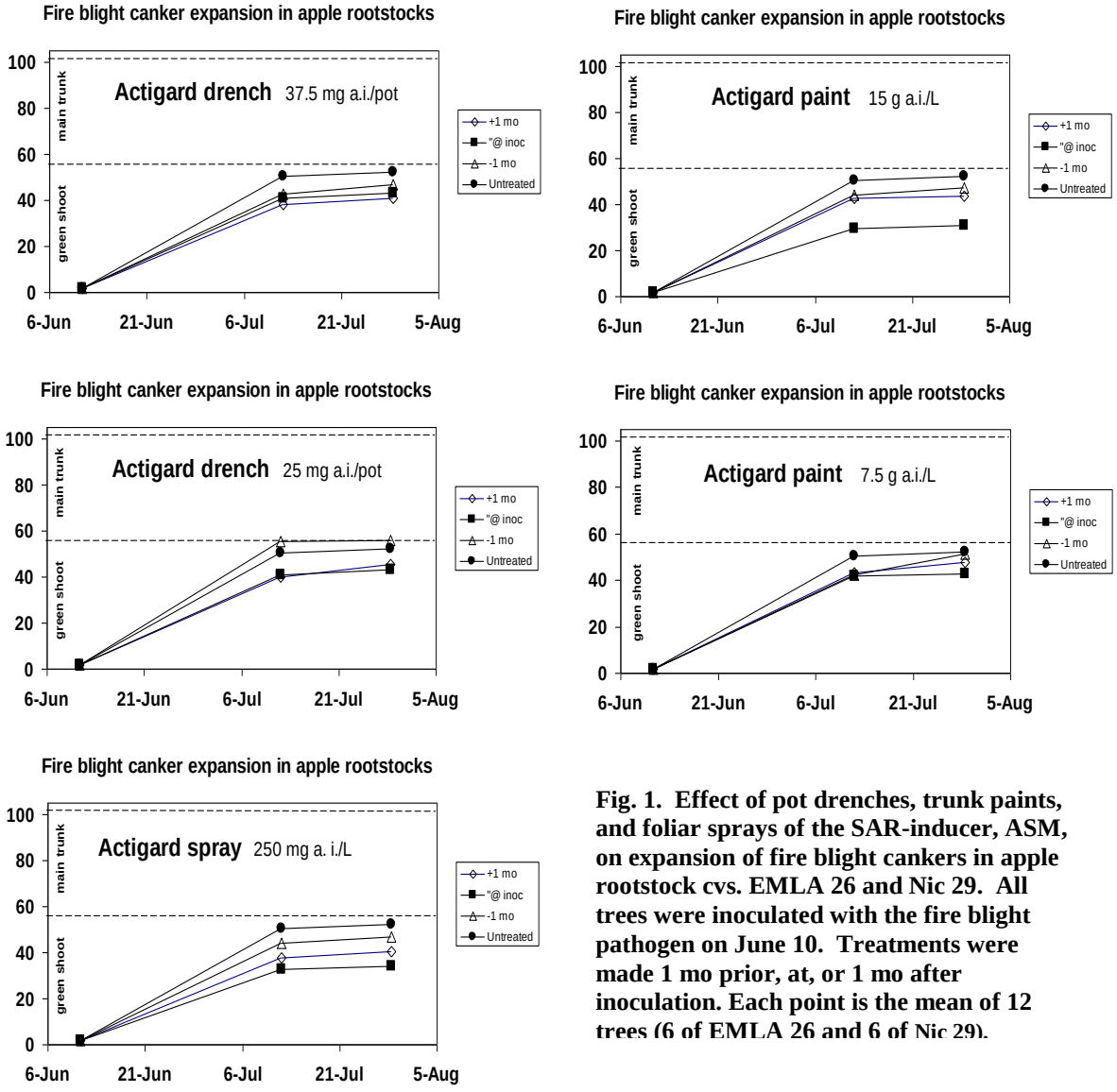
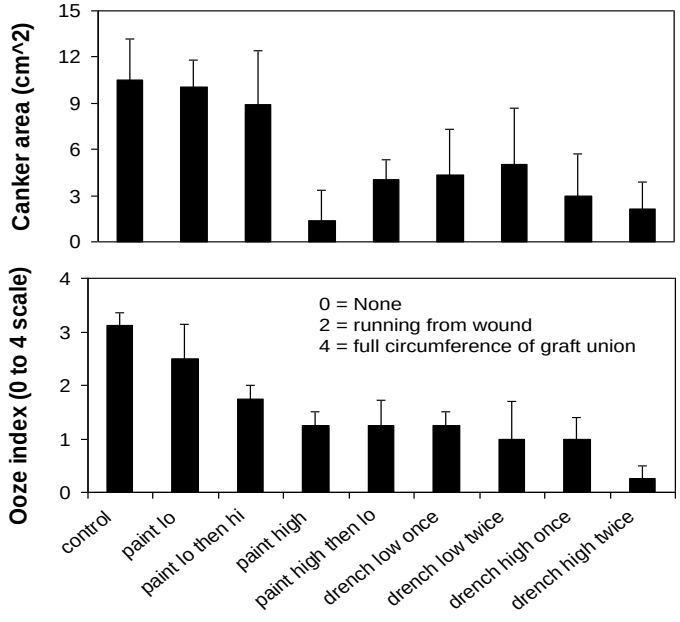


Fig. 1. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expansion of fire blight cankers in apple rootstock cvs. EMLA 26 and Nic 29. All trees were inoculated with the fire blight pathogen on June 10. Treatments were made 1 mo prior, at, or 1 mo after inoculation. Each point is the mean of 12 trees (6 of EMLA 26 and 6 of Nic 29).

3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

b). Rootstock Scion experiment. Drenches of ASM, but also some ASM-paint treatments, provided significant control of canker expansion in wound-inoculated, EMLA 26 rootstock (under Pacific Gala). In particular, two drenches (May 21 and July 2) of ASM (50 mg a.i. per plant) provided nearly complete suppression of canker development. By October, scions of several of the untreated trees were dead as a result of the girdling fire blight canker in the rootstock. In contrast, none of the ASM-treated trees were girdled; these trees were overwintered and will be re-assessed in spring 2011.



Ooze of the fire blight pathogen on untreated EMLA 26 (Gala).

Discussion:

Kasumin. When registered, Kasumin will enhance control and broaden the effective tool box for conventional fire blight management. Kasumin is not used in human medicine, and shows no cross resistance to streptomycin or oxytetracycline. **Note: the PNW industry should plan to comment on the proposed label during EPA comment period (likely timing: August 2011), as some proposed label restrictions are more restrictive than prior labels for antibiotic materials** (e.g., no alternate row spraying). An EPA registration for Kasumin is on track for spring 2012.

Organic control. Consistency of control from Blossom Protect requires further evaluation, and we need to obtain a better understanding for how Blossom Protect (and the buffer) suppresses the fire blight pathogen. Our working hypothesis (which is providing the outstanding results) is that the yeast protects the nectary from infection and best used late in bloom (like oxytetracycline or Serenade Max); direct evidence, however, to support this hypothesis is lacking. An EPA registration for Blossom Protect is on track for spring 2012.

SAR. Like pears, fire blight-susceptible apple rootstock cultivars respond to drenches, paints and sprays of the SAR inducer, acibenzolar-*S* methyl, resulting in slowed canker expansion in diseased trees. The effect of ASM was most dramatic when woody tissue of EMLA 26 was inoculated with *E. amylovora* just below the graft union. We are making progress in understanding the effective rates of ASM for the various methods of application. Whether or not SAR will be practical in the field is going to depend on the cost of effective rates of ASM. ASM costs ~80¢ per gram; material costs for the range of treatments in the above experiments were 5-15¢ per tree. Effective rates for larger trees will likely be higher.

CONTINUING PROJECT REPORT **EXTENSION**

Project Title: Identification of powdery mildews attacking apples and cherries in WA

PI:	Dean Glawe	**Co-PI (2):**	Chang-Lin Xiao
Organization:	Washington State Univ.	**Organization:**	Washington State Univ.
Telephone/email:	206-616-9554/ glawe@wsu.edu	**Telephone/email:**	(509)663-8181 Ext 229/ clxiao@wsu.edu
Address:	Forest Resources, Box 352100	**Address:**	WSU-Tree REC
Address 2:	University of Washington,	**Address 2:**	1100 N. Western Avenue
City:	Seattle	**City:**	Wenatchee
State/Zip	WA 98195	**State/Zip:**	WA 98801

Cooperators: Gary Grove, Washington State Univ.
Growers across the state

Total Project Request: **Year 1:** $37,860

Other funding sources: None

WTFRC Collaborative expenses: None

Budget 1
Organization Name: Washington State Univ. Contract Administrator: Cheryl Hagelganz
Telephone: 509-335-4852 Email address: chagelganz@wsu.edu

Item	2010	2011 (extension)	
Salaries[1]	0		
Benefits[1]	0		
Wages	$18,122		
Benefits	$1,740		
Equipment	0		
Supplies[2]	$5,700		
Travel[3]	$4,298		
Miscellaneous[4]	$8,000		
Total	$37,860	No request	

Footnotes:
[1]hourly worker (Tess Barlow)
[2]laboratory supplies (reagents, DNA extraction kits and associated materials, plastic ware, supplies for microscopy, collecting bags, misc)
[3]travel (7 trips from Seattle to collect research specimens in orchards, 3 nights, 4 days, per diem, est. at $614/trip)
[4]Gene sequencing (estimated 800 runs @ $10 each)

OBJECTIVES

1) To develop accurate, up-to-date information on the species of powdery mildews attacking apples in Washington.
2) To develop preliminary information on the species of powdery mildews attacking cherries in Washington.

Activities include:

- Collecting specimens from about 10 sites within apple orchards and 10 sites within cherry orchards in each of five fruit-production regions, including: Brewster to Okanogan area (north); Wenatchee area (north central); the greater Royal Slope area (the Columbia Basin); Yakima area (south central); Tri-Cities; and Walla Walla area (south).
- Characterizing about 300 specimens using bright-field and differential interference microscopy
- Generating ITS and 28S rDNA sequences from about 50 apple powdery mildew specimens (10 from each production region)
- Generating ITS and 28S rDNA sequences from about 50 cherry powdery mildew specimens
- Analyzing data by various methods including comparing morphological data with published descriptions of powdery mildew species and comparing DNA sequence data against reference sequences in GenBank
- At the conclusion of the study specimens will be deposited in the Mycological Herbarium at Washington State University and sequence data will be deposited in GenBank

Anticipated timeframe of activities and accomplishments (April 1, 2010-March 31, 2011)

Activities	Time period	Personnel
Identify collecting sites representing diversity of environments and apple and cherry cultivars in the state.	Winter and early Spring, 2010	Xiao, Grove, and Glawe
Assemble a collection of about 600 specimens of apple and cherry powdery mildew.	April-October, 2010	Xiao, Grove, and Glawe
Characterize the apple specimens and representative cherry specimens.	April, 2010-March, 2011	Glawe and Barlow
Generate ITS and 28S rDNA sequences.	June, 2010-March 2011	Barlow and Glawe
Determine the modern name or names to apply to the collected powdery mildews on the basis of morphological and sequence data.	October, 2010-March, 2011	Glawe
Report on research findings to WTFRC.	December, 2010 [January, 2011]	Glawe, Xiao, and Grove
Deposit specimens in Mycology Herbarium at WSU, deposit gene sequences in GenBank, submit manuscript for publication.	March, 2011	Barlow and Glawe

SIGNIFICANT FINDINGS

This project is still in progress because of the nature of the research. A one-year extension of this project without additional funds has been approved by the WTFRC.

Summary of significant findings:

- To date, only *Podosphaera leucotricha* has been detected on apple and only *Podosphaera clandestina* has been found on cherry.
- No variation in ITS and 28S rDNA sequences have been detected in *P. leucotricha* (from apple)
- No other species of powdery mildews known to attack apples have been detected
- No variation in ITS and 28S rDNA sequences have been detected in *P. clandestina* (from cherry)
- A wild species of *Prunus* was found to host *P. clandestina* but this strain differed in 4 of 620 bp when compared to ITS in specimens collected from commercial cherries

METHODS

For the duration of the study the same methods for extracting DNA, amplifying rDNA sequences, and assessing variability will be used. Preliminary results suggest that it may be possible to use IGS sequences to assess further variability of these powdery mildews; IGS sequences are known to be more variable than ITS or 28S rDNA sequences. Morphological characterization will continue to use brightfield and differential interference microscopy.

RESULTS & DISCUSSION

So far we have compiled data on 562 apple powdery mildew specimens collected from 35 sites. Sequencing currently is in progress. Specimens sequenced so far were selected to maximize geographic diversity. To date, only *Podosphaera leucotricha* has been detected. The following information summarizes the apple varieties collected, the number of collecting sites ("# sequencing completed" is the number of sites from which DNA sequences have been obtained), and the number of specimens from which variety that were collected.

Variety	**# Collecting Sites (# sequencing completed)**	**# Specimens**
Fuji	7(4)	82
Gala	13(7)	150
Ginger Gold	2(0)	22
Golden Delicious	2(2)	23
Granny Smith	7(5)	82
Honey Crisp	9(2)	87
Jonathan	1(1)	10
Pink Lady	9(1)	25
Rubens	1(1)	10
Variety to be confirmed	5(3)	71

88 cherry powdery mildew samples were collected from 10 sites. Sequencing currently is in progress, specimens sequenced so far were selected to maximize geographic diversity. To date, only *Podosphaera clandestina* has been detected. The following information summarizes the cherry varieties collected, the number of collecting sites ("# sequencing completed" is the number of sites from which DNA sequences have been obtained), and the number of specimens from which variety that were collected.

Variety	**# Collecting Sites (# sequencing completed)**	**# Specimens**
Bing	6(3)	61
Rainier	1(0)	4
Sweetheart	1(0)	9
Van	1(1)	1
Wild *Prunus*	1(1)	3
Variety to be confirmed	1(0)	10

In work so far we have detected only *Podosphaera leucotricha* on apple and *Podosphaera clandestina* on cherry. Although much work remains to be done, if these results hold, the information from this study may facilitate exporting apples and cherries to other countries.

The discovery of a distinct strain of *P. clandestina* on wild cherry raises some questions of significance to the industry. Can this strain attack commercial cherry varieties or breeding lines? How diverse are *P. clandestina* strains on wild hosts? Do they increase the likelihood that resistance to powdery mildew could be overcome in future varieties? Can wild strains play a role in the emergence of fungicide-resistant strains in orchards? Additional work to collect on wild hosts and test the host ranges of P. *clandestina* strains on them would help answer these questions.

Results so far suggest that ITS and 28S regions from strains of powdery mildews attacking apples and cherries are invariable. If work in progress confirms the preliminary results it suggests that these regions are potentially very useful for PCR-based approaches to detecting these species.

Collecting cherry powdery mildew was hampered this year because incidence was lower than in previous years, and the development of populations was much slower than for apple powdery mildew. Consequently the plan to collect both apple powdery mildew and cherry powdery mildew on the same collecting trips proved to be less than successful. For future projects it would be prudent to plan separate collecting trips if both diseases are being studied.

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

Project Title: Identification of critical physiological targets in codling moth

PI: Stephen F. Garczynski
Organization: USDA-ARS
Telephone: 509-454-6572
Email: steve.garczynski@ars.usda.gov
Address: 5230 Konnowac Pass Road
City: Wapato
State/Zip WA / 98951

Cooperators: Dr. Amit Dhingra, WSU; Dr. Kevin Wanner, Montana State University

Total Project Request: **Year 1:** $47,785 **Year 2:** $49,000 **Year 3: $34,204**

Other funding sources

Agency Name: USDA-CSREES National Research Initiative
Amount awarded: $365,042 (2008-2011)
Notes: This grant was awarded based on results generated from my 2006-2007 WTFRC funded research to identify codling moth chemosensory receptors.
Notes: Due to teaching commitments, Dr. Lavine will not be participating in year 3 of this project.

Budget 1
Organization Name: USDA-ARS **Contract Administrator:** Charles Myers
Telephone: (510) 559-5769 **Email address:** Chuck.Myers@ars.usda.gov

Item	**(2009)**	**(2010)**	**2011**
Salaries	14,000	15,000	16,000
Benefits	1019	1024	1204
Wages			
Benefits			
Equipment			
Supplies	12,000	12,000	12,000
Travel			
Miscellaneous	5000	5000	5000
Total	$32,019	$33,024	**$34,204**

Footnotes:
[1]Salary will be used to support Ms. Jennifer Stout, a part time GS-7 Technician (½ year)
[2]The miscellaneous funds requested are to help defray the cost of equipment maintenance which includes autoclaves, Real time PCR machine, yearly Laminar Flow hood (biosafety cabinet) certification, and Pipette calibration, repair and certification.

OBJECTIVES:

The goal of this project is to provide fundamental biological information on codling moth physiology that can be used to identify new targets for enhanced pest control. Identification of critical physiological targets in codling moth will provide information and methods that will allow us and other researchers to develop new strategies and tools for control of this major pest of apple.

1) Characterize pheromone biosynthesis activating neuropeptide (PBAN) and its receptor (PBANR) in late 5th instar larvae and adult females. Pheromone biosynthesis activating neuropeptide (PBAN) is a polypeptide (short protein) hormone that stimulates the production of pheromones in insects by interacting with its receptor (PBANR). Both PBAN and PBANR are potential protein targets that if blocked could inhibit pheromone biosynthesis (including codlemone), thus having great potential to enhance the effectiveness of mating disruption for codling moth control.

2) Characterize diapause hormone (DH) and its receptor (DHR) in eggs, neonate and 5th instar larvae. Diapause hormone (DH) is encoded by the same gene as PBAN. DH, along with other physiological factors, in some moth species regulates diapause through signals sent by its receptor (DHR). Both DH and DHR are potential targets that if altered may disrupt the codling moth's ability to enter or leave diapause, and allow researchers to take advantage of this physiological pathway for codling moth control.

3) Identify potential targets in eggs and neonate larvae by analyzing the transcriptome, and determine those that may be critical for insect survival. Because of the codling moth's life cycle, eggs and neonate larvae are accessible to control measures in the orchard. Sequencing the transcriptome (a compilation of genes that are being actively expressed) of eggs and neonate larvae will allow us to identify potentially critical protein targets in these codling moth life stages. After identification, we will characterize potential protein targets to gain a further understanding of the basic physiology of eggs and neonate larvae and assess their usefulness for codling moth control.

4) Identify potential targets in adult males and females by analyzing the transcriptome, and determine those that may be critical for insect survival. Adult males and females are also accessible to control measures in the orchard. Sequencing transcriptomes made from chemosensory organs (mouthparts, antennae, and legs) will allow us to identify smell and taste receptors expressed in males and females. Further characterization of these receptors and their signal transduction pathways will help us to gain a further understanding of physiology as it is related to host and mate finding. Proteins important in these physiological pathways are potential targets for enhanced insect control measures.

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS):

Year 1

- A gene transcript encoding the codling moth PBAN receptor has been cloned.
- A gene transcript encoding the codling moth Insulin receptor has been cloned.
- A gene transcript encoding the codling moth short neuropeptide F receptor has been cloned.
- Transcriptomes from codling moth eggs, neonate larvae, and adult male and female chemosensory organs have been made.
- Full length transcripts of heat shock proteins (HSP) have been cloned and the expression profiles for the small heat shock proteins in various codling moth stages have been determined.

Year 2

- Transcripts encoding chemosensory proteins (13 unique), odorant binding proteins (10 unique), General odorant binding proteins (3 unique), and pheromone binding proteins (5 unique) have been identified in transcriptomes of codling moth chemosensory organs.
- Transcripts encoding 25 unique heat shock proteins have been identified in the transcriptomes of codling moth eggs, larvae, and male and female chemosensory organs.
- A membrane receptor that interacts with codlemone has been tentatively identified via a cell based assay.
- A common odorant receptor with homology to the pheromone/kairomone receptor family has been detected in the genomes of codling moth, oblique-banded leafroller, *Pandemis* leafroller and oriental fruit moth.

Manuscripts In Press or accepted for Publication

Garczynski, S.F., T.R. Unruh, C. Guédot and L.G. Neven. (In Press) Characterization of three transcripts encoding small heat shock proteins expressed in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). Insect Science.

Garczynski, S.F., K.W. Wanner and T.R. Unruh. Identification and initial characterization of the 3' end of gene transcripts encoding putative members of the pheromone receptor sub-family in Lepidoptera. Accepted for publication in Insect Science

METHODS

1) Transcriptome generation and analysis.
    A) RNA was isolated from various chemosensory tissues, eggs, and neonate larvae.
    B) RNA was converted to cDNA, and the cDNA was sequenced using 454 technology.
    C) Nucleotide sequences of cDNAs were assembled and then annotated using bioinformatic software packages.
    D) Annotated sequences were analyzed, and potential targets identified. These included chemosensory proteins, odorant binding proteins, pheromone binding proteins, and heat shock proteins.
2) Microplate Assays
    A) CHO cells were genetically modified to express codling moth “pheromone” receptors.
    B) CHO cells stably expressing pheromone receptors were incubated with codlemone.
    C) Detection of responses to codlemone were recorded on a microplate fluorometer.
3) Amplification of 3’ Untranslated region of codling moth “pheromone” receptors
    A) Sequence specific primers were designed.
    B) PCR reactions to amplify the 3’ untranslated region were performed.
    C) Amplification products were cloned, sequenced and analyzed
4) Detection of “Pheromone” receptors in other tortricid moths
    A) Genomic DNA was extracted from codling moth, oblique banded leafroller, Pandemis leafroller, Oriental fruit moth, and Strawberry leafroller.
    B) Primers specific for each of the codling moth pheromone receptors were used to amplify PCR products in the genomes of the tortricids above.
    C) PCR products generated were cloned, sequenced. and analyzed.

RESULTS AND DISCUSSION

The overall goal of this project is to identify and characterize targets in the codling moth that can potentially be used in the control of this insect pest. The main targets are proteins important in pathways that regulate critical physiological functions in the insect. Four physiological systems, endocrine (hormones), chemosensory (smell and taste), reproductive (egg formation and development), and digestive, are being examined in this study. In the past year, several gene transcripts encoding proteins involved in the above physiological pathways have been identified in the codling moth.

Transcriptome annotations of chemosensory tissues have led to the identification of chemosensory, odorant and pheromone binding proteins expressed by codling moth (Table 1). 13 chemosensory proteins, 10 odorant binding proteins, 3 General odorant binding proteins, and 5 pheromone binding proteins were identified among the sequences determined in the transcriptome. Chemosensory proteins are important for recognizing environmental compounds, including detection of food sources. General odorant binding proteins and Pheromone binding proteins are important for sex pheromone and kairomone detection, and odorant binding proteins allow the insect to sense chemical "smells" in their environment. Identification of these proteins will allow future characterizations to determine the specific compounds recognized by the codling moth chemosensory system, and lead to research giving us a better understanding of how this insect pest detects mates and host plants.

Table 1. Chemosensory Proteins, Odorant Binding, General Odorant binding, and Pheromone Binding proteins identified in Codling Moth Transcriptomes

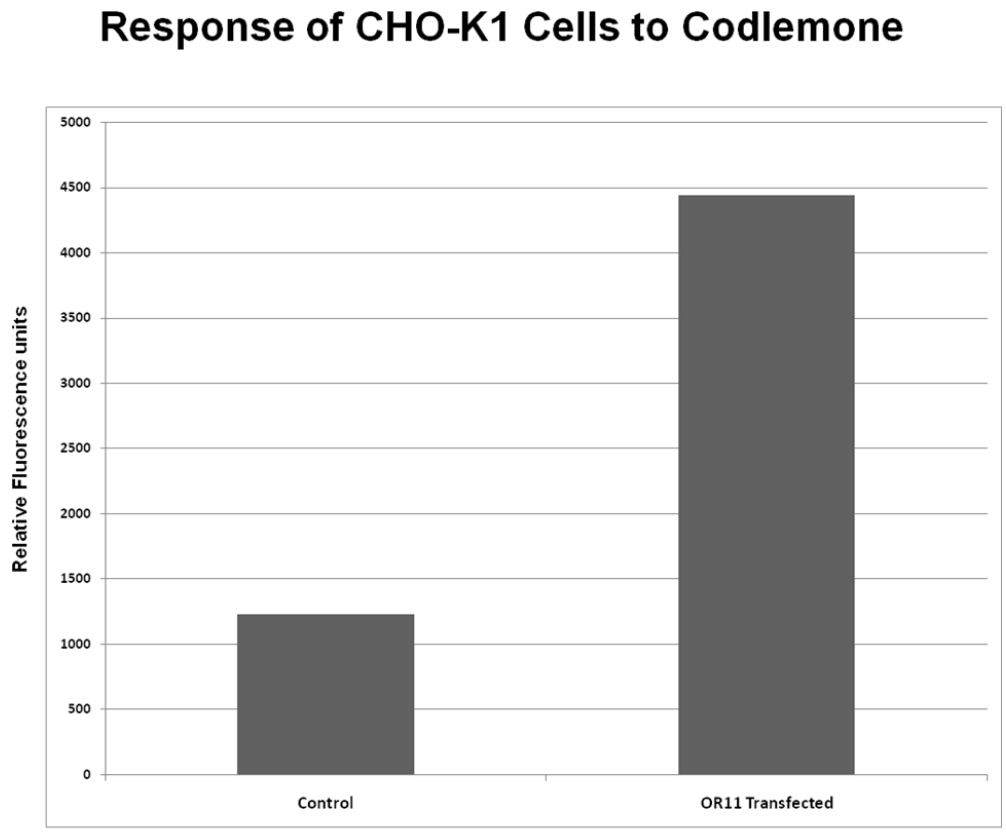
Male Antennae	**542**	**4**	**8**	**3**	**4**
Female Antennae	**475**	**7**	**9**	**3**	**4**
Male Legs and Mouthparts	**431**	**5**	**1**	**0**	**2**
Female Legs and Mouthparts	**350**	**6**	**0**	**0**	**1**
Eggs (Embryos)	**660**	**4**	**0**	**0**	**0**
Neonate Larvae	**617**	**5**	**0**	**0**	**1**
All Tissues	**2267**	**13**	**10**	**3**	**5**

Chemosensory proteins = CSP, Odorant binding proteins = OBP, General odorant binding proteins = GOBP, Pheromone binding proteins= PBP

Heat shock proteins were also detected in the codling moth transcriptomes. We previously identified 3 small heat shock proteins using a degenerate primer/PCR approach (Garczynski et al. In Press). The transcriptome data revealed the presence of at least 12 additional small heat shock proteins, and several other larger heat shock proteins (Data not shown). Heat shock proteins are expressed in response to environmental stressors, including insecticide exposure, cold, and

atmospheric treatments. Plans to monitor expression of codling moth heat shock proteins in response to insecticides, and controlled atmosphere conditions are currently being planned to determine their potential role in codling moth responses to these different stress treatments.

Cell lines expressing "pheromone" receptors cloned from codling moth were generated for a cell based assay system to determine their ligands. Initial studies have been done, and CpOR11 appear to be responsive to codlemone (see figure below). Because CpOR11 is a highly expressed male-biased receptor, it is potentially the codling moth sex pheromone receptor. These is exciting to us in that it lays the ground work for the further characterization of pheromone receptors and provide a high-throughput assay system that can be useful for finding more potent agonists or antagonists that can be used for codling moth control. Work this upcoming year will include further development of this assay system.



Relative fluorescence differences in detection of intracellular Calcium.
Ca_i was detected in cell lines loaded with Fluo-4AM. Microplates were seeded with CHO-K1 cells (Control) or CHO-K1 Cells transfected with plasmids encoding CpOR2, CpOR11 and CpGq (OR11 Transfected). Cells grown on microplates were treated with or without 10μM Codlemone, and relative fluorescence was measured on Fluoroskan Ascent FL. Graph represents RFU differences measured for control or transfected cells in response to codlemone. Because OR11 is highly expressed , male biased, and seems to be responsive to codlemone, could it be the major codling moth sex pheromone receptor?

Another potentially exciting result is the detection of codling moth "pheromone" receptors in other tortricid pests that can use apple as a host. DNA sequences encoding a portion of CpOR124 and CpOR121 (members of the "pheromone" receptor family) have been amplified in PCR reactions (data not shown) using genomic DNA from various tortricids (oblique banded leafroller, Pandemis

leafroller, and Oriental fruit moth). This result could indicate a potential importance of these receptors in the detection of kairomones or other volatiles, perhaps leading these insects to tree fruit hosts. Work this upcoming year will be to confirm the presence of these receptors in each of the tortricid pests, and to determine expression and ligands in cell based assays.

A mechanism that potentially controls pheromone receptor placement in the nerve membrane has been uncovered in our analyses. By altering the length of the 3'untranslated region of "pheromone" receptor transcripts the insect can potentially regulate how much receptor protein gets made. This would be important in codling moth detection of sex pheromone and would allow for males to "hone in" on females as pheromone concentration increases. Having transcripts with a variety of 3'UTR lengths can potentially regulate the number of receptors on a nerve membrane, or work to produce increased receptor numbers in response to pheromone detection. This rare mechanism has been previously cited as a means for translational control of gene transcripts in mammals, but has yet to be reported for insects. This upcoming year we will be exploring this further, perhaps explaining how mating disruption "works".

CONTINUING PROJECT REPORT **YEAR** 2 of 3
WTFRC Project Number: CP–09-904

Project Title: Improving the Management of Two Critical Pome Fruit Diseases

PI: Timothy J. Smith
Organization: Washington State University
Telephone/email: 509-667-6540 / smithtj@wsu.edu
Address: 400 Washington Street**,**
City: Wenatchee,
State/Zip WA 98801

Research Tech: Esteban Gutierrez

Cooperators: Travis Allan, Allan Bros. Fume Trial Site; Mike Conway, Trident Agricultural Products.

Total Project Request: Year 1: $18,294 **Year 2:** $18,760 **Year 3: $19,071**

Other funding sources

Trident Agricultural Products has provided in-kind support (fumigation) $9000 value, and grants totaling $7000 to date. 2010 grants totaling $18,800 were received from private companies for fire blight and replant disease work. Over the past two years, grants and in-kind support of this project from sources other than the Washington Tree Fruit Research Commission total $40,300.

WTFRC Collaborative Expenses: None

Budget
Organization Name: WSU **Contract Administrator:** Jennifer Jansen
Telephone: 509-335-2867 **Email address:** jjansen@wsu.edu

	2009	2010	**2011**
Salaries	11,493	11,951	12,429
Benefits	5,401	5,617	5,842
Wages			
Benefits			
Equipment	100		
Supplies	100		
Travel	1,200	1,200	800
Miscellaneous			
Total	$18,294	$18,760	**$19,071**

Footnotes: Salaries and benefits are in support of 0.34 FTE of a full time technician. Travel is to plot sites.

SUMMARY OF SIGNIFICANT FINDINGS

- For the third season in apple and pear fire blight control material trials, a dried yeast product, *Aureobasidium pullulans*, called "Blossom Protect" in Europe, provided blossom protection similar or superior to antibiotics. The effort to find an acidifying buffer to replace the bulky buffer now recommended for use with Blossom Protect was not successful. However, acidifying buffers applied as a blossom treatment controlled fire blight 38%, which is not a control break-through, but may lead to inexpensive control enhancement after further testing.
- The antibiotic kasugamycin, which recently was granted a special label for use in Michigan, protected apple blossoms from a streptomycin susceptible strain of blight bacteria to a degree similar to the protection provided by streptomycin (AgriStrep, etc.), and both were superior to oxytetracycline (Mycoshield, etc.). While this work is not finished, the addition of oxytetracycline to kasugamycin did not improve performance.
- A specific proprietary copper compound formulation once again provided blossom protection equal or superior to antibiotics. The standard (Kocide 3000) copper compound used as a comparison in the trials did not adequately protect the flowers from infection, a result common in past trial copper treatments. The new copper compound did not appear to russet apples, D'Anjou or Bartlett pears when applied during primary bloom, though it must undergo much more extensive fruit safety tests during the critical post bloom infection period. The manufacturer is still working on the formulation, so registration is not near.
- This season's most effective treatment in both apple and pear trials was two applications of acibenzolar–s-methyl (ASM, Actigard) in pre-bloom, followed by an antibiotic at time of inoculation. This treatment also reduced the severity of damage to infected test portions of the trial trees. This will be tested more extensively next year. Application of this product to the soil under the test tree reduced blight infection, but not much.
- The "CougarBlight" fire blight infection risk model was upgraded this year by conversion of the temperature risk values to relate directly to the hourly growth rate of E. amylovora on the tips of apple stigmas. Local research by Dr. Larry Pusey, USDA-ARS Wenatchee provided the data used for this upgrade. After a "beta" test year, the new version, CougarBlight 2010, will replace the current Cougarblight version (2000).
- In the "Radar Hill" fumigation trial, tree growth in the treated trees continued to be quite acceptable, though there is more variation in height than evident in year 1. The untreated trees grew much less well, especially in trunk cross section (or caliper). Caliper of untreated trees was 58% the average of treated trees. In other trials, lesser trunk caliper to this degree in early years of tree growth has led to much lower yields over the life of the orchard. While height and cross section of the trunk differ only between treated and untreated (greatly), there was some developing variation among the treatments in total lateral shoot growth. This is almost certain to lead to significant yield differences between some treatments in the fourth leaf, the first planned production season (2012.)

FIRE BLIGHT PROJECT (75% of effort in 2010)

OBJECTIVES- ***Fire blight of apple and pear, as stated in the proposal:***

1. We will continue to assess efficacy of new or inadequately tested sprayed fire blight control products in the orchard, on both apple and pear.
2. To increase confidence in the biological organism that appeared promising in the 2008, and 2009 trials, we will significantly expanded our testing in 2009 and 2010 to include a range of alternative spray timings, rates, and buffers.

3. We will further study the relationship of temperatures to fire blight infection risk, with the intention of changing the temperature assumptions used in the Cougarblight model, if these studies show that changes are necessary.

METHODS:

The methods used in this trial are standardized under the European and Mediterranean Plant Protection Organization protocol on efficacy evaluation of bactericides, 2002, OEPP/EPPO, Bulletin 32, 341- 345.

Objectives 1 and 2, Fire blight control product efficacy: Two fire blight control material efficacy trials were carried out, one on Red Delicious apples at WSU TFREC, and the other on D'Anjou pears at the WSU Smith Tract research unit. These sites and cultivars were chosen due to their low sensitivity to the necessary fire blight exposure and infection.
About 400 blossom clusters, 100 per replicate, were treated by back-pack mist sprayer at various timings and rates relative to stage of blossom development, then inoculated by spraying a known concentration of a streptomycin-susceptible laboratory strain of *Erwinia amylovora* to assure a high degree of infection, dependant on degree of protection by the tested substance. Efficacy was evaluated by counting the number of blighted vs. unblighted fruit clusters on the inoculated area on the tree. This method skews the data to indicate a higher percentage of blossom infection than would ever be likely in an orchard, but is very useful when comparing the relative efficacy of one product to another. The reader should not extrapolate the percent control achieved in these trials to the percent control likely in the orchard under natural infection conditions.

Objective 3, The Redesign of the CougarBlight risk model:
Overview: Contamination of flowers by *E. amylovora* does not necessarily lead to infection. After infesting the flower, populations of the pathogen have only a few days to grow to at least 100,000 to 1 million live bacteria prior to the potential infection event. This pathogen multiplies on the flower stigmas, slowly at temperatures below 70ºF, moderately at temperatures between 70 and 75ºF, and rapidly at temperatures between 75 and 93ºF. Optimum population size growth rate occurs between 82 and 90ºF. At temperatures over 95ºF, growth rapidly decreases to zero and populations decline in size at any temperature over about 99ºF. The temperature measurements used in the CougarBlight model were previously described as degree days above 60ºF. This has never been an accurate description, particularly when describing the 2010 version.
Methods: The new "temperature risk value" units were developed from unpublished data (P.L.Pusey, UDSA-ARS) for population growth of *E. amylovora* on stigmas. Crab apple flowers were inoculated with *E. a.* using a suspension of 10^7 CFU/ml, (10,000,000 live bacteria per ml, or 50 million per teaspoon), resulting in a starting population of about 300 live bacteria per flower. The flowers were held at 15 different temperatures between 39 and 102ºF for 24 hours. The resulting population size was divided by 24 to estimate the increase in population per hour. That number was then divided by 1,000 to make the temperature value numbers smaller and more practical to manage. These numbers were used to develop a population growth curve, and fill in missing values for each half degree of temperature Celsius between 4ºC and 35ºC and the equivalent range in Fahrenheit (39 and 95F). (Note: models in both F and C versions now use the same temperature threshold numbers.) These temperature values are used to compile heat units per hour relative to the potential population size of blight bacteria colonies. Then, for forecasting blight risk, a table of average daily temperature risk values related to the daily high temperature was developed. More than 2500 days in April, May and June at numerous sites and several years in central Washington State, USA, were assigned a value for every actual hourly temperature. These values were summed for every 24-hour period, and

sorted into groups relating to daily high temperature. While the temperature risk values usually tend to fall very close to the average, there can be significant variation away from this average for any specific actual day. Due to this inevitable variation, average risk values taken from the table and thresholds are considered as forecast estimates and guidelines. These average daily risk values may be used to run the simple form of the model, and will be used in the forecasting mode of any automated CougarBlight model system. To accurately determine the actual daily temperature risk values, hourly temperatures must be monitored and assigned an individual corresponding risk value, which is summed with others for the day. Computer automation is almost required for this task. The hourly specific values are published and available. The new "CougarBlight 2010" will run as a beta option in 2011.

RESULTS & DISCUSSION:

Two non-antibiotic materials performed very well in the 2009 trials. The copper compound, which will be referred to as "copper product TS (Trade Secret)," reduced fire blight infection as well as, or better than, standard and test antibiotics, and to a far greater degree than any other copper material or copper/fungicide combination tested by the author in this or previous trials. The company manufacturing this product requested that the product remain unnamed at this time. The formulation of this product is being adjusted to reduce phytotoxicity on citrus, and new product was not available for inclusion in this year's pear trial. However, it was applied to both D'Anjou and Bartlett pears as a russet trial. There was no russet on the fruit skin observed at harvest, even on the usually russet-prone D'Anjou pears. The 2009 formulation seems more effective than the 2010 version, but results were still impressive at the high recommended rates.

The biological product is a mixture of two strains of *Aureobasidium pullulans*, a yeast, which is applied in combination with a pH 5 acid buffer. This genus and species of yeast is commonly found in the Pacific Northwest as a natural colonizer of apple and pear flowers so will probably thrive and spread to newly opened blossoms under PNW conditions. It is not likely that this organism is producing its own antibiotic to achieve antibiotic-like performance in inoculated trials, as this is not typical of yeasts. It is possible that another mechanism, such as successful competition for resources on the stigma surface or within the nectary, serves as a control process. In order for control to occur, it appears that this organism must be in place soon after each flower opens so as to become well-established on the flower before the introduction of *Erwinia amylovora*, the fire blight pathogen.

Buffering spray water to acid pH is recommended when applying antibiotics. The company that will market Blossom Protect in the USA recommended the addition of a large quantity of a specific acid buffering additive (9.35 lb. per 100 gal./A), called "Buffer A" to be applied along with the yeast. The addition of this pH 5 buffer has consistently improved control. This season, the Buffer A and another acidifying buffer, "Tech Spray Mg," were tested to test the effect of flower nectary acidification. Tech Spray Mg was also tested as an alternative to the bulky Buffer A. Both buffers when applied to flowers had a low, but significant effect on reducing the infection percentage, but the alternative did not effectively replace Buffer A as an acidifier for the *A. pullulans* "Blossom Protect."

Note: Some of the products reported above are not yet registered for use in orchards. They are reported only to report the results of research. Check the label prior to use.

FIRE BLIGHT CONTROL PRODUCT EFFICACY – PEARS

Product	Rate	Timing	% Infection	% Control
Actigard Pre-bloom, Sprayed on. + Strep 100% bloom	Actigard 0.2 gram per tree, twice Strep. 200 ppm	Actigard 20 and 50% bloom Strep. 100% Bloom	0.8	98.2a
Kasumin 2L + oxytetracycline	2 qt./A, 100 ppm 1 lb/A, 200 ppm	100% bloom	7.9	82.4b
"Blossom Protect" A.p. Yeast (full rate) + Buffer A ½	1.34 lb/100gal/A 4.7 lb/100/A	20, 50 & 100% bloom	7.9	82.4b
A.p. Yeast (half rate) + Buffer-A ½	0.68 lb/100gal/A 4.7 l b/100 A	20, 50 & 100% bloom	7.9	82.4b
A.p. Yeast (full rate) + Buffer A	1.34 lb/100gal/A 9.35 lb. /100/A	20 & 50% 100% bloom	8.5	81.1b
Streptomycin 17%	1 lb/A, 200 ppm	100% bloom	9.0	80.0b
Kasumin 2L (1x)	2 qt./A, 100 ppm	100% bloom	9.2	79.5b
Oxytet. (FireLine)	1 lb/A, 200 ppm	100% bloom	10.0	77.7b
Kasumin 10L + oxytetracycline	6.4 oz/A, 50 ppm 0.5 lb/A, 100 ppm	100% bloom	14.5	67.7c
A.p. Yeast (full rate) + alt. buffer	1.34 lb/100gal/A to pH 5	20, 50 & 100% bloom	16.7	62.8c
Kasumin 10L (1x)	12.8 oz/A, 100 ppm	100% bloom	17.1	61.9c
Kocide 3000	0.5 lb/100/A	80& 100% bloom	21.4	52.3d
Actigard soil treatment	0.2 gram per tree twice	TC and 50% Bloom	26.7	40.5e
Acid Buffer (pH 5)	2 qt./A	20, 50 & 100% bloom	27.6	38.5e
Buffer A	9.35 lb. /100/A	20, 50 & 100% bloom	27.9	37.9e
No treatment, inoculated check	**0**	**NA**	**44.9**	**0 f**
No treat No inoc.	0	NA	0.0	NA

Table 1. ***Pears***: Summary of data. Values followed by the same letter should not be considered different. Least Significant Difference in percent control = 9.2

*Streptomycin was effective in this trial because a streptomycin susceptible lab strain of the blight bacteria, *Erwinia amylovora*, was used to inoculate the flowers.

FIRE BLIGHT CONTROL PRODUCT EFFICACY - APPLES

Product	Rate	Timing	% Infection	% Control
Actigard Pre-bloom, Sprayed on tree + Streptomycin	Actigard 0.2 gram per tree, twice Strep. 200 ppm	Actigard 20 and 50% bloom Strep. 100% Bloom	2.9	92.8a
Copper Product 2009 formulation	83 fl.oz./A	80% and 100% bloom	4.0	90a
Streptomycin 17% (treated standard)	1 lb/100/A, 200 ppm	100% bloom	7.7	80.8b
A.p. Yeast (half rate) + Buffer 1/2	0.68 lb/100gal/A 4.7 l b/100 A	20, 50 & 100% bloom	8.3	79.3b
Copper Product 2010 formulation	95 fl.oz./A	80% and 100% bloom	9.0	77.5b
Kasumin 2L (1x)	2 qt./A, 100 ppm	100% bloom	9.1	77.3b
Copper Product 2009 formulation	28 fl.oz./A	80% and 100% bloom	9.7	75.8b
Copper Product 2010 formulation	32 fl.oz./A	80% and 100% bloom	11.2	72.0b
A.p. Yeast (full rate) + Buffer A	1.34 lb/100gal/A 9.35 lb. /100/A	20 & 50% 100% bloom	11.4	71.5b
Oxytetracycline (FireLine)	1 lb/100/A, 200 ppm	100% bloom	15.0	62.4c
A.p. Yeast (full rate) + alt. buffer	1.34 lb/100gal/A to pH 5	20, 50 & 100% bloom	15.3	61.8c
Kocide 3000	0.5 lb/100/A	80% and 100% bloom	15.6	61.0c
Acid Buffer (pH 5)	2 qt./A	20, 50 & 100% bloom	18.5	53.8c
Buffer A	9.35 lb. /100/A	20, 50 & 100% bloom	25.7	35.8d
Actigard soil treatment	0.2 gram per tree, twice	Tight cluster & Petal fall	28.0	30.0d
No treatment, inoculated check	**0**	**NA**	**44.9**	**0 e**
No treat. No inoc.	0	NA	0.0	NA

Table 2. ***Apples***: Summary of data. Values followed by the same letter should not be considered different. Least Significant Difference in percent control = 9

*Streptomycin was effective in this trial because a streptomycin susceptible lab strain of the blight bacteria, *Erwinia amylovora*, was used to inoculate the flowers.

ORCHARD REPLANT DISEASE SOIL FUMIGANT PROJECT (25% of effort in 2010)

OBJECTIVES as stated on the proposal:
We will demonstrate the positive effect on soil fumigation on the productivity and quality of apples grown under a very modern production system.

METHODS, *The replant disease treatment trials:*
Establishment: In the fall of 2008, block of land south of Othello, Washington that had recently supported an apple orchard (with one fallow season) was selected as a site for the fumigant trial. The land was ripped thoroughly and smoothed prior to fumigation. On October 27, 2008 a replicated fumigation trial was established, with four treatments and untreated checks. Each replicate was approximately 0.8 to 1 acre, with a total of about three acres for each treatment. Fumigant application was by Trident Agricultural Products, Inc. Application depth was 16 inches. Shank spacing was 20 inches. At the base of each shank were 4 inch wings where the fumigant was emitted. Maximum spacing of fumigation outlets was 12 inches. The soil temperature and moisture were well within the optimum range. Treatments applied are as listed in table 3.

Treatment	Rate chloropicrin per acre	Rate 1,3 DCP per acre
Pic-Plus	10.9 gal. = 150 pounds	0
Pic-Clor 60, 20 gpa	10.5 gal. = 144 pounds	9.5 gallons = 94 lbs.
Telone C-35, 25 gpa	7.0 gal. = 97.5 pounds	18.0 gallons = 178 lbs.
Telone C-17, 30 gpa	3.7 gal. = 51 pounds	26.3 gallons = 259 lbs.
Untreated	0	0

Table 3. Soil fumigant rates applied in the 2009 Radar Hill soil fumigation/orchard replant disease trial. The rate range of current orchard replant site fumigation is 50-100 lbs. chloropicrin + 20-30 gal. per acre of 1,3 Dichloropropene.

This block was planted by Allen Brothers Fruit Company to Cripp's Pink "sleeping eyes" at 8.5 x 3 feet in the spring of 2009. These trees are being trained to a five wire upright trellis. At the proposed tree spacing, approximately 5000 trees were planted in each treatment, split into three replicates. The untreated checks are much smaller than the treatment areas in deference to the valid concerns of the orchard owners and manager. Approximately 40 – 50 trees will be growing well away from treated soil in the interior of each of the three untreated areas, which in the author's experience will be sufficient for valid statistical analysis.

Judging by adjacent and nearby blocks of orchard under similar management, the system promises to produce very well, reaching full production in five years or less. An orchard managed in this manner will offer great advantages as a replant trial for at least two reasons: 1. the rapid return to full production will reduce the number of necessary evaluation years from the seven to ten common in the past down to five, and 2. As this orchard system is very modern, it will help us determine if planting trees at high densities, drip irrigating and fertigating reduces the economic impact of this root-damaging disease complex.

***Evaluation**:* Year 2: Cross sectional area of the trunk at 4 inches above the graft union, tree height, (2010. Below). Year 3 (4 and 5): Cross sectional area of trunk, (fruit per tree, fruit size, and yield (probably starts 2012.).

RESULTS & DISCUSSION: REPLANT TREATMENT TRIALS
Radar Hill Trial:

Treatment:	***PicPlus (150 lbs./A Chloropicrin) 0 DCP***	***PC60 (144 lbs./A Chloropicrin) 94 lb/A DCP***	***Telone C-35 (25 GPA, 98 lb/A cloropic) 178 lb/A DCP***	***Telone C-17 (30 GPA, 51 lb/A cloropic) 260 lb/A DCP***	***Untreated***
Average Height (in inches)	86a	85a	86a	88a	74b
% of check	116	115	116	119	100
Average Trunk Cross Section in mm^2	249a	249a	236a	253a	139b
% of check	179	179	170	182	100
Average Total Inches Lateral Shoots	155ab	120c	139bc	185a	29d
% of Check	534	414	479	638	100

Table 4. Average inches height and cross section area of trunk 4 inches above the graft union in second season Cripp's Pink apple "sleeping eye" on M9 planted after fumigation on a replant site.

The height and trunk size of about 100 trees in each treatment was measured in October 2010 after second season growth stopped. All of the young trees were planted as a fall 2008 budded "sleeping eye." Since then, they have been tied to bamboo or string leading upwards on the trellis wire, promoting vertical growth. Growth across the orchard and within the fumigated treatments was relatively uniform, but some variation is apparent in zones, perhaps due to soil quality variation. However, the unfumigated check height averaged 74 inches, both statistically and visually less than the treated trees. There are a few areas within the fumigated treatments where the young trees grew less well than the average, especially along the eastern edge where the soil texture appears sandier. In the unfumigated checks, the growth was much more variable and height averaged about 13 inches, or 16.6% less tall than the treated average. The difference in average trunk cross-sectional area, which relates to total vegetative growth, and is very relevant in relation to early tree yields, was even more divergent. The untreated check averaged 139 mm^2 trunk cross-section, 57% the size of the treated trees. *This degree of growth suppression in untreated checks vs. treatments in past replant trials has resulted in very significant yield differences in subsequent seasons yields.*

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-09-900

Project Title: Pheromone technology for management of codling moth and leafrollers

Co-PI(1):	Jay Brunner	**Co-PI(2):**	Larry Gut
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City/State/Zip:	Richland/WA/99354	**City/State/Zip:**	Wapato/WA/98951

Cooperators: Mike Doerr, WSU-TFREC; Peter McGhee, Michigan State University.

Total Project Request: **Year 1:** $120,797 **Year 2:** **$**100,434 **Year 3: $123,635**

Other funding sources

Agency Name: Pheromone Companies

- Shin-Etsu: $40,000 ($20K each to WSU and MSU) to help fund evaluation of new dispenser technology.
- Private Company: $60,000 ($30K each to MSU and WSU) for assessment of release rates, video recording and flight tunnel of behavior, and attraction of dispensers to codling moth.
- Private Company: $24,000 to MSU for assessment of a novel attract-and-kill formulation.
- Private Company: $30,000 ($20K to MSU and $10K WSU) for assessment of a novel mating disruption formulation.
- In 2011 there may be agreements with some of the companies listed above for continued work.
- There is also a possibility of two more companies interested in collaborating on discovery or development aspects associated with the objectives of this project.

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	2009	2010	2011
Salaries [1]			
Technical – M. Doerr (3 month)	5,278	5,489	15,833
Res. Analyst III (Hebert prog.)	8,714	9,063	9,426
GRA – 9 mo appt @ 0.50FTE	22,014	0	0
Benefits			
Technical – M. Doerr (3 months)	1,689	1,757	5,051
Res. Analyst III (Hebert prog.)	4,270	4,441	4,619
GRA – 9 mo appt @ 0.50FTE	1,881	0	0
Wages (temporary labor)[2]	7,000	8,000	14,400
Benefits (15%)	1,260	1,440	2,160
Supplies	5,000	5,000	5,500
Travel [3]	2,085	2,085	2,085
Total	$59,191	$37,275	**$59,074**

Footnotes:
[1] Technical – Mike Doerr for 3 months, Research Analyst III @ 0.2 FTE per year.
[2] Temporary labor to assist with data collection and entry. Three students @ $10/hour, 40 hours/week for 12 weeks.
[3] Expenses of operating vehicles allocated to the project – fuel, maintenance, and repairs and travel at $.0385/mile x 1000 miles per field season (ten 100 mile trips).

Budget 2:
Organization: Michigan State Univ. **Contract Administrator:** Emily Flanner
Telephone: 517-355-5040 x256 **Email:** flanner@cga.msu.edu

Item	2009	2010	2011
Salaries	26,187	26,981	27,782
Benefits	12,847	13,506	14,107
Wages	3,000	3,000	3,000
Benefits	172	172	172
Supplies	1,000	1,000	1,000
Travel	1,000	1,000	1,000
Total	$44,206	$45,659	**$47,061**

Budget 3:
Organization: USDA-ARS, Wapato **Contract Administrator:** Chuck Myers
Telephone: 510-559-5769 **Email:** Chuck.Myers@ARS.USDA.GOV

Item	2009	2010	2011
Salaries	0	0	0
Benefits	0	0	0
Wages	14,000	14,000	14,000
Benefits (10% of labor)	1,400	1,400	1,400
Supplies	1,000	1,000	1,000
Travel	1,000	1,000	1,000
Total	$17,500	$17,500	**$17,500**

Project objectives:

1. Improve hand-applied dispenser mating disruption systems for codling moth by determining an optimized dispenser release rate and density.
2. Characterize adult moth behavior that leads to optimization of an attract and kill (A&K) technology for codling moth and leafrollers.

Significant findings 2010

Optimization of hand-applied dispensers

- Pheromone meso-dispensers at 32/acre provided trap shutdown similar to a hand applied dispensers, Isomate Flex at 320/acre and a Puffer treatment at 1/acre.
- Release of sterile moths from the Canadian SIR program provided a good tool to challenge/evaluate large and small plot pheromone treatments.
- Large plot field trials demonstrated that the Isomate Flex dispenser was as good as the industry standard for hand-applied dispensers, the Isomate C Plus dispenser.
- The ***Tangler*®** pheromone delivery technology provided good control of CM relative to Isomate C Plus despite not being optimized for pheromone release rate. Application times for this technology were a fourth that of a hand applied dispenser using the launcher technology developed for the Tangler and half that without using the launcher.

Moth behavior and attract and kill research

- The MSU micro-trap captured large numbers of CM but not as many as the delta trap in direct comparisons.
- In MI the MSU micro-trap provided increased trap shutdown as the density increased above 50/acre, similar to a hand applied pheromone treatment in small plot trials.
- In WA the MSU micro-trap provided trap shutdown equal to delta traps when both were applied at a density of 300/acre in small plot trials.
- In large plot trials in MI the MSU micro-trap provided trap shutdown superior to an Isomate Flex treatment.
- The attract-and-remove approach looked promising as a tactic to control leafrollers in MI.

Methods:

Methods used in this project were outlined in last year's new project proposal (2009). Specific methods used in 2010 are outlined in the Results and Discussion section.

Results and Discussion:

Optimization of hand-applied dispensers. The first objective of this project was to improve hand-applied mating disruption systems for codling moth. A team effort focused on understanding the impact of pheromone release rates from different devices on codling moth behavior. Behaviors were assessed with pheromone-baited traps, in small plot and field-cage studies, and with video recordings.

Field Trials - Puffers, Rings and Flex. Three codlemone dispensing technologies were evaluated in 2010: CheckMate CM-O Puffer, Isomate (Ring) meso-type dispenser, and Isomate Flex (Flex) dispenser. The Puffer contained 55.5 mg A.I. released codlemone as a mist in 40ul puffs every 15 minutes from 5PM – 5AM over a 160-day period. The Ring dispenser (roughly equal to 5 Isomate CTT - twin tubes) was loaded with 2095 mg A.I. Thirty-two Rings released about the same amount of codlemone as a single Puffer over 160 days. The Flex was a new dispenser developed in part because of our research and released about the same as the Isomate C plus dispenser.

All treatments were placed in large blocks (10 acres for Ring and Flex dispensers and ≈40 acres for Puffers) at three different locations. Treatments were Puffers at 1/A, Flex at 320/A, and Rings at 1, 2, 4, 8, 16, and 32/A. Sterile moths obtained from the Okanogan Kootenay Sterile Insect Release Program (SIRP) were used to challenge treatments. SIRP moths were released, 400 weekly from mid-

June through August. Moth release was from a single point equidistant from the nearest pheromone dispensers in each treatment. Pheromone traps (12) baited with 0.1mg lures (female mimics) were placed around each moth release point (Fig. 1). Treatments were evaluated by comparing the relative capture of SIR moths in each treatment.

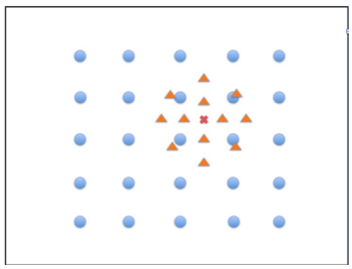

Fig. 1. Release site (small square) to nearest dispensers (circles) and traps (triangles).

There was no difference in SIR moth capture between the single Ring (Meso) dispenser treatment and the non-pheromone control (Fig. 2). Trap shutdown increased as the density of Ring dispensers increased up to 32/A, where trap shut down averaged 93.7 ± 2.0%.

There was little difference between the best Ring treatment (32/A) and the Flex and Puffer treatments. Trap shutdown was highest and had the smallest variance in the Flex 80, 94.9 ± 0.8%, but not statistically different from the Puffer, 92.8 ± 2.0%, and the Ring, 93.7 ± 2.0%, treatments.

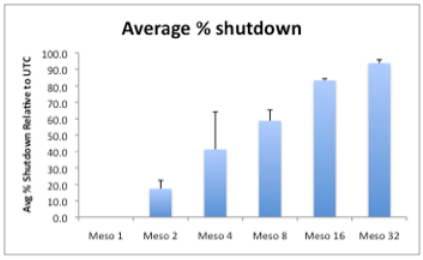

Fig. 2. Percent trap shutdown relative to non-pheromone control.

Additional information gleaned from these trials was the strong dispersal capacity of the SIR moths. We consistently captured SIR moths in traps placed by consultants around our study locations, as far as several hundred meters away.

In MI Rings and Flex dispensers were compared in six orchards over two years. Treatments were Flex (F) at 40 and 400/A and Rings (R) at 4 and 40/A. The average accumulation of CM/trap/generation is shown in Fig. 3. The Flex at 400/A provided the greatest reduction in trap captures. There was little difference between other treatments, including the Rings at only 4/A.

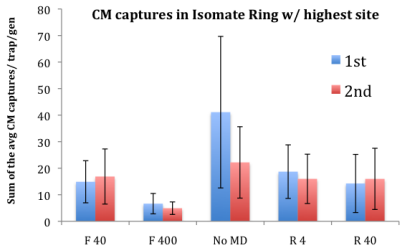

Fig. 3. Average moth capture per trap per generation.

Interpretation. Three very different approaches of dispensing CM pheromone were challenged with release of SIR moths in WA. The highest rate of the Rings provided very good trap shut down, comparable to the Puffers and Flex treatments. SIR moths represent a good tool to challenge pheromone treatments where wild CM densities are too low to obtain adequate results. In MI where wild moth populations were higher than WA the Flex dispenser at 400/A was superior to the Ring at 40/A.

Field Trials - Isomate C Plus and Flex. We worked a second year with reduced release rate dispensers. In WA we compared Isomate C Plus, Isomate Flex (Flex), Flex 50 and Flex 25, all at 400 dispensers/A. The release rates from these dispensers is still being analyzed, but last year release rates were proportional to the load, that is Isomate C Plus ≈ Flex, and Flex50 and Flex25, equal to 50% and 25% release of Flex. The four treatments were compared at two locations, Brewster and Quincy. Treatment blocks were 10 acres in size. Treatments were monitored with nine traps baited with standard Trécé L2 lures placed in a grid in the center 3-acres. Beginning on June 16, 2009 SIR male moths were released at four locations between monitoring traps weekly through August 25.

One location, Brewster, had high CM (wild) captures during the first generation indicating high potential for crop damage. Captures were lowest in the Isomate C Plus and Flex treatments, 0.4 and 0.2 moths/trap, and higher in the Flex 50 and Flex 25 treatments, 2.0 and 1.4 moths/trap. The Quincy site had very low CM and there was no difference in capture of wild moths between treatments.

SIR moth captures at the Brewster site were lower in the Flex, 4.8 moths/trap, than the Isomate C Plus, 8.6 moths/trap, which was similar to the Flex 50, 8.8 moths/trap. The Flex 25 treatment had the highest moth capture, 18.1 moths/trap. At the Quincy site moth captures were generally higher in all treatments and the pattern among treatments was different. All the Flex treatments had high capture of SIR moths with the Flex 50 being the highest.

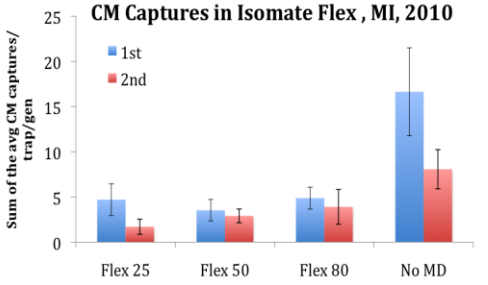

Fig. 4. Average capture of CM in different Flex dispenser treatments compared to a non-pheromone control.

A similar trial was conducted in MI at three locations. In this trial the same three Flex treatments, Flex 80 = Flex, Flex 50 and Flex 25, were compared to a non-pheromone control. Each treatment consisted of 20A at each location. Captures of CM were relatively low in apple orchards throughout Michigan in 2010. Highest captures were in the No MD control plots with mean captures of *ca.* 20 and 7 moths in 1st generation and 2nd generation respectively (Fig. 4). All three Isomate treatments reduced moth captures compared to the No MD control with average captures of *ca.* 5 and 4 moths per trap for 1st and 2nd generation. Flex 25 and Flex 50 releasing reduced amounts of codlemone, performed similarly in suppressing moth captures compared to the commercially available Flex dispenser.

SIR Moths. The behavior of SIR moths was very encouraging. They were strong dispersers and readily found and were captured in pheromone-baited traps. These moths represent a tool to use in future studies comparing pheromone or other treatments for CM control.

Interpretation. The new Isomate Flex dispenser provided CM moth suppression levels similar to that of Isomate C Plus. The low load Flex dispensers (Flex50 and Flex25) provided good suppression of CM captures but results were more variable than in 2009.

Field Trials - Tangler, Isomate: The ***Tangler*®** is a newly-developed mating disruption technology designed to automate the application process and reduce cost, especially the application cost. The Tangler technology was developed by Ridge Quest, a Michigan-based company. It consists of a module loaded with pheromone and a launcher that enables a rapid application of numerous modules to the upper tree canopy (Fig. 5). The bola design results in propelled modules becoming tangled in tree branches. Capsules are clipped together and fed into a mechanical launcher operated by compressed gas.

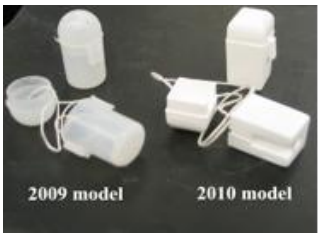

Fig. 5. Tangler technology

Field trials to determine the efficacy of the ***Tangler*®** system were conducted at five sites in MI. Field plots consisted of 15-25 acre apple blocks subdivided into three plots with buffers. Treatments were the Tangler (400 capsules/acre), Isomate CM Flex (400 dispensers per acre), and a no pheromone check. Captures of males in pheromone-baited traps and fruit injury counts at mid-season and prior to harvest was used to assess treatment effects.

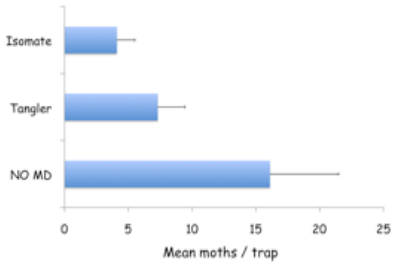

Fig. 6. Tangler field trial results, 2010.

The experimental Tangler formulation provided disruption equivalent to the hand-applied dispensing system, Isomate CM Flex (Fig. 6). The two pheromone treatments provided approximately 70% disruption. This is a good start, but our aim is for the Tangler to outperform currently registered

formulations. Some technical difficulties were encountered that we believe hampered the performance of the Tangler and prevented the system from having a greater impact on codling moth mating behavior.

Pheromone release rates from the Tangler modules averaged 0.33µg/hr and ranged from 0.15-0.66µg/hr. This is below our target level, 1.2 µg/hr, needed to obtain a high level of disruption. Laboratory tests are ongoing to increase the release rate. A design change in the capsules proved problematic for the automated launching system. Therefore a direct comparison of the time required for hand application of the Tangler (without the launcher) versus Isomate ropes was conducted on four farms. The average time for hand application of the Tangler was 2x less than that for Isomate.

A newly designed launcher and Tangler module were developed over the summer, and the efficiency of the automated system was evaluated late in the fall. The average application time was 16.88 minutes/acre. The average time to apply 400 Isomate 'ropes' was 60 minutes/acre. Thus, automated deployment of the Tangler modules was nearly 4x faster than hand application of 'ropes'.

Pheromone release rates: We have evaluated various experimental and commercial pheromone dispensers to determine their release rates. Results are currently being analyzed.

Interpretation. The ***Tangler*®** continues to show promise as a technology to automate application of pheromone dispensers. Application times for this technology were a fourth that of a hand applied dispenser using the launcher developed for the Tangler and half that without using the launcher. Optimization of the technology, pheromone release rate, and launcher compatibility will be addressed in 2011.

Kairomone lures. USDA experiments with kairomone attractants showed that eight-component blend capture two times more moths than the AA+3-methyl-1-butanol lure (Fig. 7). Collaborations with a private company evaluating kairomones showed promise as a CM attractant capturing 3 to 6 times more moths than a pear ester lure.

Interpretation. There are new kairomones or combinations that hold promise for capturing CM, including females, which would be very important in A&K research.

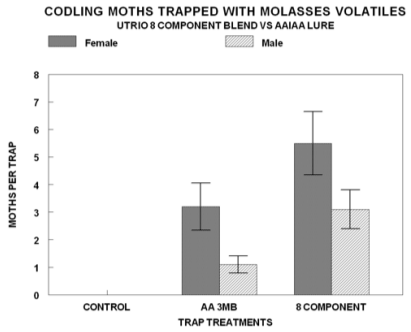

Fig. 7.

Moth Behavior and Attract and Kill (A&K) Research.
The second objective of this project was to characterize behavior of adult codling moth and leafroller in order to optimize development of A&K technologies. A team effort focused on assessing moth behaviors in different environments and to different A&K technologies.

Trap/lure Comparisons. The relative attractiveness of six trap by lure combinations was assessed during the second CM flight in WA. Traps used were the large delta and the MSU micro-trap. Lures were a Flex 25 dispenser, an L2 lure and a 0.1 mg lure. The MSU micro-trap caught fewer CM (DF 1, F 32.6763, P>F 0.001), 17.7, 27.7, and 24% relative to the delta traps using Flex25, L2, and 0.1X lures, respectively (Fig. 8). A significant lure effect was noted with both trap styles (DF 2, F 8.7878, P>F 0.001) with the L2 and 0.1X lures being the most attractive. There was no significant trap by lure interaction.

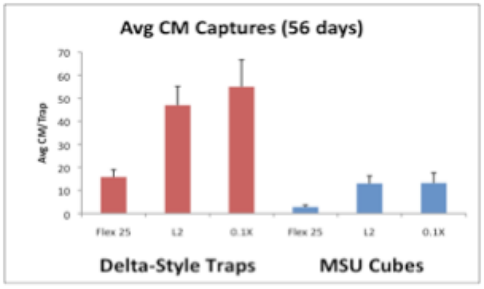

Fig. 8.

Field Trials - Codling Moth. Three different and coordinate field studies using different treatments were conducted in WA and MI in 2010.

The effects of varying the density of MSU micro-traps on inhibition of CM catch in a central monitoring trap was evaluated in a small plot study in MI. Plots consisted of 25 apple trees in a 5x5 spacing (ca 0.2 ac). The experimental design was a RGB with four replicates. Treatments included a no pheromone check, Isomate Flex at 200/A, micro-traps at 50/A, 100/A, 200/A, and 400/A. All micro-traps were baited with L2 lures. A single monitoring trap baited with an L2 lure was placed centrally in each plot to assess treatment effects.

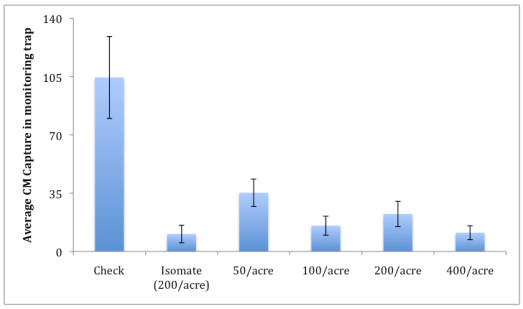

Fig. 9. Average moth capture per treatment.

All pheromone treatments reduced the capture of CM in the central monitoring trap. The three highest micro-trap application rates significantly reduced CM capture in the monitoring trap compared to the lowest micro-trap rate (Fig. 9). CM capture rates were statistically equal between the mating disruption standard at 200/acre and the micro-trap rates of 100/acre and 400/acre.

In MI a large plot attract-and-remove trial was conducted to compare a standard mating disruption control program to a trap-out control scenario for reduction of CM male activity. Treatments included a no pheromone control, Isomate Flex, non-sticky micro-traps, and sticky micro-traps. Treatments were applied to 0.5-acre plots at an application rate of 200/A. All micro-traps were baited with L2 lures for first flight and 0.1mg lures for second flight. Two monitoring trap baited with an L2 lure were placed in each plot to assess treatment effects.

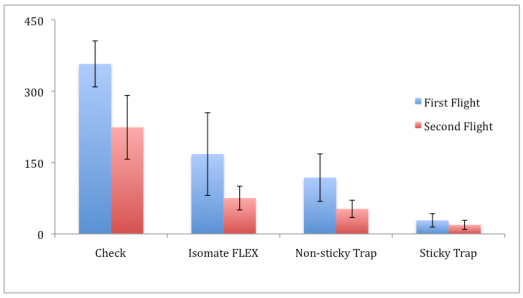

Fig. 10. Average moth capture per treatment.

All pheromone treatments were effective at reducing the capture of CM in central monitoring traps (Fig. 10). During first flight, the attract-and-remove approach was more effective at reducing CM capture in the monitoring traps than either of the other treatments. In the second flight the attract-and-remove approach was statistical superiority to the commercial mating disruption standard, even with a use of lower load lures, 0.1 mg.

A similar attract-and-remove small plot trial was conducted in WA. In the first generation three densities of removal traps (large Delta baited with 0.1 mg lures) were used, 100, 200 and 300/A plus an untreated control (UTC). All treatments were 0.15 acres in size and replicated three times. A trap in the center of each plot was designated as the monitoring trap. In the second generation the high density (300 traps/A) was used to compare the Delta and MSU micro-trap with an untreated control.

In the first generation significantly more CM were removed in the 300/acre trap treatment than the 150/A or 100/A trap treatments. Moth captures in the monitoring traps were reduced by 79.3, 86.0, and 91.2% relative to the UTC in the 100, 150, and 300 traps/A treatments, respectively. The test orchard had a very high CM density, but the attract-and-remove treatments did have less fruit injury (reduction of 23.9-50.2%) than the untreated UTC.

In the second generation trial the delta traps and the MSU micro-traps removed a high number of CM when both were applied at 300/A. The delta traps and the MSU micro-traps reduced CM captures in the monitoring trap relative to the untreated control by 85.8, 68.4%, respectively (Fig. 11).

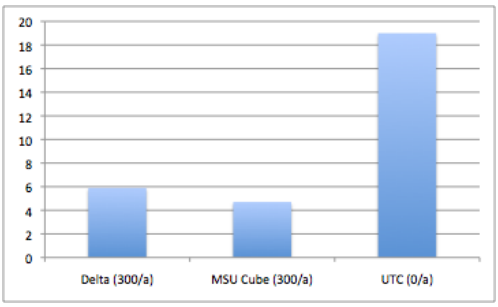

Fig. 11. Average moth capture per treatment.

In USDA trials implemented use of PVC pipe kills stations baited with 0.1 mg pheromone or AAPE (acetic acid+pear ester) lures at rates of 10 and 50 per acre. Kill stations used an insecticide, Assail, formulated in a grease as the toxicant. There was no effect when 10 stations per acre were used. When 50 stations/acre were used male numbers were reduced 74% with the pheromone lure and 30% with the AAPE lure. There was no effect on females in the AAPE baited kill stations.

Interpretation. All of the field trials point to a good potential for use of attract-and-remove or attract-and-kill as a strategy for CM management. There is developing technology that could make this approach economical. The micro-trap is much smaller than the delta trap, yet still catches 25% as many moths. This is encouraging considering it was designed to be deployed at densities of 100/A.

Video Monitoring. The approaches and captures of CM in the MSU micro-traps and a Pherocon® IV traps was evaluated using video recordings. In the second generation when low load lures were used, 0.1 mg, the micro-traps captured 23% of all approaches compared to 22% for the Pherocon traps.

Interpretation. These data provide further evidence of the efficiency of the micro-trap in attracting and capturing CM.

Field Trials - Leafroller. A small plot attract-and-remove trial was conducted in MI to compare a standard mating disruption treatment to a trap out program for OBLR. Treatments included a no pheromone check, Isomate OBLR/PLR +, and Pherocon IIB traps baited with standard lures as the attract-and-remove treatment. Treatments were applied to 0.5-acre plots at 200-point sources per acre. Both pheromone treatments were effective at reducing the capture of OBLR leafroller in monitoring traps. During both flights, the attract-and-remove approach was more effective at reducing OBLR capture in the central monitoring traps than the commercial mating disruption standard (Fig. 12).

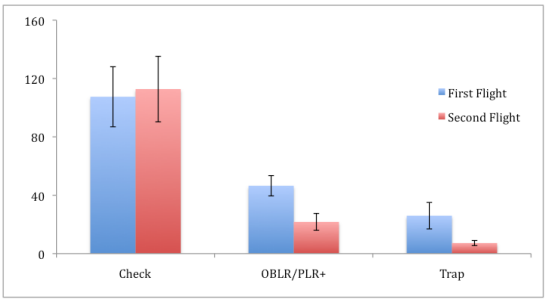

Fig. 12. Average number of OBLR per treatment.

Interpretation. The attract-and-remove approach for OBLR appears to have greater potential compared to a mating disruption approach. Developing a cheap device to remove leafroller males from orchards could reduce the need for chemical control treatments or, when combined with soft chemical controls like Bt, enhance biological control in IPM programs.

FINAL PROJECT REPORT
WTFRC Project Number: CP08-802

Project Title: Control of postharvest fruit rots in apple

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Cooperators: Selected packinghouses across central Washington State

Other funding sources: None

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

Budget History:

Item	2008	2009	2010 (extension)
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	0

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:

- Residual protection of apple fruit by preharvest Pristine was still evident five months after harvest but declined after the fruit were stored at room temperature for one week after cold storage.
- Preharvest Pristine plus postharvest BioSave further reduced blue mold incidence during cold storage. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage, suggesting that both Pristine and BioSave only suppress blue mold during cold storage.
- On Fuji and Red Delicious fruit, both Scholar and Penbotec on drenched fruit exhibited very good residual protection of fruit from infection by *Penicillium expansum*. BioSave alone applied at packing reduced blue mold incidence, but the effectiveness declined when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit.
- All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure, a DMI fungicide) and pyraclostrobin (a strobilurin fungicide). Pyrimethanil-resistant mutants were resistant to cyprodinil (Vangard), indicating that there is cross resistance between pyrimethanil and cyprodinil and that use of Vangard in the field could promote the development of resistance to pyrimethanil (Penbotec), a key postharvest fungicide.
- Pyrimethanil-resistant isolates of *Botrytis cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil. Pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards where Topsin M and Vangard had been used in the past. The results indicate that there is no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil. Development of *B. cinerea* populations resistant to TBZ and pyrimethanil in the field could compromise the efficacy of postharvest use of TBZ and pyrimethanil for gray mold control.
- In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit from a packinghouse in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil.
- Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold, indicating that pyrimethanil resistance in *P.*

expansum reported in this study can result in failure of blue mold control in apples with pyrimethanil.

- The finding of the occurrence of pyrimethanil resistance in *P. expansum* suggests that further research is needed to monitor the frequency of pyrimethanil-resistant populations, understand the biological characteristics of pyrimethanil resistance in *P. expansum*, determine whether the level of pyrimethanil resistance results in the failure of blue mold control with Penbotec, and develop relevant measures to manage pyrimethanil resistance.
- Octylgallate alone, or in combination with Scholar, did not control blue mold on apple fruit caused by fludioxonil-resistant *P. expansum*.

Methods:

Pristine was applied to Fuji fruit one week before harvest. Fruit were stored in CA for five months. Fruit were removed from CA, washed and brushed through a research packing line, wounded and inoculated with *P. expansum*, and treated with either BioSave or a fungicide. Inoculated fruit were stored at 32°F in air for eight weeks and one additional week at room temperature. Decay was evaluated.

Fruit of Red Delicious and Fuji from commercial orchards were drenched with either Scholar or Penbotec and stored in CA. The fruit were removed from CA five and seven 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 were stored at 32°F in air for eight weeks and one additional week at room temperature. Decay was evaluated.

Blue mold-decayed fruit were collected from grower lots that had been drenched with Penbotec or Scholar from packinghouses. Isolations of *P. expansum* from decayed fruit were attempted. Isolates of *Penicillium* spp. were identified to species. Isolates of *P. expansum* were screened for resistance to pyrimethanil, fludioxonil and thiabendazole (TBZ). A subset of isolates was also tested to determine EC_{50} values of the fungicides.

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:

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

This experiment was conducted on Fuji in both 2008-09 and 2009-10 seasons. Pristine was applied to Fuji apples seven days before harvest. Fruit were removed from CA five 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 were stored at 32°F in air for eight weeks and one additional week at room temperature. The results from the 2008-09 trial were reported last year. The results from 2009-10 season are summarized in Table 1. The results from the two seasons were consistent.

Residual protection of apple fruit by Pristine was still evident five months after harvest but declined significantly after the fruit were stored at room temperature for one week after CA storage (Table 1). The loss of residual protection by Pristine at room temperature was likely due to that Pristine only suppresses but not eradicate *P. expansum*. At room temperature, Pristine residues may decline, thus *P. expansum* resumes growth. BioSave alone reduced blue mold incidence to approximately 38% during

the eight-week cold storage. Preharvest Pristine plus postharvest BioSave further reduced blue mold incidence to approximately 3% during cold storage. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage.

Table 1. Preharvest Pristine in combination with postharvest BioSave for blue mold control on Fuji apples in 2009-10 season.

Preharvest Treatment	Fungicide applied 5 months after harvest	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[z]	36.0a	100.0a	72.7a
	Scholar	0.0f	0.0d	0.0c	0.0d
	Penbotec	0.0f	0.0d	1.3c	13.0c
	TBZ	100a	32.7a	100a	70.5a
	BioSave	37.5d	11.9b	98.8a	37.5b
Pristine	No Fungicide	53.8c	12.3b	98.8a	37.6b
	Scholar	0.0f	0.0d	0.0c	0.0d
	Penbotec	0.0f	0.0d	0.0c	0.0d
	TBZ	76.3b	13.2b	100a	39.7b
	BioSave	2.5e	6.9c	78.8b	12.3c

[z] Values within the same column followed by the same letter are not significantly different according to the Waller-Duncan K-ratio *t* test at K ratio = 100 ($P = 0.05$).

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 conducted on the 2008 crops and results were presented in the 2009 research report. We repeated the two trials on the 2009 crops and finished in spring 2010. The results are presented in Table 2 and Table 3. On Fuji, both Scholar and Penbotec on drenched fruit showed very good residual protection of fruit from infection by *P. expansum* (Table 2). BioSave alone applied at packing reduced blue mold incidence to 33-43% on the 2008 crops during cold storage but not on the 2009 crops (Table 2). The effectiveness of BioSave was lost when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit. The results suggest that Scholar and Penbotec have long lasting residual protections against *P. expansum* on drenched apple fruit.

Table 2. Postharvest drench in combination with BioSave applied at packing for blue mold control on Fuji apples in 2009-10 season.

		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	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage
Nontreated	No fungicide	95.0	97.5	100.0	100.0
	Scholar	0.0	0.0	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
	TBZ	2.5	8.8	0.0	5.0
	Bio-Save	90.0	95.0	95.0	100.0
Scholar	No fungicide	0.0	1.3	0.0	8.8
	Bio-Save	0.0	1.3	1.3	7.5
Penbotec	No fungicide	0.0	0.0	0.0	2.5
	Bio-Save	0.0	0.0	0.0	0.0

On Red Delicious, both Scholar and Penbotec on drenched fruit also showed very good residual protection of fruit from infection by *P. expansum* during the eight-week cold storage, but Scholar's residual protection declined slightly when the fruit were move to room temperature (Table 3). BioSave alone applied at packing reduced blue mold incidence to 44-76%, but the effectiveness was lost when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit. The results suggest that residues of Scholar and Penbotec on drenched Red Delicious apple fruit protect apple fruit for several months post-drenching.

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

Previously we documented that when Scholar and Penbotec are applied to Red Delicious fruit as a postharvest drench treatment, they can provide long protection of the fruit from infection by *P. expansum*. In this project, we conducted similar trials on Fuji to examine whether this also occurs on Fuji apples. The results have been reported. In summary, when Fuji fruit were drenched with Scholar or Penbotec, excellent residual protection against *P. expansum* was still evident five and seven months after harvest. These results are consistent with what we observed on Red Delicious. Taken these together, our research suggests that residues of fludioxonil and pyrimethanil on/in apple fruit are persistent during cold storage and that residual protection of apple fruit by the two fungicides can last for at least seven months under apple-storage conditions.

Table 3. Postharvest fungicide drench in combination with BioSave applied at packing for blue mold control on Red Delicious apples in 2009-10 season

		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	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage	% blue mold at 8 weeks at 0ºC post packing	% blue mold at one additional week at room temperature after storage
Nontreated	No fungicide	98.8	98.8	100.0	100.0
	Scholar	0.0	1.3	0.0	0.0
	Penbotec	0.0	0.0	0.0	0.0
	TBZ	10.0	17.5	0.0	2.5
	Bio-Save	43.8	98.8	76.3	100.0
Scholar	No fungicide	0.0	7.5	0.0	10.0
	Bio-Save	0.0	5.0	0.0	12.5
Penbotec	No fungicide	0.0	0.0	0.0	0.0
	Bio-Save	0.0	0.0	0.0	0.0

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 4), 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 4). Fludioxonil-resistant and pyrimethanil-

resistant mutants and their parental wild-type isolates of *P. expansum* were sensitive to pyraclostrobin (a strobilurin fungicide) (data not shown).

We obtained 12 pyrimethanil-resistant *Botrytis cinerea* isolates from orchards where Vangard (cyprodinil) and Topsin M had been used in the past. Sensitivity of these isolates to selected fungicides was tested. All pyrimethanil-resistant isolates of *B. cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil (data not shown). All 12 pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards. The results indicate that there is no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil.

Table 4. 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.

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

Isolates of *P. expansum* were obtained from decayed apple fruit collected from packinghouses in 2008 and 2009. These isolates were tested for resistance to pyrimethanil, fludioxonil, and TBZ.

In 2008, 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. 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. 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. 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.

In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit. In total, 186 and 16 isolates of *P. expansum* were collected from Penbotec-drenched and Scholar-drenched apple fruit, respectively (Table 5). One isolate from Penbotec-drenched fruit showed significant resistance to pyrimethanil. EC_{50} values of pyrimethanil on mycelial growth and

conidial germination for the resistant isolate were 9.9 and 3.1 µg/ml, respectively, which were 7.4-fold and 16.5-fold higher than the means of the baseline population. Whereas EC_{50} values of pyrimethanil for a subset of 37 pyrimethanil-sensitive isolates ranged from 0.632 to 1.518 mg/L, with a mean of 1.07 mg/L, which is within the baseline sensitivity.

All isolates were sensitive to fludioxonil. Of the 202 isolates tested, 35 were resistant to TBZ, indicating that TBZ-resistant strains remained in *P. expansum* populations even after TBZ was not used.

In a decay-control study, all inoculated fruit in the nontreated controls were decayed. Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold (Fig. 1). This is the first report of pyrimethanil resistance in *P. expansum* from decayed apple fruit collected from commercial packing houses. The pyrimethanil-resistant isolate was obtained from a packing house in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil. Our results also indicate that pyrimethanil resistance in *P. expansum* reported in this study can result in failure of blue mold control in apples with pyrimethanil.

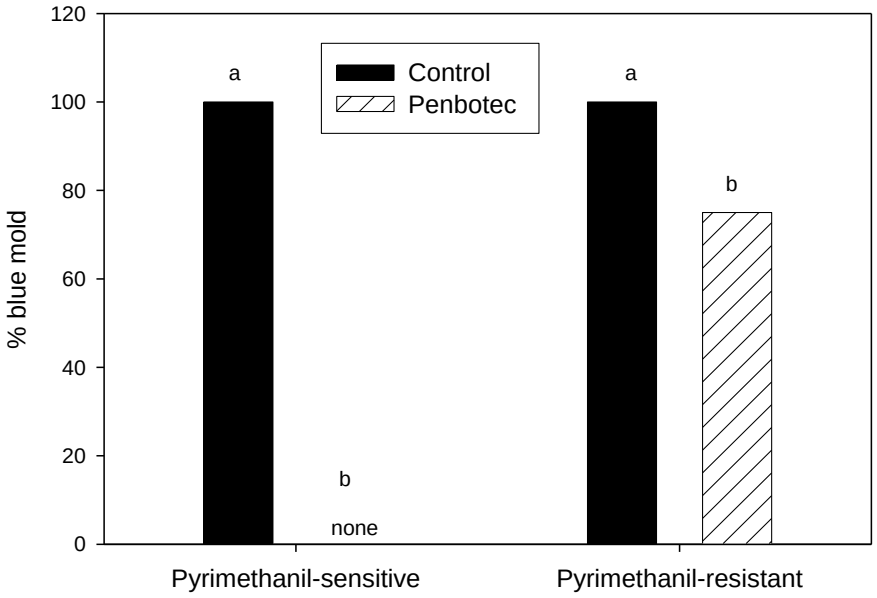

Fig. 1. Control of blue mold incited by pyrimethanil-sensitive and pyrimethanil-resistant strains of *Penicillium expansum* with Penbotec.

Table 5. Monitoring of resistance to postharvest fungicides in *Penicillium expansum* from apples

Source	Drench Treatment	# isolates of *P. expansum*	# isolates resistant to Penbotec	# isolates resistant to Scholar	# isolates resistant to TBZ
Packinghouse 1	Penbotec	8	0	0	2
Packinghouse 2 –Lot 1	Penbotec	34	0	0	6
Packinghouse 3 –Lot 1	Penbotec	29	0	0	5
Packinghouse 3 –Lot 2	Penbotec	4	0	0	4
Packinghouse 3 –Lot 3	Penbotec	14	0	0	1
Packinghouse 3 –Lot 4	Penbotec	97	1	0	12
Packinghouse 2 –Lot 2	Scholar	16	0	0	5
Total isolates from Penbotec-drenched fruit		**186**	**1**	**0**	**30**

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 on Petri dishes, octylgallate showed the potential as a promising chemosensitizing agent to overcome fludioxonil resistance in *P. expansum*. We further evaluated octylgallate in combination with Scholar (fludioxonil) for control of blue mold caused by two different fludioxonil-resistant strains (FR2: resistant to fludioxonil but sensitive to TBZ; FR3: resistant to both fludioxonil and TBZ) (Table 6). Octylgallate alone, or in combination with Scholar, did not control blue mold caused by fludioxonil-resistant *P. expansum*. It appears that in vitro and in vivo test results were not consistent. It is not known what causes the difference in results between the two tests, but compounds present in apple fruit flesh may affect the activity of octylgallate against fludioxonil-resistant *P. expansum*.

Table 6. Efficacy of octylgallate and Scholar for controlling blue mold caused by fludioxonil-resistant isolates (FR2 and FR3: fludioxonil resistant; W2: fludioxonil sensitive) of *Penicillium expansum*

Treatment	% Blue mold 8 wk at 32 F		% Blue mold 1 week at room temperature
	FR3	W2	FR2
CK	76.3	100	100
Octylgallate 0.15 mM	81.3	96.3	100
Octylgallate 1.0 mM	78.8	100	100
Octylgallate 0.15 mM + Scholar 230 SC	88.8	2.5	100
Octylgallate 1.0 mM + Scholar 230 SC	93.8	3.8	100
Scholar 230 SC	85	1.3	100

Executive Summary

This report is a summary of a two-year project conducted in 2008 and 2009. Part of the research was completed in 2010 because of the postharvest nature of the project. The goals of the project were to develop integrated programs using recently registered reduced-risk fungicides and a biocontrol agent to control major postharvest diseases in apples and to monitor and characterize resistance of *Penicillium expansum* to recently registered postharvest fungicides.

Blue mold caused by *Penicillium expansum* and gray mold caused by *Botrytis cinerea* are major postharvest diseases of apples. In the present project, we evaluated various pre- and postharvest integrated programs or pre- and post-storage integrated programs for decay control. Residual protection of apple fruit by preharvest Pristine was still evident 5 months after harvest but declined after the fruit were stored at room temperature for one week after cold storage. Preharvest Pristine in combination with postharvest biocontrol agent BioSave was more effective than Pristine alone in reducing blue mold at storage temperature. However, the effectiveness of these treatments declined after the fruit were stored at room temperature for one week after cold storage, suggesting that both Pristine and BioSave only suppress blue mold during cold storage.

When Penbotec and Scholar were applied as postharvest drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Fuji fruit in CA storage conditions. This observation is similar to what we previously observed on Red Delicious. On Fuji and Red Delicious fruit, both Scholar and Penbotec on drenched fruit exhibited very good residual protection of fruit from infection by *Penicillium expansum*. BioSave alone applied at packing reduced blue mold incidence, but the effectiveness declined when the fruit were stored for one additional week at room temperature after cold storage. BioSave did not provide additional protection for Scholar-drenched or Penbotec-drenched fruit.

All fludioxonil-resistant and pyrimethanil resistant mutants and wild-type isolates of *P. expansum* were sensitive to triflumizole (Procure, a DMI fungicide) and pyraclostrobin (a strobilurin fungicide). Pyrimethanil-resistant mutants were resistant to cyprodinil (Vangard), indicating that there is cross resistance between pyrimethanil and cyprodinil and that use of Vangard in the field could promote the development of resistance to pyrimethanil (Penbotec), a key postharvest fungicide.

Pyrimethanil-resistant isolates of *Botrytis cinerea* were sensitive to fludioxonil, indicating no cross-resistance between pyrimethanil and fludioxonil. Pyrimethanil-resistant isolates were resistant to TBZ, but TBZ-resistant isolates that were sensitive to pyrimethanil also were observed among the isolates obtained from the same orchards where Topsin M and Vangard had been used in the past. The results indicate that there was no cross resistance between TBZ and pyrimethanil, but use of Topsin M and Vangard in these orchards resulted in *B. cinerea* isolates resistant to both TBZ and pyrimethanil. Development of *B. cinerea* populations resistant to TBZ and pyrimethanil in the field could compromise the efficacy of postharvest use of TBZ and pyrimethanil for gray mold control.

In 2009, for the first time we detected pyrimethanil resistance in *P. expansum* obtained from decayed apple fruit from a packinghouse in which pyrimethanil had been used as a postharvest drench treatment in each of four consecutive years, suggesting that pyrimethanil-resistant individuals are emerging in *P. expansum* populations in Washington State after repeated use of pyrimethanil. Pyrimethanil applied at label rate completely controlled blue mold incited by a pyrimethanil-sensitive isolate, but 75% of the fruit that were inoculated with the pyrimethanil-resistant isolate and treated with pyrimethanil developed blue mold, indicating that pyrimethanil resistance in *P. expansum* reported in this study can result in failure of blue mold control in apples with pyrimethanil. The finding of the occurrence of pyrimethanil resistance in *P. expansum* suggests that further research is needed to monitor the frequency of pyrimethanil-resistant populations, understand the biological characteristics of pyrimethanil resistance in *P. expansum*, determine whether the level of pyrimethanil resistance results in the failure of blue mold control with Penbotec, and develop relevant measures to manage pyrimethanil resistance.

FINAL PROJECT REPORT

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

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State/Zip WA / 98951

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

Other funding sources: None

Total Project Funding: 49,110

Budget History:

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

ORIGINAL OBJECTIVES

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

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

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

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

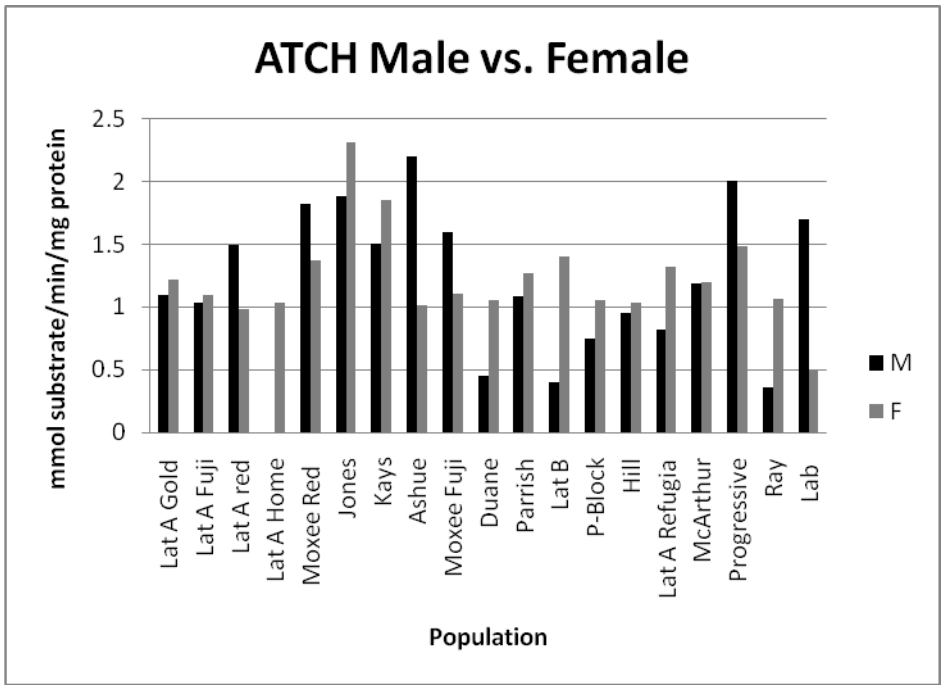

Figure 1. Acetylcholinesterase activity in male and female Codling Moth collected in the Yakima Valley.

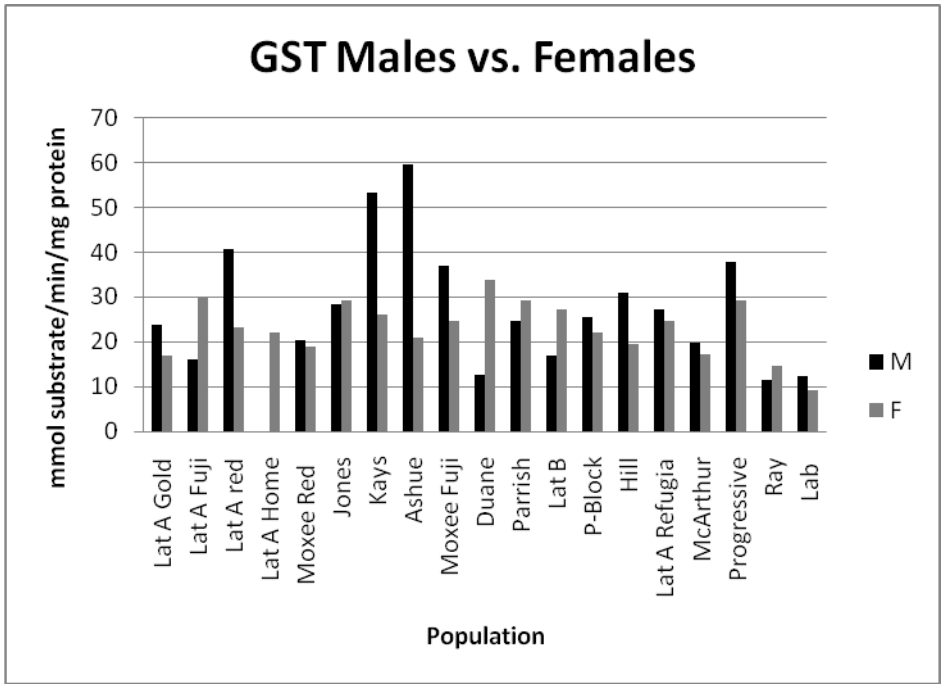

Figure 2. Glutathione S-Tranferase activity in male and female Codling Moth collected in the Yakima Valley.

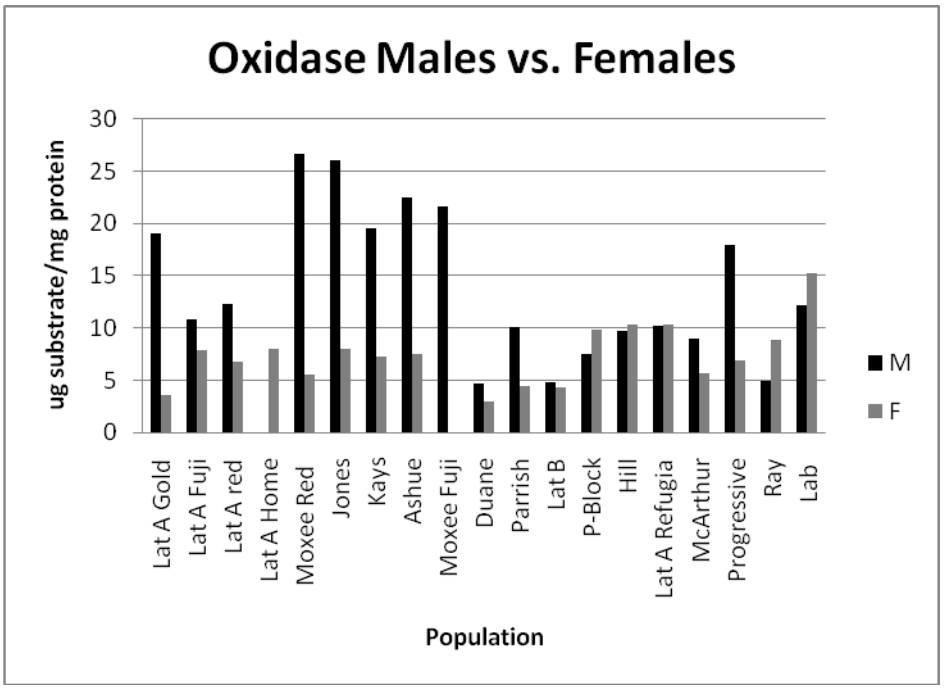

Figure 3. Mixed-Function Oxidase activity in male and female Codling Moth collected in the Yakima Valley.

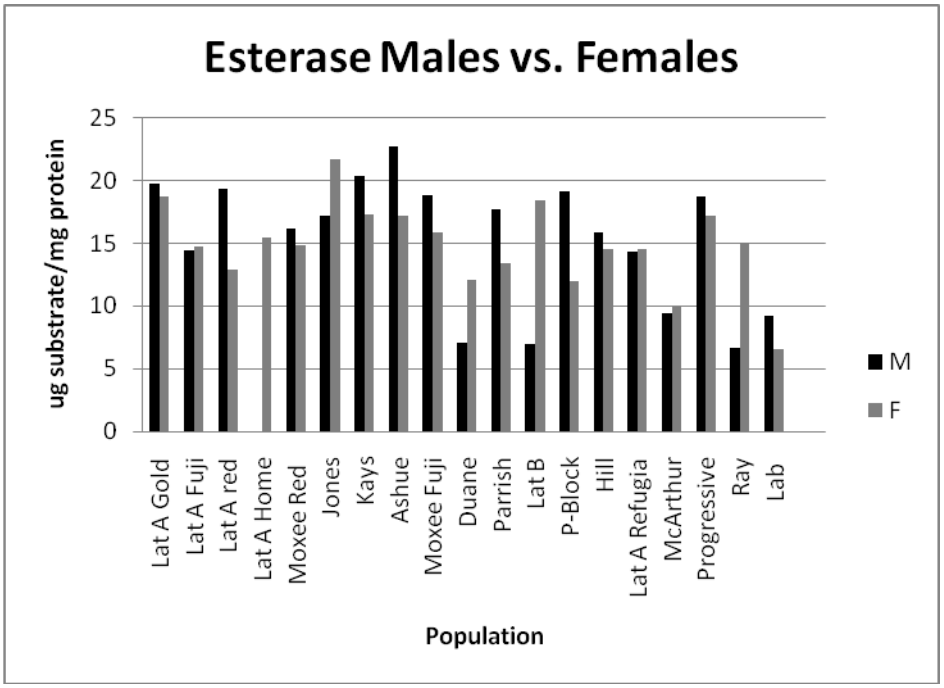

Figure 4. Non-specific Esterase activity of male and female Codling Moth Collected in the Yakima Valley.

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

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

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

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

EXECUTIVE SUMMARY

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

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

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

FINAL PROJECT REPORT **WTFRC Project Number:** CP-08-800

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

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

Other funding sources

Agency Name: USDA-CSREES SCRI
Amount awarded: $2.244M

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

Budget History:
Organization: WSU-TFREC **Contract Administrator**: ML Bricker, Kevin Larson
Telephone: 509-335-7667, 509-663-8181x221 **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

[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 History 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	43,638
Benefits	11,635	11,169	18,702
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	62,840

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

Budget History 3:
Organization: Agriculture & Agri-Food Canada **Contract Administrator**: Karen St. Martin, Goewin Demmon
Telephone: 250-494-7711 **Email**: KSM stmartink@agr.gc.ca, GD demmong@agr.gc.ca

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

[1] Summer student wages with 20% benefits plus inflation in year 2.
[2] Supplies in year 2 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, beat-tray sampling, and attractant-trapping.
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 and Accomplishments:

- WTFRC funding for this project was leveraged to bring in substantial additional funding ($2.2M) through a CSREES Specialty Crops grant; moreover, data collected for the WTFRC project were included as preliminary results in the justification section of the SCRI grant proposal.
- Beating trays have severe limitations for sampling natural enemies, but do show that our aphid feeding ladybird beetles and spiders tend to have only a single generation in the orchard; lacewings, and some of the predatory bugs show 2+ generations.
- The phenology model for the lacewing *Chrysopa nigricornis* is nearly complete and works on apple, cherry, and walnut. Pesticide impacts on pears obscure the phenology in that crop, but more analysis will be performed.
- Pesticide distortion of phenology may become a very sensitive way to evaluate pesticide impacts on natural enemies.
- We evaluated release rates of semiochemical-based attractant lures over time in both laboratory and field conditions and now have a lure system that gives a consistent release rate for at least 30 days with all materials; the release rates are constant even in direct sunlight over that period.
- We completed 14 different attractant studies over the past three years and now have lures for at least three species of lacewings, three species of syrphid flies, and a broad range of parasitoids. We can also "design" attractant blends so that we can enhance or reduce capture of certain species to make trap checking easier.
- Lab studies on four ground dwelling predators showed that earwigs and daddy-long legs fed on free-living fifth instar codling moth larvae but not cocooned (overwintering) larvae; wolf spiders and a predatory ground beetle fed on both life stages
- Gut content analysis of field collected predators showed that 6, 2, 4 and 10% of the daddy-long legs, earwigs, spiders, and ground beetles, respectively, scored positive for having fed on codling moth larvae over the previous 24-hour period.
- Adult tachinid parasitoids (*Nilea erecta*, *Nemorilla pyste*) were both heavily impacted by the normal field rate of Esteem® residues. Full field rates of Intrepid® reduced *Nemorilla* survival, but not *Nilea* survival.

Significant Progress:

Objective 1. Our sampling produced phenology data from three data sources: (1) overwintering bands that were placed in orchards last fall and brought to outdoor shaded shelters by early December; (2)

Table 1. Comparison of the abundance and phenology of the lacewing *Chrysopa nigricornis* measured in Washington apple orchards using beat-tray samples and attractant traps during 2009.

	Initial Capture		Last Capture		Total Captured	
Orchard	Beat Tray	Attractant	Beat Tray	Attractant	Beat Tray	Attractant
1	22-Jul	24-Apr	22-Jul	15-Oct	1	7,158
2	24-Jul	13-May	4-Sep	6-Oct	4	13,770
3	none	18-May	none	6-Oct	0	9,108
4	11-Aug	21-May	8 Sept	19-Oct	5	3,195
5	18-Aug	4-Jun	10 Sept	30-Sep	2	717

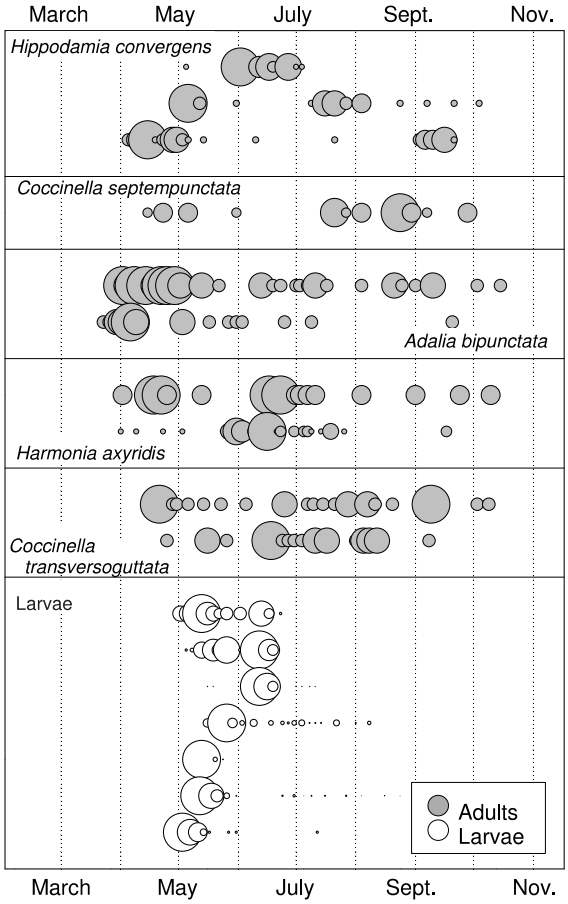
beating tray samples collected in eight orchards in the Wenatchee and Yakima areas from early spring to fall; and (3) attractant-baited trapping in the same eight orchards.

We caught 33,948 *Chrysopa nigricornis* lacewing adults using four attractant traps in five orchards. In contrast, beat-samples conducted concurrently in the same orchards captured 12 lacewings. This is the second year in which beating trays have severely underestimated lacewing abundance and/or activity (see Table 1).

At each site, the attractant baited traps caught *C. nigricornis* both earlier and later in the season, and in dramatically higher numbers (Table 1). This information shows that adult lacewings emerge earlier in the spring (≈75 days earlier) and that they continue to fly later in the fall than expected based on beat samples (≈43 days late). Our data show that while beat-samples may have its place for estimating populations of certain natural enemies, it is very clear that certain other species are not effectively monitored using trays. For example, looking at the *C. nigricornis* data from beat-samples, it would appear that this predator is rare, when it is actually extremely abundant.

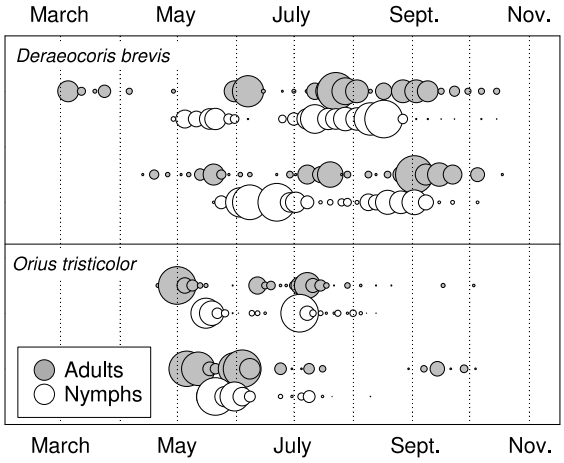
While beating trays are much less effective at capture of some natural enemies, they also provide information that most attractant traps don't provide, namely the presence of both adult and immature stages. We collected beat tray samples in eight orchards (4 in Yakima area, 4 in Wenatchee area) over the first two years of this grant. Dave Horton has taken the lead on analysis of this data and several interesting things appeared in the analysis. First, all aphid-feeding ladybird beetles appear to have only a single generation per year (Fig. 1). Spiders also showed only a single generation per year (data not shown).

Fig. 1. Beat tray data of the capture of several species of adult ladybird beetles in WA apples. Each row of circles is a separate orchard. Size of circle is



Phenological results for ladybird beetles and spiders suggest that these predators may thus recover only very slowly from disruption caused by pesticides. Our lacewings tend to have 2+ generations per year, a life history trait shared by some of the predaceous bugs (*Orius* and *Deraeocoris*) (Fig. 2). We are currently analyzing these data in conjunction with pesticide use patterns and surrounding vegetation surveys to determine if there are landscape level determinants of natural enemy species abundance and diversity.

Fig. 2. Beat tray data of the capture of *D. brevis* and *O. tristicolor* in WA apples. Size of the circle is proportional to the number collected; each row of



Objective 2A. *Determine release rates of semiochemicals.* We investigated a total of 14 lures for longevity and release rate over time in both lab and field settings at WSU-TFREC. The polyethylene tubing lures we developed provided a stable release rate for each compound that is a function of membrane thickness and temperature. In year two, we finished field-testing lures that were deployed inside a normal white delta trap.

Our work in Objective 2B showed that we could reduce unintended honeybee capture and increase capture of other natural enemies by switching to yellow sticky panels. The switch to yellow sticky panels would result in the lures being directly exposed to the sun – thus we needed to evaluate lure longevity in the direct sun. This past summer, we tested 21 different lures for 13 different attractants. Lure release rate was slightly higher when exposed, and lure depletion was only an issue with two of the attractants. In those two cases, we were able to increase the lure load and bring lure longevity back to the normal 30+ days.

Objective 2B. *Evaluate field effectiveness and spectrum of activity of the different attractants*. Over the past three years, we have run 14 field trials to evaluate and improve our attractant traps. In 2010 alone, we ran five tests that: (1) evaluated sixteen different attractant blends (a 2 × 2 × 2 × 2 factorial experiment) using all possible combinations of the presence or absence of acetic acid (AA), acetophenone (AP), phenylacetaldehyde (PAA), and 2-phenylethanol (PE) in three different orchards to get the spectrum of activity of these new lures; (2) tested 8 blends (a 2 × 2 × 2 factorial experiment) using all possible combinations of the presence or absence of methyl salicylate, acetic acid, and PAA for lacewing attraction; (3) examined (as a 2 × 2 × 2 factorial experiment) AP, geraniol (GER), and PE with yellow sticky panels, focusing on improving trap catch of syrphid flies (key predators of aphids); (4) evaluated (as a 2 × 2 × 2 factorial experiment) AP, GER, and the *Campylomma* pheromone, in an attempt to increase efficiency of the *Campylomma* pheromone; and (5) analyzed season-long phenology (objective 5).

Results*:*

AA×AP×PAA×PE study. This factorial experiment was run in two apple orchards in the Wenatchee area and one in the Yakima area. In an attempt to evaluate the importance of these attractants in mixtures, we ran an all-possible combinations experiment (factorial) - this required us to test 16 different treatment combinations (each replicated 4 times per orchard). This trial was also run in the SCRI project in pear and walnut orchards. To date, only the apple data are complete enough to evaluate effects on specific natural enemies.

The complete factorial design is very cumbersome and expensive, but it provides us a very clear picture of how the different attractant combinations work in the field. For the syrphid fly, *Eupeodes volucris*, it was clear that its response was similar in all apple orchards, with the best attractant being

2-phenylethanol (PE) by itself (Fig. 3). Addition of any of the other compounds did not improve capture, and many combinations actually decreased the capture over PE alone. This is in contrast to the lacewing *Chrysoperla plorabunda*, which responds to a wide range of blends and nearly always responds better with two or more of the attractants. For *C. plorabunda*, the top binary blends were not significantly different from either the three or four component blends.

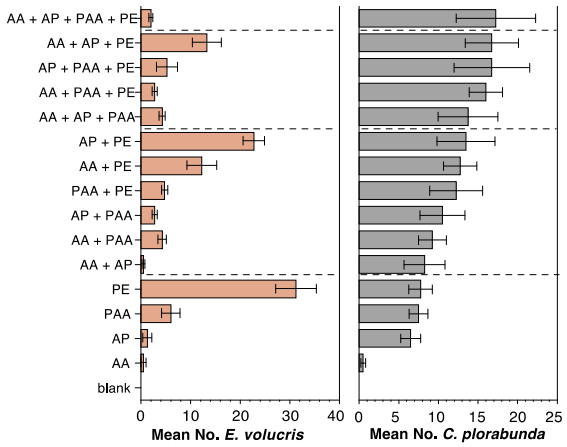


Fig. 3. Trap capture of the syrphid fly, *Eupeodes volucris* and the lacewing *Chrysoperla plorabunda* to all possible combinations of acetic acid (AA), acetophenone (AP), 2-phenylethanol (PE), and phenylacetaldehyde (PAA). Dotted lines separate 1

AP×MS×AA. This study was run to evaluate an attractant blend that was developed in Europe for the lacewing *Chrysoperla carnea*. The addition of acetic acid to the blend was thought to be a major factor in attraction and the authors suggested that it might be so for other members of the genus *Chrysoperla*, which are not predators as adults (adults are pollen feeders). We found that AA was not attractive by itself, but synergized the activity of PAA and was super additive when combined with MS and PAA for capture of *C. plorabunda*. For *E. volucris*, similar to the above studies, the single component of PAA was better than the combination of multiple attractants.

AP×GER×PE Yellow Card Study. The yellow panel study showed that trap catch of *C. plorabunda* was not improved significantly by addition of multiple components over use of PE alone. This is similar to the results from the AA×AP×PAA×PE study above if the results of that study are restricted to the AP×PE elements. The lack of activity of GER alone and the lack of either MS or AA (which tend to act as a synergist) breaks the general trend of *C. plorabunda* responding best to a mixture. The results with the syrphid *Eupeodes* were similar to the other tests where the single component PE was not significantly different than any mixture.

Overview of trapping experiments

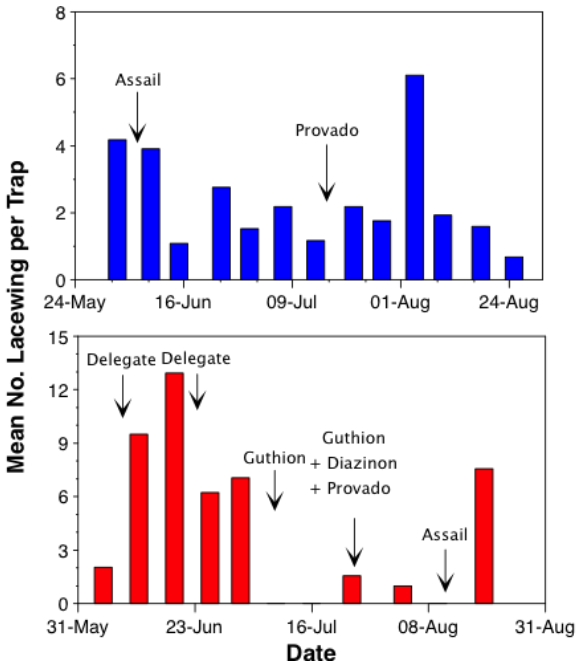
Running full factorial experiment allows us to custom tune a blend to attract a range of natural enemies and reduce capture of those of less interest. Once the data sets from the SCRI data are completed for walnut and pear, we will design blends that attract the desired range of natural enemies and test them in the field next year. For example, in our apple data, we could use several of the three component blends (*e.g.*, AA + AP + PAA or AA + AP) to limit catch of *E. volucris*, while still capturing *C. plorabunda*. We will also run one more factorial this coming year on our SCRI project that will combine the best of attractants that we tested in 2009 and 2010 to finalize our blends down to 3-4 attractants targeting key natural enemy taxa.

Evaluations of insecticide impacts: The efficacy of our attractants permits us to document how natural enemy populations are influenced by insecticide sprays. Focusing on lacewing populations, our data last year showed that reduced-risk insecticides had less impact on the lacewings, while organophosphates appear to decimate lacewing populations (Fig. 4). In one orchard near Quincy with a minimal pesticide program, we found that lacewing populations experienced typical oscillations but never "crashed" (Fig. 4 top). A different orchard in the same area saw its lacewing population decline sharply and remain low for approximately four weeks, during which two applications of azinphos-methyl (Guthion) were applied (Fig. 4 bottom). During this same four week period at other Quincy orchards, our trapping indicated that lacewing populations were rising. The trapping data show that the attractant lures can be used to evaluate pesticide applications and these lures have been used in

apple, cherry, walnut and pear to evaluate pesticide impacts on natural enemy populations in the SCRI project this year.

Objective 3A. In 2008 and 2009, video monitoring of predation on CM cocoons deployed in the field proved technically problematic but suggested that most predation was caused by vertebrates and not insects. Observations of 192 camera hours in 2008 identified three predation events by birds. Recordings of 1,114 hours in 2009 showed 11 predation events by mice (7), ants (3), and an earwig (1). This suggests that predatory arthropods on the orchard floor may have less impact on CM than vertebrates. To better understand this, the Unruh lab conducted predator-feeding studies in the laboratory in 2010 using likely CM predators. Gut content analyses (in objective 3b) will validate whether these predators do indeed discover and eat CM larvae in the field. In 2011, additional visual field measurements of predation on CM will be presented.

Fig. 4. Comparison of the trap catch of *Chrysopa nigricornis* in an orchard with minimal pesticide



Results: Four arthropod taxa that were large enough to be capable predators of free living or cocooned CM larvae were collected by pitfall trapping and tested in the lab. These were harvestmen (Opiliones or "Daddy long legs"), several large wolf spiders (Lycosidae), the predatory ground beetle *Pterostichus melanarius* (Carabidae), and the European earwig (*Forficula auricularia*). Each predatory species was offered either late instar CM larvae or cocooned CM larvae and their behavior and prey consumption were monitored in 70-100 replicates/species/host types. The studies showed earwigs (93%) and daddy long legs (95%) ate free CM larvae but no larvae in their cocoons (0% for both species). In contrast, the carabid beetle, *P. melanaria* and wolf spiders ate both free 5th instar larvae and cocooned larvae equally (>95% for both host types for both species). The carabid and the wolf spiders were voracious predators; they attacked and consumed the CM larvae within one hour of presentation; cocooned CM consumption took several hours. The daddy long legs and earwigs were slower at prey consumption and took many hours (4–24) to attack and consume the free-living larvae. Some daddy long legs were injured by the free living CM.

Objective 3B. *Gut content analysis methods to replace PCR:* The loop-mediated DNA amplification (LAMP) protocol has been the focus of the lab for the last two years; however, this approach was dropped because of repeated lab contamination by reaction products (a problem described by many others using LAMP). This problem caused us to prepare samples in a teaching lab at Heritage University (courtesy of Dr. N. Barcenas) to separate samples from areas where reaction products were produced. Even with these precautions, we concluded LAMP was poorly suited for gut content analysis (GCA) where a bioassay that can be conducted in one lab is desirable. The GCA is ongoing with a revised PCR method that detects a CM odorant receptor gene and a recently developed buffer system that supports amplification of "dirty" (whole-body homogenate) samples.

Results: Limited numbers of GCA have been conducted for Opiliones (18), earwigs (167), spiders (129), and the carabid, *P. melanaria* (48). We found that 6, 2, 4 and 10% of the Opiliones, earwigs, carabids, and spiders (respectively) tested positive for consumption of codling moth within the previous 24 hours (after 24 hours, the codling moth DNA break down and are not detectable). Specimens were collected daily from pitfall traps between mid-August to mid-September. The results

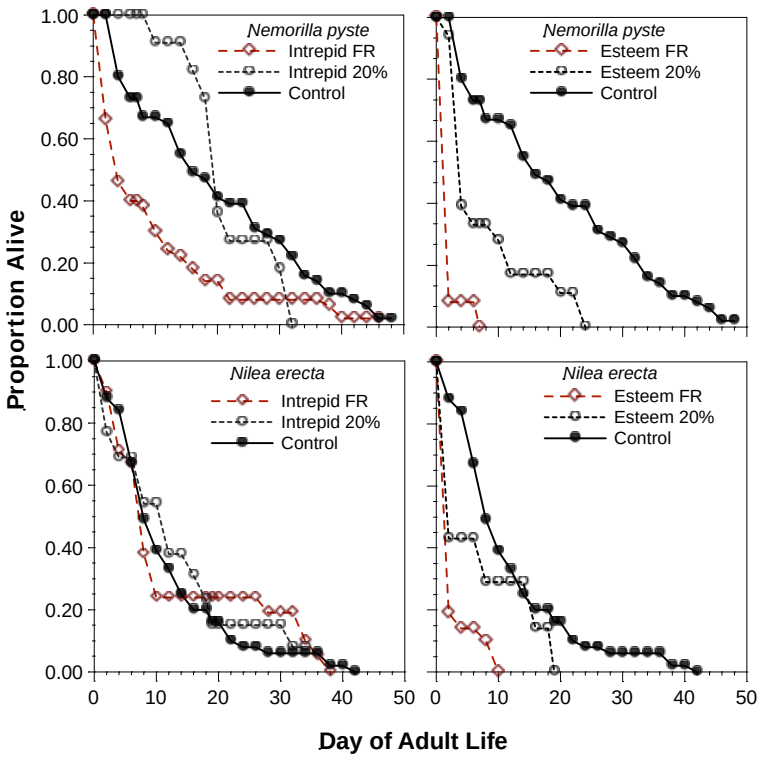
to date do not completely correspond to the feeding studies: low predation rates were observed by carabids and higher than expected results were observed for daddy long legs. These results suggest that large spiders are the most potent predators of CM, which is consistent with their very aggressive behavior in the laboratory. PCR studies are ongoing and larger sample sizes should be available for the oral report. Additionally, GCA will continue through 2011 under SCRI and results will be provided to TFRC at that time.

Objective 4. *Determining the impact of IGR's used for leafroller control on tachinid adults.* Last year we presented data that demonstrated the effect of Esteem (pyriproxyfen) and Intrepid (methoxyfenozide) residues on longevity of *Nilea erecta*, and for *Nemorilla pyste* we presented data for Esteem only. Last year's objective was to finish evaluating the effect of Intrepid (methoxyfenozide) residues on longevity of *N. pyste* and to increase replications for the two IGRs with *N. erecta.* To obtain the desired residues, the IGRs were applied at field or 20% field rates to plastic deli cups that were then air-dried. Cups were provisioned with 10% honey-water solution. Cohorts of male and female *Nilea erecta* and *Nemorilla pyste* were placed in the treated cups individually upon the day of emergence to the adult stage, with 10% of the cohort reserved for untreated (control) cups. Cups were monitored daily to determine the day of death.

Results: The control mortality curves differed in shape between the two fly species (Fig. 5). *Nemorilla* mortality was roughly linear over their entire adult life, whereas *Nilea* mortality curves were characterized by higher initial mortality rates, which then decreased over time. When exposed to the field rate of Intrepid, the mortality curves of *Nemorilla* flies showed an increased early mortality compared to controls (≈40% by day 10), while *Nilea* mortality rates were unaffected. Reduced rates of Intrepid (20% of the field rate) had no affect on either species.

Esteem was highly toxic to both species. At field rates, maximum life expectancy was roughly 25% of untreated controls in both *Nemorilla* and *Nilea* and cohort survival was reduced to 8-20% (i.e., 92-80% mortality occurred). Even at 20% of the field rate, Esteem caused extreme early mortality, killing more than half of the cohort in 2-3 days. Clearly these parasitoids are highly susceptible to Esteem, and even reduced rates do not allow sufficient longevity to include time for mating and attacking hosts.

Fig. 5. Effect of pesticide residues on longevity of the tachinid leafroller parasitoids *Nemorilla pyste* and *Nilea erecta*.



While these trials examined the affects of IGR's on mortality, exposure to these compounds may also affect behaviors or physiological processes that are necessary for successful mating and reproduction. However, from our results we can conclude that Intrepid at field rates has a strong negative effect on populations of *Nemorilla*, which is the most common and abundant of the two flies. However, Intrepid is less

problematic for biological control compared to Esteem. Esteem was highly toxic to both species at low concentrations, suggesting that this compound is entirely antagonistic with leafroller biological control. The mechanism by which the flies are affected by residues is unclear, although it likely entails ingestion of the compounds from surfaces through grooming and/or sponging which is a common occurrence in both species.

Objective 5. We have analyzed the squalene trapping data now from apple (7 orchard sites over two years), sweet cherry (3 orchard sites over one year), pear (10 orchard sites over two years) and walnut (6 orchard sites over two years). The trapping data, along with an unpublished manuscript on temperature development of *C. nigricornis* by former USDA-YARL entomologist R. Fye, has allowed us to develop a phenology model for this key natural enemy. We found the number of flights in WA varies from 2 (cherries) to 3+ generations in warmer apple orchards. Our work on the four different crops shows that a single model works on all crops (Fig. 6), but there are some variations in flight that can be attributed to spray programs. Specifically, the first generation in cherries is completely suppressed by spray programs aimed at other pests (*e.g.*, black cherry aphid, western cherry fruit fly) or by diseases (oil applications) (Fig. 7). In pear, pesticide applications so distort the phenology that without spray records, the emergence of the different generations are difficult to ascertain. Our studies also suggest the differences in phenology can be used to help understand pesticide impacts as well.

Fig. 6. Phenology of the lacewing *Chrysopa nigricornis* adults in apple, cherry, and walnut

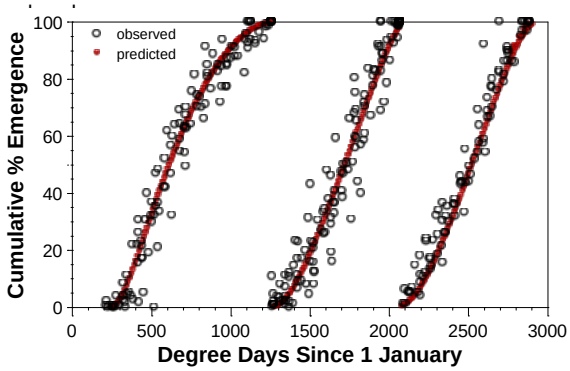
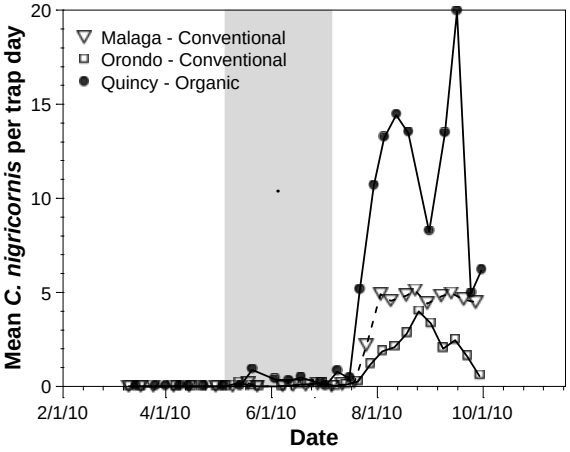


Fig. 7. First generation of *C. nigricornis* in cherries should occur in gray box, but is suppressed by insecticides and fungicides applied during that



This year, we also ran season-long phenology studies in eight apple orchards (4 in the Yakima area, 4 in Wenatchee area) using four attractants (1) squalene for *C. nigricornis*, (2) GMP (geraniol + methyl salicylate + PE) – a general lure for lacewings, syrphids, and parasitoids), (3) AP – targeted at mainly lacewings, western flower thrips and parasitoids, and (4) PAA – specifically targeted for hymenopterous parasitoids in the families Scelionidae (some species are key stinkbug parasitoids) and Eulophidae (same family as *C. florus*, the parasitoid of OBLR and PLR).

Our data from the long-term phenology data should provide enough information to develop phenology models for another lacewing, *Chrysoperla plorabunda*, which is common in both the Wenatchee and Yakima areas on apples. Likewise, with our data combined that from the different SCRI crop systems (walnut, pear) and the cherry data this coming year we should be able to develop phenology models for at least two species of syrphid flies, *Campylomma* bug, and hopefully the parasitoid of the woolly apple aphid, *Aphelinus mali*. These models should begin to appear on WSU–DAS next winter as each is developed and we can start making changes to management timings to reduce impact on natural enemies while maintaining the necessary efficacy against the pests.

Executive Summary:

This grant served as the basis for our $2.4M SCRI grant to enhance biological control in western apple, pear, and walnut orchards. It not only helped to provide matching funds, but it also supplied the initial data sets that showed the SCRI grant panel that the work was possible and had a strong applied basis. In addition, the work done in the first year of this grant also served to convince our colleagues in California and Oregon that we had technologies in apple that could be useful in pear and walnut orchards and that it would be to our mutual benfit to work on these areas (as well as others) to enhance biological control in western orchards.

The results of experiments performed in this grant have radically changed our understanding of the diversity and abundance of natural enemies in apple orchards. We have developed new sampling methods using Herbivore-Induced Plant Volatiles (HIPV) released in a consistent fashion by lures developed by this project. Over all the studies with HIPV lures that we have performed in the last 3 years, we have now developed a highly active and specific attractant for *C. nigricornis* (squalene), a very general attractant (Geraniol + Methyl Salicylate + 2-phenylethanol) for lacewings, parasitoids, and some syrphid flies. In addition to these two attractants, our testing protocols showed us that attraction to certain natural enemy groups could be almost completely shut down by the addition of a particular component and use of different trap types, which can also enhance capture of specific natural enemy groups. Some of our blends are already highly attractive to Scelionid (many stinkbug parasitoids) and Eulopid parasitoids (same family as the leafroller parasitoid *C. florus*), and several blends that are also attractive (although not strongly so) to predaceous hemipterans. Further studies on HIPVs will still be needed to refine some of the blends, and we hope to finish most of this work using the remaining research funding from the SCRI grant.

The combination of the banding, beating trays, and HIPV lures has given us the data set to develop natural enemy phenology models that will be used to improve timing of our management actions. We currently have a phenology model for the lacewing *Chrysopa nigricornis*, which was the most abundant predator collected in our studies. Combined with our data taken in the USDA-SCRI grant, we should also have the data to develop at least five more natural enemy models to help refine management programs.

The work with the Tachinid flies shows how an important leafroller natural enemy can be greatly impacted by "reduced-risk" insecticides. Esteem was particularly harsh on newly emerging flies and caused 80-92% mortality within 2 days. Even at 20% of the field rate, Esteem caused extreme mortality of both species, killing more than 50% of the flies by 2-3 days. Intrepid is much less toxic, having no real effect on *Nilea*. However, *Nemorilla* flies experienced increased mortality over untreated flies by roughly 40% on day 10. Our current recommendations on WSU–DAS should reduce the impacts of both insecticides, because we recommend no sprays during the last two leafroller instars when the tachinids are most active. However, these data do show that even with those caveats, that pesticide choice is still a key component of leafroller management.

In terms of future work, we see a great potential to using our natural enemy lures to evaluate the impact of different pesticides on natural enemies and the resulting pest supression in the orchard. In particular, we see the possibility of manipulating natural enemy spatial distributions in the orchard by deploying lures into high pest areas to "jump-start" the egg laying and predation in a particular area. We feel that it is unlikely a reasonable decision to place the lures into an area for all season, because it would disrupt the ability of the natural enemies to use the HIPV to locate their prey and may act similar to mating disruption and reduce the reproductive rate of the natural enemy. However, for this to be successful, we need to know more about the attractive range and evaluate which lures are the best for suppression of key pest groups. This approach is one of the components of the new grant being submitted by Jones and Chambers this winter.

FINAL PROJECT REPORT

Project Title: Wooly Apple Aphid resistance in advanced rootstock selections

PI:	Gennaro Fazio	**Co-PI:**	Betsy Beers
Organization:	USDA ARS PGRU	**Organization:**	WSU
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City:	Geneva, NY 14456	**City:**	Wenatchee, WA 98801

Total Project Request: **Year 1:** $12,000

Budget 1
Organization Name: USDA ARS PGRU-FAZIO

Item	**2010**		
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies **	2,000		
Travel			
Miscellaneous			
Total	2,000		

Footnotes: ** DNA genotyping supplies.

Budget 2
Organization Name: WSU TFREC-BEERS

Item	**2010**		
Salaries			
Benefits			
Wages **	7,000		
Benefits	1,500		
Equipment			
Supplies***	1,500		
Travel			
Miscellaneous			
Total	10,000		

Footnotes: **Temporary labor help for greenhouse inoculations and data collection * Greenhouse supplies, fees.**

Background and Objectives: the woolly apple aphid (WAA), *Eriosoma lanigerum* has become a more severe pest in Washington apple production in the past few years. Milder winters have promoted overwintering survival on the aerial parts of the tree. In addition new plantings are rarely made on resistant rootstocks, and a very low percentage of the acreage is currently on resistant rootstocks. The transition from organophosphate insecticides to either insect growth regulators or neonicotinyl insecticides may also be contributing to higher pressure. It may be possible to control this pest significantly by utilizing genetically resistant rootstocks in new apple plantings. Resistant rootstocks would significantly reduce the ability of WAA to overwinter in the root zone and therefore decrease their survival and recurrence during the growing season. A previous greenhouse test of 8 clonally propagated rootstocks and 2 seedling rootstocks demonstrated that several of the new Geneva rootstocks to have virtual immunity to a Washington strain of woolly apple aphid due to the presence of the Robusta 5 derived *Er2* WAA resistance gene located on Linkage Group 17 of the apple genome. We have closely linked markers to this gene, however the ultimate test of the presence and efficaciousness of this gene can only be revealed by a good phenotypic replicated test. Although not part of this proposal we intend to clone the WAA resistance gene by probing a BAC library of G.41 possessing the Er2 gene. The proposed objectives were: 1. Test an array of Elite Geneva rootstocks and other commercially available rootstocks in a replicated greenhouse test for resistance to Wooly Apple Aphid (WAA) and disseminate knowledge to growers for planting recommendations.
2. Test the same array and as well as parents and other apple rootstocks for the presence of markers linked to wooly apple aphid the resistance genes.

Significant Findings
Objective 1: Nine rootstocks were categorized as resistant (3010, 3067, 4010, G.214, 4288, 4292, 4809, G.87, G.210), while five of the rootstocks were categorized as susceptible (2006, 3902, 4011, 4088, and M.9 Pajam). The final group (5890, 4814, 6969, 2406) were categorized as unsure; while the percentage of replicates rated as susceptible was low, there was enough evidence of woolly apple aphid establishment to take them out of the resistant group. These clones may exhibit an intermediate level of resistance to WAA.
Objective 2: Most published DNA markers linked to the different sources of wooly apple aphid resistance were difficult to work with in a high throughput marker assisted selection setting. Published markers linked to *Er2* were also difficult to ascertain and we had to rely on in-house developed markers to continue work on this resistance gene. Hence we developed additional markers like MalSSR2952 that were better suited at distinguishing the resistance in the progeny of Robusta 5 (the source of resistance).

Results and Discussion

OBJECTIVE 1

Despite repeated infestation, the first rating indicated only a moderate level of infestation in the susceptible 'M.9 Pajam' (ratings of 0-2). For this reason, the aphid populations were allowed to develop for an additional 6 weeks. At this time, most of the susceptible rootstocks were rated as 2 or 3.

For the purposes of summary, rootstocks rated as ≤1 were considered resistant, and those rated >1 were considered susceptible (Table1014.1). The percentage of reps falling into 'Resistant' or 'Susceptible' was calculated, and an overall rating assigned.

Nine of the rootstocks (3010, 3067, 4010, 4214, 4288, 4292, 4809, 5087, 6210) were considered 'Resistant' to woolly apple aphid, in that none aerial portions of the replicates had any rating >1, and only 1 replicate had a root system rated as susceptible (5087 was rated 1 and 3 for the

top and roots, respectively). Given the consistency of the other replicates, this replicate may have been mislabeled.

Five of the rootstocks (2006, 3902, 4011, 4088, and M.9 Pajam) were categorized as susceptible, with 43 to 100% of either the tops or roots rated as susceptible. This group included the known susceptible 'M.9 Pajam'.

Four of the rootstocks (5890, 4814, 6969, 2406) were categorized as 'Unsure'; while the percentage of replicates rated as susceptible was low, there was enough evidence of woolly apple aphid establishment to take them out of the 'Resistant' group. These clones may exhibit an intermediate level of resistance to WAA, or the infestation levels are the result of phenotypic variation.

Only two of the rootstocks in the 2010 evaluations were also in the 2009 evaluations (exp. 0912). One, '5087', was considered 'Resistant' in both evaluations. The other '4011', was considered 'Intermediate' in 2009, and susceptible in 2010. However, it was represented by only 4 replicates in 2009, and 8 in 2010, so the latter rating is felt to be more reliable.

Correlation of greenhouse experiments with molecular marker information

We have synthesized DNA markers listed on table 2 and assessed their presence/absence in an array of 96 apple rootstock DNAs that includes genotypes in Table 1 as well as commercial apple rootstocks and parents in the breeding program. We have discovered that several of the SCAR markers in Table 2 were very hard to amplify by PCR and therefore not suitable for large scale Marker Assisted Selection. A sample of one of the many DNA marker gel images generated during this study is in Figure 1.

Table 2. A list compiled by Bus et al. (2008) represents some of the markers that were used to tag resistance to WAA.

Marker name	Marker type	Original RAPD/EST	WAA gene	Forward primer	Reverse primer	Product size (bp)	PCR conditions [a]	Linkage group
NZsc_C20	SCAR	OPC20	*Er1*	TCTCTAACTCAATAACTCCCAAGAC	ACTTCGCCACCATTATCACTCCTGA	2,000	Td 70–60	8
NZsc_GS327	SCAR	GS327	*Er1*	GCCAAGCTTCAATGTCGGAGTAGAT	CAAGCTTCCCCTAAGGCTATTGCCA	1,600	Ta 60	8
NZsc_O05	SCAR	OPO05	*Er1*	CCCAGTCACTAACATAATTGGCACA	CCCAGTCACTGGCAAGAGAAATTAC	1,700	Ta 60	8
NZsn_O05	SNP	OPO05	*Er1*/*Er3*	AACGTCATGTCAATAT	CCCAGTCACTGGCAAGAGAAATTAC	880	Td 70–60	8
NZsc_E01	SCAR	OPE01	*Er3*	CCCAAGGTCCGAACACAAATGAGAG	CCCAAGGTCCAAAACTATCCCGAAG	1,350	Ta 60	8
NZsc_A01	SCAR	OPA01	*Er3*	CAGGCCCTTCAGCAAAGAGGTGTCT	CAGGCCCTTCACTACTAATAAGAAC	1,250	Ta 60	8
NZms_EB106753	SSR	EB106753	*Er1*/*Er3*	TCTGAGGCTCCCAAGTCC	TAGGAGCAGAAGAGGTGACG	175	Td 65–60	8
NZms_EB145764	SSR	EB145764	*Er2*	TTCCAGCGATCCAAAACAAT	GCTCAGGAACACCTCGTTCT	198	Td 65–60	17

The SCAR and SNP primers were derived from RAPD markers (Operon Technologies, Alameda, CA), and the SSR primers were designed from ESTs of apple.

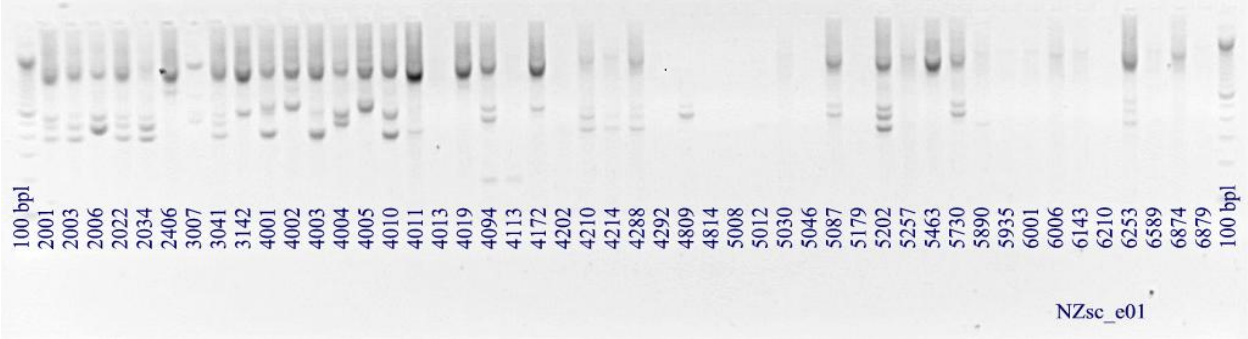

Figure 1. An example of a gel image of SCAR marker NZsc_E01 where the array of Geneva elites are tested for the presence of the resistance gene *Er3*.

With regards to the resistance gene Er2 derived from Robusta 5 we tested several markers located on chromosome 17 of apple that were reported to be linked. The markers and genomic location in parenthesis were: NZms_EB145764 (1,585Kb), MR1133 (3,576Kb), MalSSR2952 (5,014Kb), CH05g03 (5,324Kb), GD153 (9,138Kb), and GD96 (11,820Kb). We found inconsistencies according to the genetic map by Bus et al. (2008) which placed Er2 between markers GD96 and GD153 (Figure 2) next to NZms_EB145764. This report led us to believe that we could use GD96 and GD153 as predictors of the presence of the resistance gene.

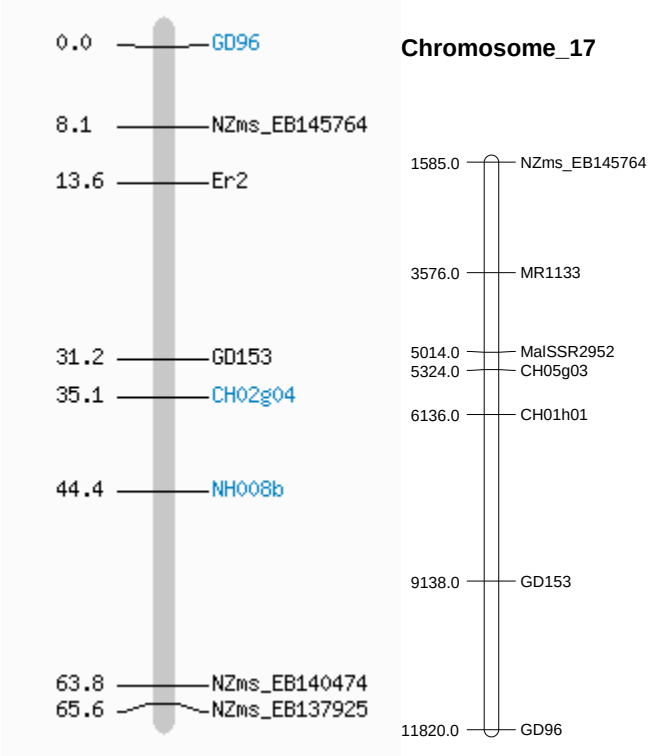

Figure 2. Putative map of the location of the *Er2* gene by Bus et al. (2008). Physical map of chromosome 17 with correct marker locations in Kilobases.

As it turns out the predictive power of GD96 and GD153 was not good and had to synthesize the NZms_EB145764 marker to detect *Er2*. While the new marker had good predictive power for most

Robusta 5 ((R5) progeny in crosses with Ottawa 3 it was not able to detect the Robusta 5 resistance allele in another family whose progeny is G.41 and G.202 which have been experimentally classified as immune to the wooly apple aphid. Further study of this marker in a segregating population indicated that it was not behaving in a true codominant manner like most SSR markers do, rather one of the alleles was null. This hinders the utilization of such marker for large scale marker assisted selection. Alternative markers are being sought for easy visualization/selection on gels. With regards to the rootstocks classified as U (unsure) in the greenhouse phenotypic experiments summarized on table one, G.969 (6969), G.890 (5890) should be resistant according to the molecular marker information. Rootstock CG.4814 does not possess any markers for resistance, however this rootstock may be a recombinant showing no markers bust still possessing the gene(s) for resistance. Rootstock CG.2406 is has a very different genetic background than the others tested here, because it is the only one with *Malus micromalus* in its pedigree. This rootstock did not seem to possess any marker allele related to the R5 resistance, however in the test for the marker NZsc_GS327 linked to the Northern Spy (NS) resistance gene on Chromosome 8 we detected a marker with similar molecular weight indicating the possibility that a similar gene might be acting, in addition we detected a unique allele for the NZmsEB106753 marker also linked to the NS resistance. Susceptible rootstocks CG.2006, CG.3902, CG.4011, CG.4088, did not possess markers for any of the detectable resistance genes. More work is needed to study the inheritance and presence of aphid resistance in potential parent material for further crosses. In the meantime the possibility of combining resistance of the Er1 gene on chromosome 8 and Er2 gene on chromosome 17 may be real on third generation crosses made recently.

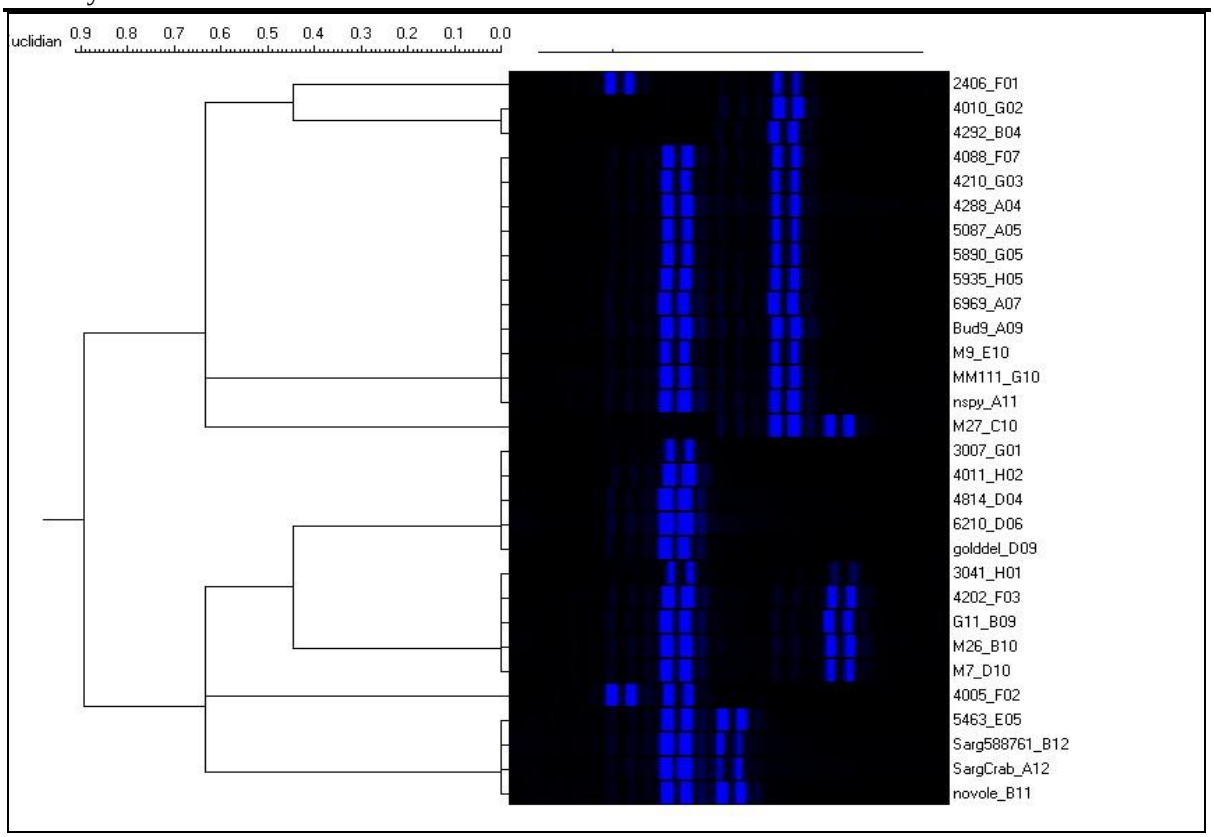

Figure 3. Marker NZmsEB106753 supposedly diagnostic for the Er1/Er3 resistance on Chromosome 8, does not show differences between resistant Northern Spy (nspy) and susceptible B.9 and M.9 rootstocks – hence it not useful as a diagnostic tool.

Table 1. Rootstock rating, 27 August 2010.

	Tops					Roots			
Rootstock	Mean rating	n reps susceptible (TOP)	n reps	% Susceptible (TOPS)	Rating	Mean rating	n reps susceptible (ROOTS)	n reps	% Susceptible (ROOTS)
3010	0.63	0	12	0.0	R	0.42	0	12	0.0
3067	0.46	0	12	0.0	R	0.21	0	12	0.0
4010	0.08	0	13	0.0	R	0.00	0	13	0.0
4214	0.40	0	15	0.0	R	0.10	0	15	0.0
4288	0.13	0	8	0.0	R	0.00	0	8	0.0
4292	0.04	0	12	0.0	R	0.00	0	12	0.0
4809	0.00	0	12	0.0	R	0.13	0	12	0.0
5087	0.17	0	12	0.0	R	0.33	1	12	8.3
6210	0.11	0	9	0.0	R	0.00	0	9	0.0
5890	0.30	1	10	10.0	U	0.45	1	10	10.0
4814	0.38	2	13	15.4	U	0.15	0	13	0.0
6969	0.77	2	13	15.4	U	0.88	2	13	15.4
2406	0.40	1	5	20.0	U	0.00	0	4	0.0
2006	1.14	3	7	42.9	S	1.31	4	8	50.0
3902	1.46	6	12	50.0	S	2.50	12	12	100.0
4011	2.10	8	10	80.0	S	1.90	7	10	70.0
4088	1.89	8	9	88.9	S	1.83	5	9	55.6
M.9 Pajam	2.18	13	14	92.9	S	1.46	8	14	57.1

R=Resistant; S=Susceptible; U=Unsure

References
V. G. M. Bus, D. Chagné, H. C. M. Bassett, D. Bowatte, F. Calenge, J.-M. Celton, C.-E. Durel, M. T. Malone, A. Patocchi, A. C. Ranatunga, E. H. A. Rikkerink, D. S. Tustin, J. Zhou and S. E. Gardiner, 2008. Genome mapping of three major resistance genes to woolly apple aphid (Eriosoma lanigerum Hausm.). Tree Genetics and Genomes 4:233-236

Previous work:

Resistance of Rootstock Selections to a Washington Strain of Woolly Apple Aphid

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Keywords: woolly apple aphid, *Eriosoma lanigerum*, host plant resistance, Robusta 5 gene

Abstract: Ten rootstock selections were tested for their ability to host woolly apple aphid aerial colonies. Differences among the various rootstocks were apparent within a few weeks of artificial infestation. After 4 wk, the susceptible rootstocks (including M.9, M.26, Bud 9, Bud 118, and seedlings from New York and Washington) were heavily infested. On MM.111 (whose resistance is derived from 'Northern Spy'), colonies established successfully, but were small and poorly developed. The majority of the replicates of the Geneva 'Robusta 5' derived resistant rootstocks (G.202, G41, and 4210) were free from infestation; but some replicates had a few very small colonies.

Host plant resistance is a little used tactic in tree fruit pest management, and the long-known resistance of certain rootstocks to woolly apple aphid is one of the few examples. This resistance is based on a naturally occurring resistance in the apple cultivar 'Northern Spy'. The characteristic was incorporated into the Malling-Merton 100 series of rootstocks in the 1920s, and these stocks were widely planted for this reason. Two phenomena occurred to effectively curtail their use. First, new rootstocks with more favorable characteristics in terms of precocity, productivity and size control were introduced. These included the Malling series (of which M.9 and M.26 have been widely planted in Washington), and the Budagovsky series. Both these series are susceptible to woolly apple aphid. The second phenomenon was that biotypes capable of overcoming the 'Northern Spy' based resistance were discovered in three areas of the world (Gilliomee 1968, Sen Gupta and Miles 1975, Klimstra and Rock 1985).

More recently, a new line of woolly apple aphid-resistant rootstocks have been introduced from the apple rootstock breeding program at Cornell's Geneva Experiment Station (Robinson et al. 2003). This resistance is based on a *Malus × robusta* selection known as 'Robusta 5'. This parent also confers a degree of fireblight resistance, and has been widely used in the Geneva program.

The objectives of this test were twofold: 1) to determine if a Washington strain of woolly apple aphid had overcome the 'Northern Spy'-based resistance, and 2) to confirm the 'Robusta 5' based resistance in our area.

Materials and Methods

Apple rootstock liners, from ¼ to ⅜ inch diameter, were planted in a soil mixture of equal parts peat, perlite, and vermiculite on 21 April. Ten rootstock types were used: The Geneva line 4210, Geneva 41, Geneva 202, Bud 9, Bud 118, M.9, M.26, MM.111, seedlings from Washington

(Willow Drive Nursery), and seedlings from New York. Ten of each type were planted. Trees were infested about one month after planting, when new shoot growth was approximately 6 cm.

Parentage of rootstocks

Rootstock	Parentage
G.41	M.27 × Robusta 5
G.202	M.27 × Robusta 5
4210	O.3 × Robusta 5
MM.111	N. Spy × Merton 793
M.9	Juane de Metz clones
M.26	M.16 × M.9
B.9	M.8 × Red Standard
B.118	Moscow pear × M.9 or M.8

Insects were collected from Mountain View Orchard, East Wenatchee. Stem sections 4-6 cm long, each with 50-200 aphids, were placed at the base of each tree on 19 May (Plate 0625.1). First instars were seen on the trees the next day. Fresh stem sections were collected on 22 May and placed on any trees that appeared to have a low number of first instars. This included all of Geneva 41, Geneva 202, 4210, and about half of the other trees. Trees were arranged on a greenhouse bench in a randomized complete block design (10 types × 10 reps) (Plate 0625.2).

Aphids had matured by 8 June and had begun to produce new first instars. Aphid densities on aerial parts of the tree were evaluated on 16 June. Two types of evaluations were performed: 1) a numerical rating system and 2) digital estimation.

Rating system: Tree were rated on scale of 0 to 4, where 0=no colonies; 1=few, small colonies; 2=few, normal colonies; 3 = heavily infested; 4 = very heavily infested with a large volume of waxy filaments.

Root evaluation. Evaluations of the root systems were performed on 14 July. Root systems were rated as infested or not infested, and with or without galls. Results are given as a percentage of the replicates which were positive for either infestation or galls.

Results and Discussion

All of the Geneva rootstocks (G.41, G.202 and 4210) were virtually immune to woolly apple aphid (Table 0625.1, Fig. 0625.1; Plate 0625.3). Only a few of the replicates had any colonies established, and those consisted of only a few aphids. By comparison, at the same point in time, the Malling, Budagovsky, and seedling rootstocks were highly infested (Plates 0625.4. 7). The MM.111 was intermediate between the two extremes (compare Plates 0625.5 and 6), however, colonies were able to establish on most replicates, but grew very slowly by comparison, never reaching the level of infestation of the susceptible rootstocks. Apparently, this is typical of the MM rootstocks, in that the resistance was never complete, but rather expressed as a marked degree of antixenosis. Based on this, there is no evidence that the Washington strain of woolly apple aphid has changed its ability to infest this resistant series over time.

Because the rootstocks in the trial were either clonally propagated or seedlings, their phenotypic expression of resistance was throughout the tree (roots and shoots). Not surprisingly, then the degree of infestation and galling on the roots mirrored that of the shoots. Neither the Geneva

rootstocks nor MM.111 had any root infestation or evidence of galls by the end of the test (56 d after initial inoculation). The susceptible rootstocks had 60-100% of the replicates with root infestation, and 10-70% had evidence of gall formation. Given the high pressure of this test, the percentages of both categories would have been higher given sufficient time. However, the trees were nearly dead at the time of root evaluation, so continuing it was not feasible.

Literature Cited

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EXECUTIVE SUMMARY

We have successfully classified rootstocks G.41, G214, G.210, G.87, G.202 as resistant to the wooly apple aphid by direct plant assays and by molecular methods. We have confirmed the susceptibility of G.935, CG.4011, CG.4088, CG.2006, CG.3902 and controls M.9, M.26, B.9 by direct plant assays and by molecular methods. Rootstocks G.890 and G.969 although exhibited the correct configuration of molecular markers for resistance were not able to be classified – the test was inconclusive in the current greenhouse test because insufficient data. Rootstock G.4814 did not possess the molecular markers for resistance and its greenhouse test was also inconclusive. Rootstock CG.2406 was also classified as inconclusive in the greenhouse test and because of a very different pedigree was not able to be classified correctly using molecular markers possessing new, unique marker types; however if classified as resistant in future studies this rootstock may be used as a novel source of resistance for future crosses aimed at combining genes for durable resistance. The 'Northern Spy' resistance to wooly apple aphid in the MM series of rootstocks was of an incomplete type when compared to the 'Robusta 5' resistance, which in the case of G.41 and G.202 and other resistant Geneva rootstocks was described as virtual immunity. This experiment has been very useful for the classification of new rootstocks. The planting rootstocks possessing a high level of resistance to the wooly apple aphid may provide the benefit of eliminating areal colonies altogether as aphids may not be able to overwinter in the root zone. This feature may be very important in organic production. Added benefits from this study are the possibility of identifying the genes/products that provide a natural barrier to wooly apple aphids – synthesizing these products and using them as more eco-friendly treatments on susceptible orchard canopies.

FINAL PROJECT REPORT

Project Title: Integrated biological control of woolly apple aphid

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Other funding sources

Agency Name: Washington State Commission on Pesticide Registration

Amount awarded: 14,338 (2010); 14,792 (2009); $12,948 (2008) (total: $42,078)

Notes: Two WSU-BioAg grants were successfully funded and completed in a supporting research area (attraction of cover crops to syrphids, important predators of woolly apple aphid), one in 2008 for $10,934, and another in 2010 for $13,539 (total $23,933). The large plot field work was enhanced by the addition of funding from the SCRI grant "Enhancing biological control to stabilize western orchard IPM systems" (Jones et al. 2008; total $2,244,274).

Total Project Funding: **Year 1:** $38,238 **Year 2:** $33,962 **Year 3:** $36,435

Budget History:

Item	2008	2009	2010
Salaries	11,007	11,448	11,448
Benefits	941	954	955
Wages	19,432	15,560	20,180
Benefits	1,858	2,178	1,917
Equipment	0	0	0
Supplies	2,000	1,000	1,000
Travel	3,000	2,822	935
Miscellaneous	0	0	0
Total	**$38,238**	**$33,962**	**$36,435**

Objectives

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

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.
- *A. mali* alone caused only a minor decrease in woolly apple aphid populations; when both predators and *A. mali* were present, woolly apple aphid populations were controlled.
- Delegate, Guthion, Lorsban, Entrust, and Sevin caused high levels of contact mortality of *Aphelinus mali*; Rimon, Warrior, Assail and Calypso were intermediate, while Altacor, cyazypyr, Kumulus, Kocide+Manzate, and Ultor were nontoxic. Rimon had no deleterious sublethal effects on *A. mali.*
- Both Rimon and Delegate appear to exacerbate woolly apple aphid populations in large-scale trials when used during the first codling moth generation; the effect is worsened when the two were used together. The effect occurred both in a block with a history of woolly apple aphid problems, and one with no prior history. The same pattern occurred where the same treatments were used two years in a row, except the mid-season outbreak was higher than the previous year. Intrepid/Altacor caused the fewest secondary pest outbreaks.

Results and Discussion

Obj. 1. Woolly apple aphid natural enemy survey. Aerial colonies of woolly apple aphid and its natural enemies (NE) were monitored in 20 orchards in 2008. The colonies appeared in May, with one or two population peaks in summer and fall, respectively (Fig. 1).

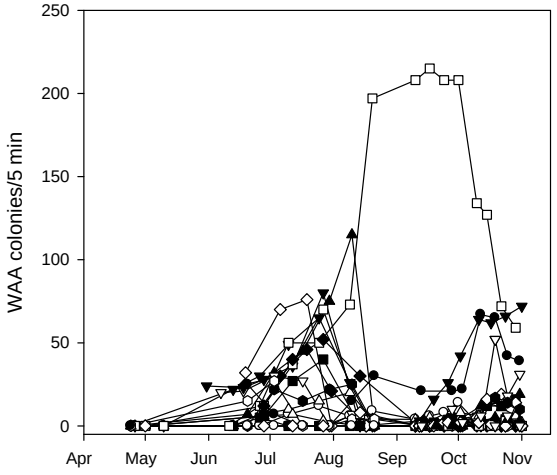

Fig. 1. Seasonal phenology of woolly apple aphid in eastern Washington, 2008.

The only parasitoid found in the colony samples was *Aphelinus mali*. Parasitism fluctuated considerably throughout the season with no apparent pattern.

The predator complex consisted of lacewings, lady beetles, syrphids, predatory bugs, and spiders. Overall, syrphids were the most abundant predator observed in our samples, which corresponds to previous studies in 2006 and 2007 (Fig. 2). All but one of the syrphid species identified from the 2008 study was *Eupeodes americanus* Wiedenmann; a single specimen of *Heringia calcarata* Loew was found at

an organic orchard in the Columbia Basin on July 1. The syrphid species, a specialist on woolly apple aphid, was not known to occur in Washington prior to this.

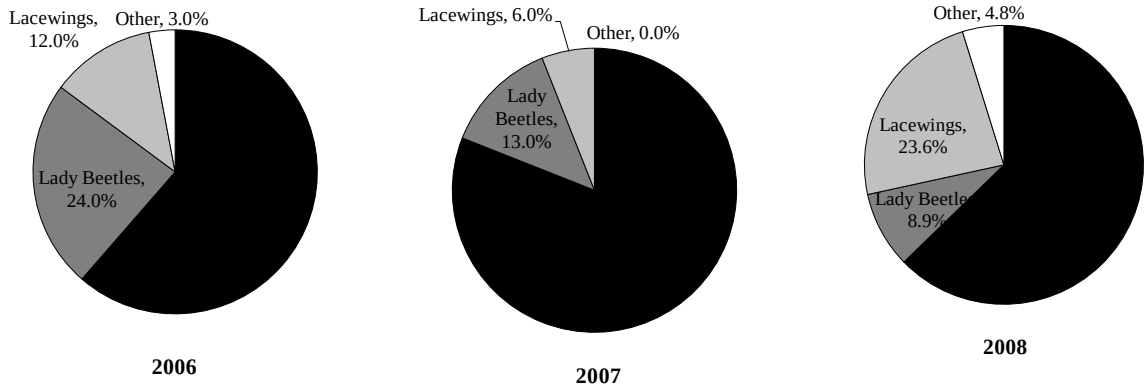

Fig. 2. Composition of woolly apple aphid predator complex, eastern Washington, 2008 (black sections are syrphids).

Obj. 2. Relative impact of the predators and parasitoids. The relative impact of predators and the parasitoid *A. mali* was demonstrated in exclusion cage studies in 2010. When both predators and parasitoids were excluded, woolly apple aphid populations continued to increase on the test trees (Fig. 3a, b). When parasitoids were allowed to attack the aphid colonies (but predators were excluded), the aphid populations increased, but at a slower rate than aphids alone. However, when both predators and parasitoids were allowed to attack the colonies, population either failed to increase (Fig. 3a) or increased slightly before declining (Fig. 3b).

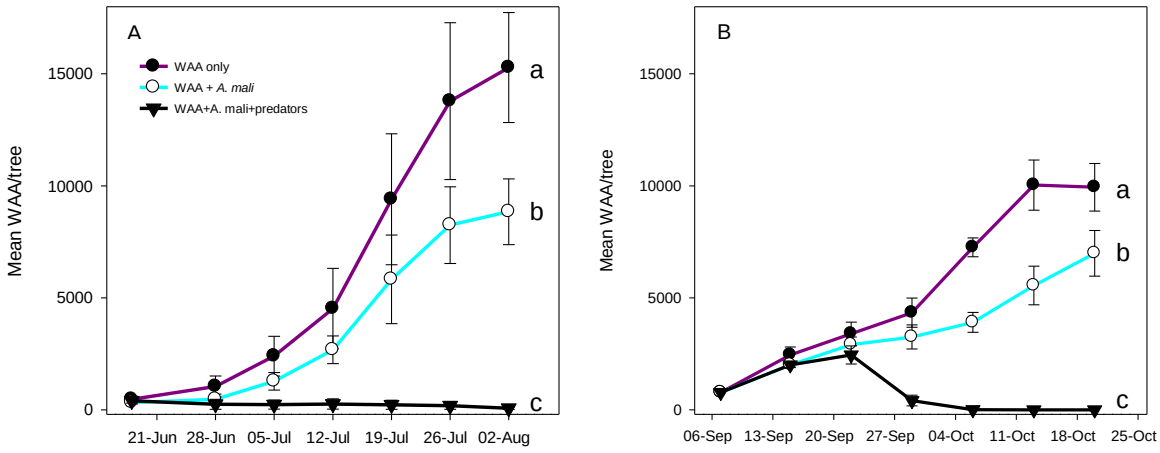

Fig. 3. Exclusion cage studies examining the effect of predators and parasitoids on population growth of woolly apple aphid. A – mid-summer; B – fall.

Obj. 3a. Laboratory tests of acute toxicity of orchard pesticides to *A. mali*. The organophosphate (Guthion, Lorsban) and carbamate (Sevin) insecticides were highly toxic to *A. mali*. Of the newer insecticides, Entrust and Delegate (spinosyns) were equally toxic (that is, close to 100% mortality at both the 1x and 0.1x rates). The neonicotinoids (Assail, Calypso) and the pyrethroid (Warrior II) were moderately to highly toxic (60-90% mortality at the 1x rate). Altacor and Cyazypyr (diamides) and Rimon (a benzoylurea chitin synthesis inhibitor) were moderately toxic (33-43% mortality at the 1x

rate). The fungicides (Kumulus, Kocide+Manzate) and Ultor (a tetramic acid derivative) were non-toxic to *A. mali* on contact.

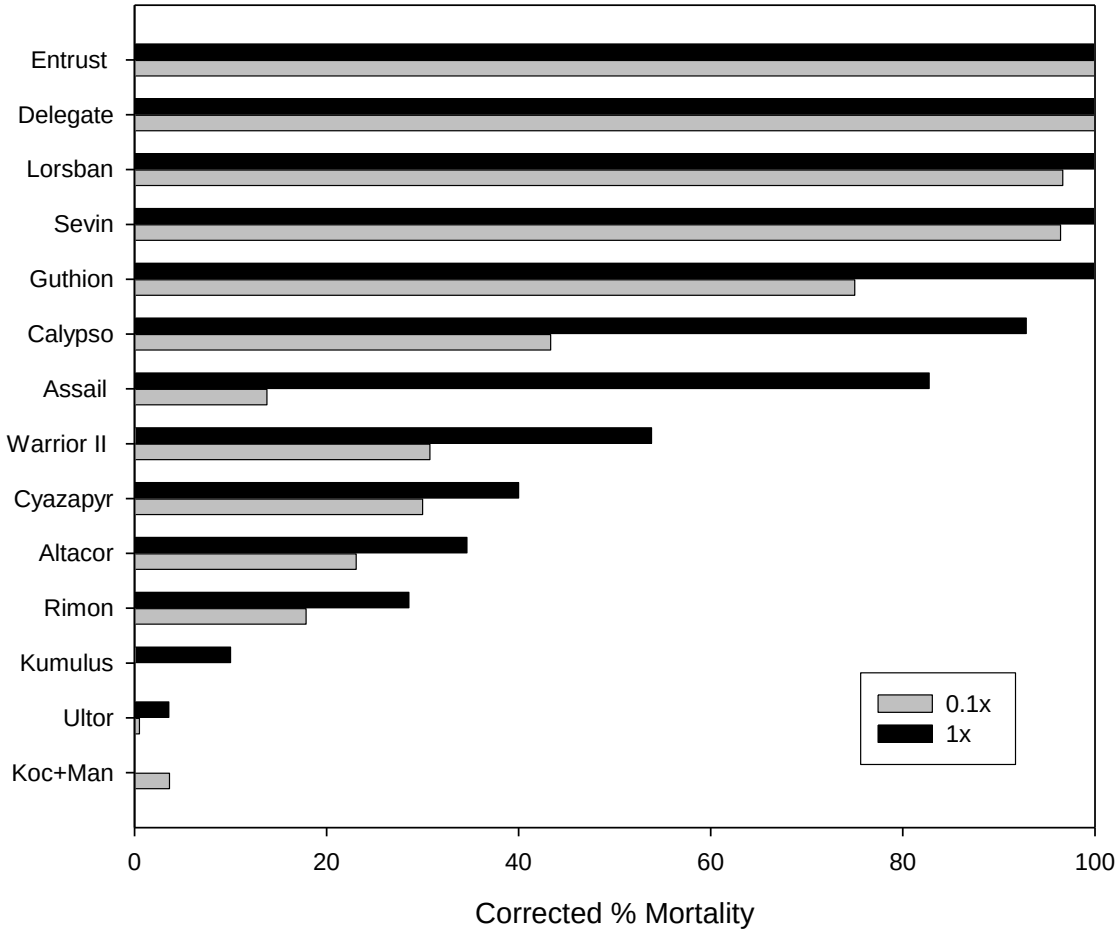

Fig. 4. Acute toxicity of the maximum label rate (1x) and 1/10 the maximum rate of various orchard pesticides on *A. mali* (Abbott's corrected mortality, 48 h).

Sublethal effects of pesticides are also under investigation; only the Rimon bioassay has been completed to date. Rimon reduced female *A. mali* fecundity and survivorship when compared to the control (Table 1). However, *A. mali* treated with Rimon produced F_1 offspring with a higher proportion of females (M:F, 0.40:0.60), which is likely why the estimate of the net reproductive rate estimate (R_0) was slightly higher for *A. mali* treated with Rimon. The higher number of females in the F_1 offspring may compensate for the reduced fecundity and survivorship of the Rimon-treated *A. mali*.

Table 1. Sublethal effects of novaluron (Rimon) on *A. mali* fecundity, sex ratio and net reproduction

Pesticide	Mean daily fecundity	% Mortality (Day 4)	Proportion females	R_0
Control	3.32	12.90	0.50	1.35
Rimon	2.90	24.24	0.60	1.55

Obj. 3b. Effect of seasonal program on populations of woolly apple aphid and its natural enemies. Large-scale tests of pesticide programs were conducted in two commercial orchards in central Washington, near Bridgeport ('Cameo') and Othello ('Delicious'). The blocks were 20-25 acres in size, with four treatments and four replicates of 1-1.5 acres in size. The treatments consisted of a petal fall (100 degree-days), delayed 1st and 2nd cover codling moth sprays (Table 1).

Table 2. Codling moth programs tested for effects on secondary pests and natural enemies

Trt. #	Treatment[1] (PF, 1st cover, 2nd cover)	Rate/acre
1	Rimon-Delegate-Delegate	32 fl oz, 7 oz, 7 oz
2	Intrepid-Delegate-Delegate	16 fl oz, 7 oz, 7 oz
3	Rimon-Altacor-Altacor	32 fl oz, 4.5 oz, 4.5 oz
4	Intrepid-Altacor-Altacor	16 fl oz, 4.5 oz, 4.5 oz

[1]Applications were made with an airblast sprayer calibrated to deliver 100 gpa.

Woolly apple aphid, green apple aphid, and spider mites were monitored from April through November, 2009, along with a broad range of natural enemies (lady beetles, syrphids, earwigs, spiders, and predatory mites). Samples were taken at 1-3 week intervals. Sample types included timed counts (both aphid species), leaf samples (mites and their predators), tap samples (various natural enemies), cardboard band traps (earwigs and spiders), and HIPV (herbivore-induced plant volatiles) attractant traps (lacewings, syrphids).

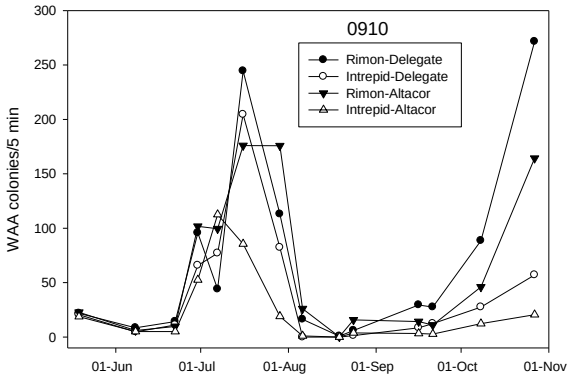

Fig. 5a. Woolly apple aphid populations, Bridgeport, 2009.

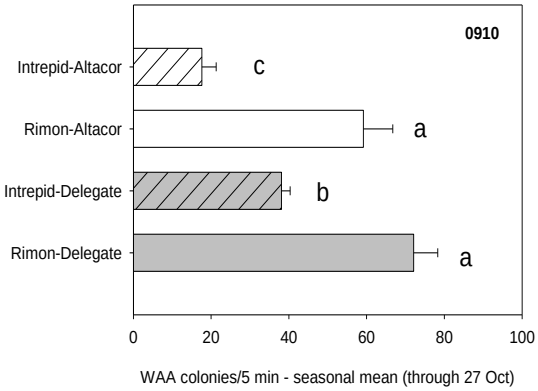

Fig. 5b. Seasonal means of woolly apple aphid timed colony counts, Bridgeport, 2009.

Bridgeport. Woolly apple aphid pressure was high in the Bridgeport plot, peaking in late July, and again in October (Fig. 5a). Significant treatment differences appeared by late June, with the Intrepid-Altacor treatment having consistently the lowest aphid densities. The highest densities occurred in the Rimon-Delegate plot, followed by the Rimon-Altacor treatment and the Intrepid-Delegate. Rimon appears to have the stronger disruptive effect on woolly apple aphid, despite the fact that it was only applied a single time at petal fall (PF). Delegate also appears to be disruptive, but somewhat less so than Rimon; when paired with Intrepid at PF, the seasonal mean aphid densities were significantly higher than when Intrepid was paired with Altacor (Fig. 5b).

Spider mite populations were moderately high in this block, peaking in late July-early August. While even the lowest treatment (Intrepid-Altacor) experienced some elevation of spider mites (ca. 11

mites/leaf), the maximum populations in the two treatments containing Rimon were significantly higher than the Intrepid-Altacor treatment on 28 July, with the Intrepid-Delegate treatment intermediate. However, this trend was reversed on the secondary peak in late August, although this may have been due to poorer leaf quality from prior mite damage. Because of this reversal, no significant differences were found in the seasonal cumulative mite days.

Earwigs were abundant in the Bridgeport plot, with densities caught in traps rising throughout the growing season, peaking in mid-September (Fig. 6a). There were clear differences among treatments by mid-June, and these differences persisted into fall. The Altacor-Intrepid treatment had the highest numbers of earwigs, while the Rimon-Delegate treatment had the fewest. Parallel to the woolly apple aphid results, Rimon and Delegate appear to be detrimental to earwigs, while Intrepid and Altacor are not (Fig. 6b). While these results are only correlations, they raise interesting questions about the role of earwigs as woolly apple aphid predators.

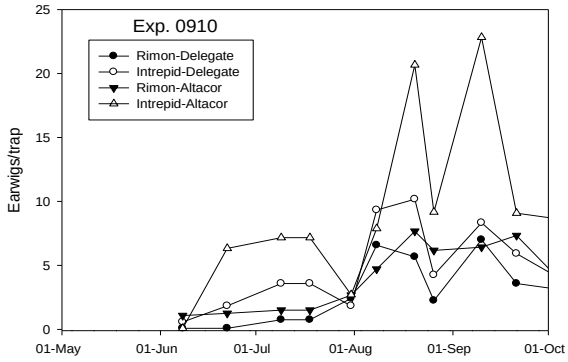

Fig. 6a. Earwig densities over time, Bridgeport, 2009.

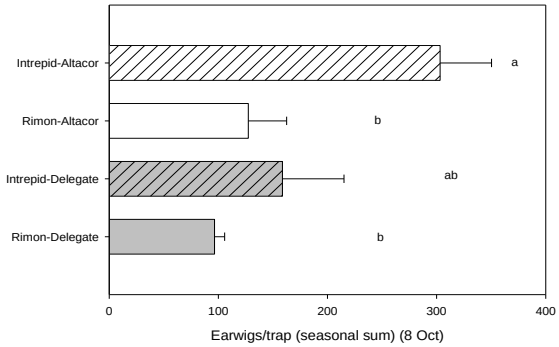

Fig. 6b. Seasonal sums of earwigs caught in traps, Bridgeport, 2009.

The HIPV traps caught large numbers of lacewings (methyl salycilate+benzaldehyde) and syrphids (geraniol) throughout the season (data not shown). There were two distinct peaks in lacewing activity, one in early July, and the second in mid-August (Fig. 7a). There was also a treatment effect in lacewing captures, with an indication that Altacor may suppress lacewing populations (Fig. 7b). Although these insects are also important woolly apple aphid predators, there was no indication that this was the primary reason for of disruption.

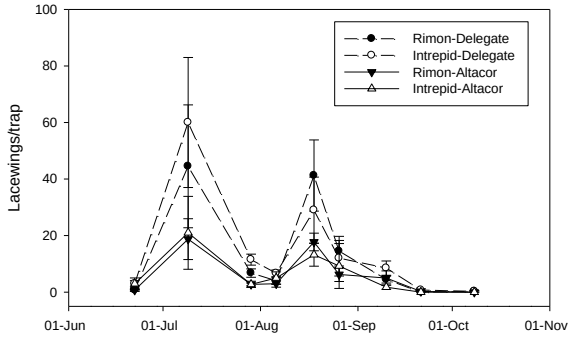

Fig. 7a. Lacewings caught in HIPV-baited Delta traps throughout the season, 2009.

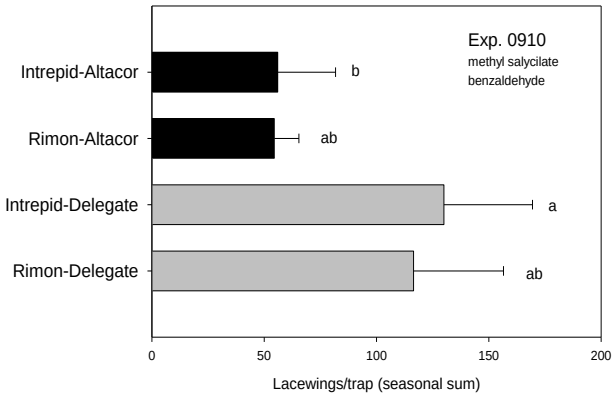

Fig. 7b. Seasonal sum of lacewing captures in Delta traps, Bridgeport, 2009.

Othello 2009. Woolly apple aphid densities were relatively low throughout the summer, but began increasing in early September, and peaking in October (Fig. 8a). Treatment effects were similar to the Bridgeport plot, in that the Rimon-Delegate treatment had significantly higher aphid densities than the other treatments. The Intrepid-Altacor treatment had the lowest levels, with the other two treatments intermediate. In this block, Delegate appeared to be slightly more disruptive than Rimon (based on the Rimon-Altacor treatment) (Fig. 8b).

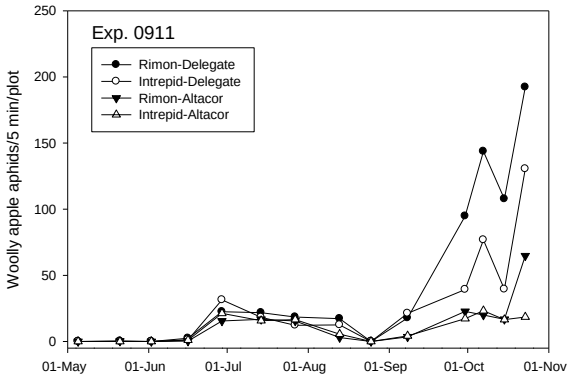

Fig. 8a. Woolly apple aphid densities, Othello, 2009.

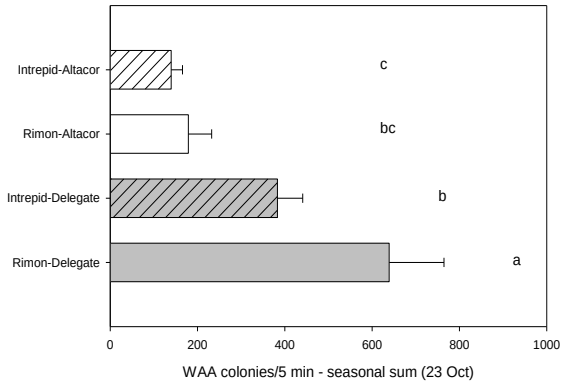

Fig. 8b. Seasonal sums of woolly apple aphid colonies, Othello, 2009.

Earwigs and spider mite densities were very low in this plot throughout the season (data not shown); thus it is difficult to ascribe strong integrated mite control disruption to any of the products tested. Similarly, since the treatment effects on woolly apple aphid were the same in the absence of earwigs, it suggests that other mechanisms may be as much, if not more, important in the outbreak of woolly apple aphid. The other notable result from the Othello site was the trend in rust mite populations (data not shown). Both of the treatments containing Rimon had significantly depressed rust mite counts, which may contribute to disruption of integrated mite control.

Othello 2010. The pattern of WAA densities in 2010 was similar to 2009 (mid- and late-season peak) except that the mid-season peak was later than 2009 (late July instead of early July) (Fig. 9a); this may be due in part to the long, cold spring in 2010, with a substantial delay in insect and tree phenology. The most surprising aspect of this experiment was that the treatments behaved in a very similar fashion to 2009, with Rimon-Delegate having the highest populations, Intrepid-Altacor having the lowest, with the other two treatments (variations on the aforementioned materials) intermediate (Fig. 9b). Also like 2009, the treatment separation occurred in the late-season peak, months after the first generation CM sprays were applied.

Unlike 2009, there was a substantial mid-season peak of tetranychids in all treatments (Fig. 10a). In general, Intrepid-Altacor had the lowest mite levels (not exceeding five mites/leaf, although (surprisingly) there were no significant differences in the tetranychid CMDs, Fig. 10b). Predator populations were the same or higher than the pest mites, and the counts are among the highest I have ever recorded. Rust mites about the same as in 2009, with a typical late June peak.

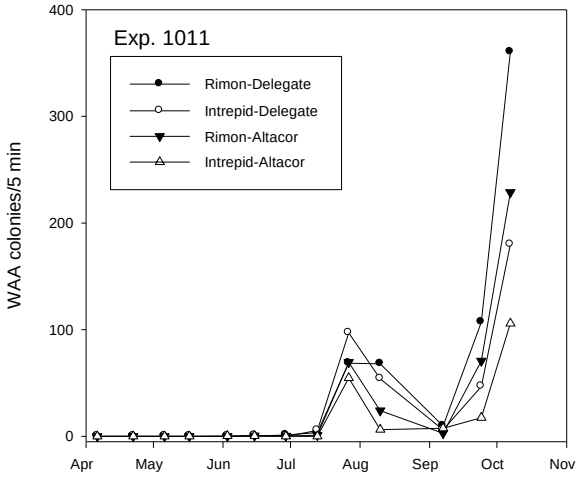

Fig. 9a. Woolly apple aphid densities, Othello, 2010.

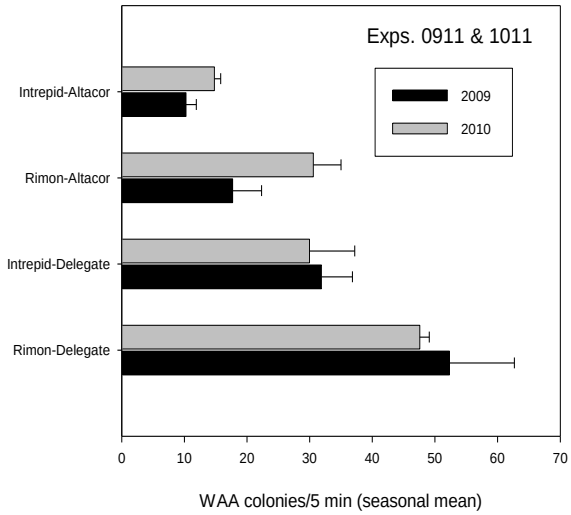

Fig. 9b. Seasonal means of woolly apple aphid colonies, Othello, 2009 vs 2010.

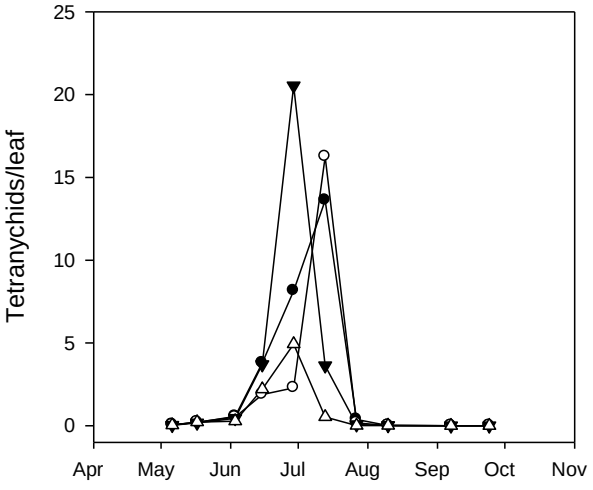

Fig. 10a. Mite densities, Othello, 2010.

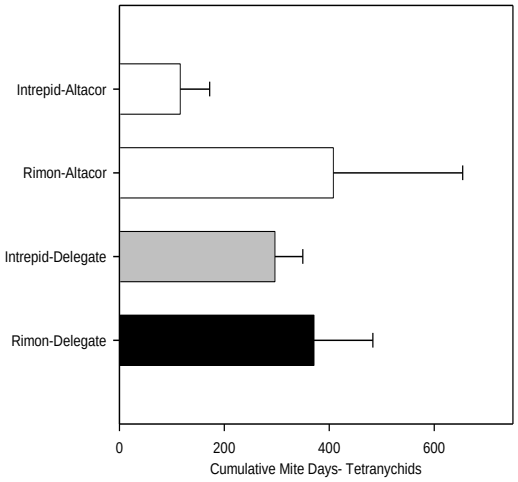

Fig. 10b. Seasonal CMDs, Othello, 2010.

Othello 2010 #2. This experiment was the first year for these treatments (Table 3). The plots were in the same block as 1011 (Othello, Bench Rd.), but 32 rows to the west. The reasoning for the treatments was to 1) take Rimon out of the PF program because it is too disruptive (replaced with all Intrepid), and re-test first generation Altacor and Delegate; 2) use Guthion as a "mite-neutral" comparison, which may also provide direct control of WAA (almost last chance before phase out!); 3) test Warrior as another possible OP replacement. All the treatments were applied first generation only; and were preceded by an oil/Lorsban in the delayed dormant. Guthion was sprayed over the entire plot second generation by the grower in response to codling moth pressure.

Table 3. Treatment schedule for large plot test, Othello #2, 2010

Trt #	Treatment (DD, PF, 1C, 2C)	Rate/acre
1	Oil+Lorsban, Intrepid, Delegate, Delegate	1.5%+4 pt, 1 pt, 7 oz, 7 oz
2	Oil+Lorsban, Intrepid, Altacor, Altacor	1.5%+4 pt, 1 pt, 4.5 oz, 4.5 oz
3	Oil+Lorsban, Intrepid, Warrior II, Warrior II	1.5%+4 pt, 1 pt, 2.56 fl oz, 2.56 fl oz
4	Oil+Lorsban, Intrepid, Guthion, Guthion	1.5%+4 pt, 1 pt, 2 lb, 2 lb

This plot produced one of the highest mid-season peaks I have yet recorded in a WAA plot, >1,000 colonies/5 min (Fig. 11a). The high populations occurred in the Delegate and Guthion plots, the Altacor was intermediate (Fig. 11b), and the lowest populations occurred in the Warrior plots. The latter comes as a surprise, since it argues that Warrior provides direct control of WAA. Another surprise is that Guthion appeared to be disruptive, and with apparently no direct toxicity; I had always assumed that all the OPs controlled/suppressed WAA, but perhaps it was really just Lorsban and Penncap). In addition to the mid-season peak, there was a secondary (fall) peak starting late September.

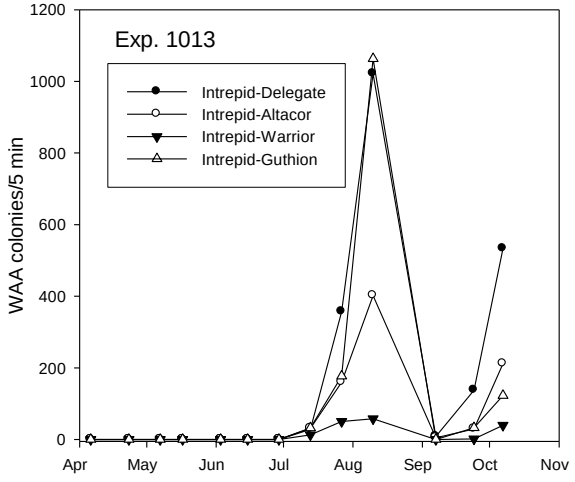

Fig. 11a. Woolly apple aphid populations, Othello #2, 2010.

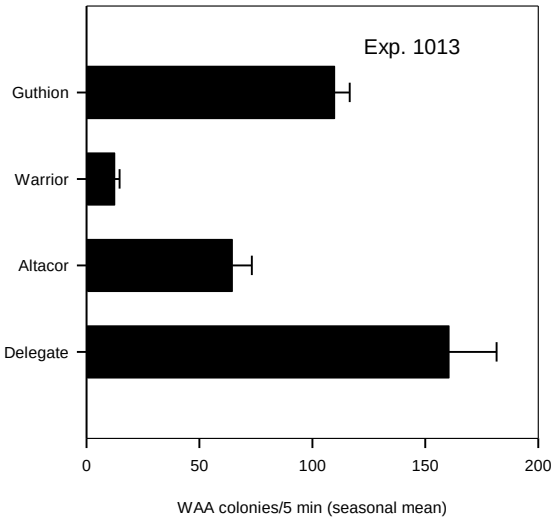

Fig. 11b. Woolly apple aphid populations seasonal mean, Othello #2, 2010.

A mid-season mite outbreak occurred in all treatments, but to a lesser extent in the Intrepid-Altacor plot (data not shown). The mite population peak was tracked by the predators responding to the availability of prey. The highest predator levels occurred in the Delegate treatment, indicating that the first generation applications did not permanently prevent predator populations from developing. The most anomalous result was that the same peak occurred in the "mite-neutral" Guthion treatment, which normally causes few mite problems. For that matter, I did not necessarily expect the peak in the Warrior treatment; this material is somewhat miticidal. In addition, the rebound of predators in the Warrior treatment is a little surprising given how toxic it is in laboratory tests.

Integrated Biological Control of Woolly Apple Aphid
Executive Summary

This project provides an assessment of three key components of biological control of an important secondary pest. The first task was to discover and quantify the natural enemy complex of woolly apple aphid, which had not been done to any extent in the past. The second task was to determine the importance of the natural enemies, regardless of their abundance. Lastly, this information needed to be tested in the context of commercial orchards with routine pesticide applications, to see if pesticides were an important factor in disrupting biological control, and how that could be minimized. This systematic approach to biological control gives eastern Washington growers specific, science-based information to better manage this pest.

The first step was the quantification of natural enemies in our area – the basis for future decision-making as to which natural enemies were most critical to conserve for woolly apple aphid control. The 2008 survey was an extension of previous limited surveys done in 2006-2007, except that the number of orchards surveyed increased from 3 (in 2006-07) to 20 in 2008, making this information robust for eastern Washington. *Aphelinus mali* was the only parasitoid found in any year or orchard. Syrphids were the most commonly found predators in colonies, but lacewings and lady beetles were also found regularly. Coincident with the predator information, colony numbers were also assessed during the survey, establishing the seasonal phenology for this pest over a broad area. Most of the orchards sampled had either one or two peaks of populations, one in mid-summer, and one in fall.

The exclusion cage studies gave a clear indication that *A. mali* can reduce populations in mid-summer and fall to an extent, but cannot prevent them from increasing steadily. However, when predators were allowed to attack the colonies along with *A. mali*, the aphid populations increased only briefly, or not at all, before being driven to low levels. Where predators were allowed access, few parasitoids were recovered, probably because parasitized aphids were consumed indiscriminately along with non-parasitized ones. Thus, the choice of selective pesticides to minimize disruption should favor.

The acute toxicity bioassays of *A. mali* indicated that a number of materials, both older and newer compounds, caused high levels of adult mortality. These included Guthion, Lorsban, Sevin, Entrust and Delegate. A number of codling moth materials, Assail, Calypso, Warrior, Altacor, cyazypyr, and Rimon caused only moderate mortality, while Kumulus sulfur, Kocide+Manzate and Ultor were non-toxic in this type of exposure. Susceptibility of the lacewings and coccinellids is currently under investigation by a regional coalition of researchers working on a SCRI grant, and results should be available in about a year. Unfortunately, the key predator (syrphids) is not currently being tested, largely because they do not lend themselves to rearing; this is an information gap that needs to be addressed. In addition, the sublethal effects of pesticides on *A. mali* are in progress, with an estimated completion date of August 2011.

The large block tests are a report card on how these pesticides affect the natural enemy-pest system in the real world. In orchards with a history of woolly apple aphid pressure, Rimon and Delegate consistently caused an increase in woolly apple aphid populations, as well as tetranychid mites. Most surprising were the far-reaching effects from early season applications (petal fall through second cover). Another interesting insight is that the mechanism of disruption can vary depending on the orchard's underlying natural enemy complex; in an orchard where earwigs were abundant, the deleterious effect of Rimon was pronounced. This finding points out the strengths and weakness of different sampling methods; earwigs did not show up in predator surveys because they are primarily nocturnal feeders. Another interesting finding was the low levels of woolly apple aphid in the Warrior blocks indicating some level of direct control; this also merits further investigation. Based on multiple large-plot tests, it appears that Intrepid (PF) and Altacor (first generation CM) is the least disruptive combination to woolly apple aphid. This corresponds to a low toxicity rating in bioassays of acute and sublethal toxicity.