2008 Apple Crop Protection Research Review
January 31-February 1
Holiday Inn Express at TRAC
Pasco, Washington

Thursday, January 31, 2008

Time	Page	PI	Title	Funding period
8:00		Hanrahan	Introduction and update	
			Final Reports	
8:15	1	Knight	Developing ULV microencapsulated sex pheromones for CM control (extension)	05-06
8:30	5	Knight	Direct control of CM with formulations of the pear ester (extension)	05-06
8:45	9	Brunner	Codling moth management with pheromones: key unanswered questions	05-07
9:15	20	Brunner	Sustainable management of leafrollers in apple orchards	05-07
9:30	30	Beers	Reinstating integrated mite control in apple orchards	06-08
9:45			**Break**	
10:00	39	Jones	Improving apple IPM by maximizing opportunities for biological control	07-09
10:30	49	Unruh	CSI in the orchard: finding the killers of 4 key apple pests (extension)	06
10:45	56	Yee	Apple maggot host attractants	07
11:00	63	Yee	DNA and morphometric diagnostics for apple and snowberry maggot flies	07
Group			**Poster Session Continuing Reports 11:15am-12:15pm**	
1	72	Xiao	Decay control and management of fungicide resistance (extension)	07
1	75	Campbell	Augmenting fungal control in apples with natural compounds	07-09
1	82	Jones	Interaction of dispersal and management of CM and OBLR	07-09
2	87	Landolt	Sprayable foam for trap and kill of cocooning codling moth larvae	06-08
2	91	Neven	Fate of codling moth in apples after harvest	07-08
2	95	Garczynski	Molecular characterization of taste, smell and feeding in codling moth	07-09
2	100	Garczynski	Identification of Bt toxin targets in codling moth larvae (extension)	07-09
12:30			**Committee lunch/project discussion 12:30pm - 2:00pm**	
			Final Reports	
2:00	102	Mazzola	Employing biological elements of orchard ecosystems	05-07
2:15	112	Mazzola	DNA microarrays for monitoring orchard soil microbial communities	07

FINAL PROJECT REPORT

Project Title: ULV microencapsulated sex pheromones for codling moth

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Cooperators: Rick Hilton, Phil Van Buskirk, Doug Light, Tom Larsen, and Bill Lingren

Other funding Sources

Agency Name: Suterra LLC
Amount awarded: $20,000
Notes:

Total Project Funding: $56,000

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007 (extension)
Salaries	14,000	14,000	0
Benefits	2,250	2,250	0
Wages	6,000	6,000	0
Benefits	1,000	1,000	0
Equipment	1,000	1,000	0
Supplies	2,000	2,000	0
Travel	1,750	1,750	0
Miscellaneous	0	0	0
Total	**28,000**	**28,000**	**0**

Significant findings

2005:

- A season-long ULV pheromone spray program (4 – 6 sprays) was as effective as the use of 300 Isomate-C PLUS™ in apple or 200 Isomate-C tt™ dispensers in pear. Overhead irrigation did not reduce the effectiveness of the ULV spray program. However, moth catch was significantly higher early in the season in overhead-irrigated ULV-treated orchards.
- Addition of pear ester MEC at 0.25% did not improve the effectiveness of either the pheromone or pheromone plus Asana ULV sprays at mid-season.
- A 6-spray ULV program with Asana was highly effective for CM management. The addition of sex pheromone did not improve this program. Cutting the rate of Asana to 3 oz per acre appeared to be effective.
- Residues of Asana were all below the accepted 2.0 ppm threshold at harvest in blocks treated with six applications of the 6 oz rate.
- The effectiveness of the Asana program was likely due to both lethal and sublethal effects. Asana residues (1 – 2 ppm) reduced the fecundity of moths > 95%, but did not effect mating success.
- ULV Asana sprays flared mites, but levels were moderate and mite predators increased late in the season.
- Four ULV applications of Assail or Intrepid plus pheromone did not significantly reduced moth catches or fruit injury compared with pheromone alone or no pheromone in grower orchards. Pest mites were not disrupted in these blocks.
- The top and bottom of ULV sprayed apple leaves remained attractive for 28 d.
- The ULV spray deposits microcapsules on the top and bottom of leaves throughout the canopy of apple orchards.
- Leaf size directly impacted the density of microcapsules deposited.
- Increasing nozzle height (smaller spray angle) increased the relative deposition of capsules on the top versus the bottom of leaves.

2006:

- The effectiveness of a 5-spray ULV Checkmate® CM-F pheromone program was similar to 400 Isomate C PLUS in replicated 10-acre apple plots.
- The addition of either Assail or Asana to a ULV pheromone 5-spray program significantly improved the effectiveness of the pheromone alone program.
- The application of insecticides alone via a low volume application significantly reduced fruit injury.
- Mite populations were low in all treatments except the air blast applications of Asana. The ratio of predator mites to two-spotted mites was higher in all ULV insecticide treatments versus the air blast treatments.
- The ULV pheromone plus Assail program was highly effective against the white apple leafhopper.
- Laboratory assays suggest that Assail and Warrior are excellent materials to use as ULV treatments for adult codling moth.
- Traps baited with artificial leaves treated with 50 – 100 microcapsules were attractive for at least 3 weeks.
- The use of a visual detector triggered by tree trunks was used to increase the clumping of capsules on leaves (PULSV).

2007:

- Adding Assail (1.7 oz/ac) to the low volume sex pheromone spray increased the number of clean fruits by 10-fold in a six-spray seasonal program.
- Reducing the rate of Assail from 1.7 to 0.85 oz/ac improved control of codling moth by 50% in a five-spray seasonal program using sex pheromones.

- Pheromone emission from microcapsules was reduced 10% in laboratory assays when they were mixed with the high rate of Assail (1.7 oz/1.25 gallons).
- The use of both Warrior (3.0 oz/ac) or Assail (1.7 oz/ac) in a pear block treated with multiple applications of kaolin clay in Medford caused mites to flare but neither rate caused any mite problems in a pear orchard in Yakima not treated with kaolin.
- Season-long applications of sex pheromone plus Warrior (3.0 oz/ac) caused spider mites to flare in one out of six commercial apple blocks.
- Thinning sprays including carbaryl were shown to be disruptive of spider mite populations in an experimental apple block, but the seasonal program of low volume sprays of Assail did not disrupt spider mites and predatory mite populations recovered to high densities.

Results and Discussion

Growers in Washington State are interested in the use of a sex pheromone formulation that can be easily applied through their standard spray equipment. Unfortunately, the microencapsulated formulations of codling moth's sex pheromone when applied with air blast sprayer technology were found to be rather short-lived and expensive to use. The development of a low volume spray approach using the GF-120 sprayer has been able to significantly improve the performance of the MEC formulation, Checkmate™ CM-F in tests conducted since 2004. The low volume and low spray pressure application deposits 6-10-fold more microcapsules than an air blast application and creates attractive point sources (leaves) throughout the canopy of the trees. We hypothesize that this approach initially creates an effective camouflage of the virgin females' signals and then is effective for several more weeks due to competitive attraction of the leaves with high numbers of microcapsules. Flight tunnel tests showed that apple leaves with large number of microcapsules can remain attractive to male codling moths for 3 to 4 weeks.

The limitations of using the low volume spray applications of the MEC formulation in a seasonal program continue to be the impact of precipitation, particularly early in the season; and its reduced effectiveness in orchards with overhead irrigation systems, and the cost of having to apply 5-6 applications to cover the entire season. Currently, the low volume applications of sprayable pheromones for codling moth are applied on nearly 3,000 acres in the western United States. The primary uses of this product have been by growers to supplement their integrated programs for codling moth both spatially and temporally. For example, growers have applied the pheromone along the borders to improve control of codling moth in this particularly difficult area within orchards. Temporally, growers have applied the sprayable pheromone at periods of peak moth flight in either generation to improve the effectiveness of the pheromone emitted from arrays of hand-applied dispensers. Also some growers apply the sprayable pheromone at the end of the season to extend the effectiveness of their programs which are based on the use of either hand-applied dispensers or aerosol puffers. Future use of the sprayable pheromone is likely to increase as growers are forced to respond to issues of insecticide drift along the borders of their orchards and insecticide residues on fruit at harvest.

The concept of adding an insecticide to the low volume pheromone spray is a useful approach for growers. Current spray programs for codling moth are focused on ovicidal and larvicidal control and generally do not target the adult stage. Interestingly, both the neonicotinyls and synthetic pyrethroid classes of insecticides have excellent activity for codling moth adults. Both classes of insecticides produce direct mortality of moths at low rates and indirect effects that strongly reduce female moth fecundity. The mobility of codling moth adults within the canopy of orchards appears to be sufficient to allow moths to contact spray residues even when they are applied at rates as low as 1.25 GPA.

Four potential problems could occur with this approach. First, we have created spider mite flare-ups in some orchards sprayed with the concentrated insecticides. With Asana we were able to avoid mite problems by reducing rates from 6.0 to 3.0 oz/ac. Similarly, parallel studies by Dr. Tom Larsen using 1.0 oz/ac of Warrior have not created spider mite problems in treated orchards. Secondly, our recent finding that a reduced rate of Assail was more effective and that the higher rate adversely impacted the microcapsules is interesting. Further studies are needed to assess the interactions of the insecticide/pheromone mixture and select the most efficacious rates. Third, the evolution of insecticide resistance by codling moth continues to be a concern for growers. Populations of codling moth in Michigan and Pennsylvania have already been reported to have developed some resistance to Warrior following growers recent, and rapid use of this product. Resistance management strategies suggest using insecticide rotation and restricting use of selected materials to only one generation per season. These approaches should also be used when implementing the low volume management program. Fourth, is the concern that concentrating insecticides increases the likelihood that residues on fruit will exceed thresholds. However, apple fruits collected in 2005 from plots treated with six sprays of Asana at 6.0 oz/1.25 GPA did not exceed the established residue limit. This likely occurred because the last spray was applied 28 d before harvest. In general, growers can avoid this problem by not using these low volume concentrated sprays late in the season and by further reductions in the rates of insecticides applied.

At present, it is not clear which insecticides can be applied as low volume concentrated sprays from the ground. Currently, Warrior is the only material that growers have used in commercial orchards; however, the label for Calypso does not restrict applications based on a minimum water volume. In addition I am working with the manufacturers of Assail to further evaluate the potential for this approach with their product. Some of these problems can be resolved by increasing the spray volumes applied from 2.5 to 5 GPA. For example, this dilution may reduce the impact of the insecticide on the pheromone material and would likely improve coverage. Furthermore, when low volume sprays are only applied along the borders this increase will still allow large orchards to be easily treated with an ATV carrying a small spray tank.

FINAL PROJECT REPORT

Project Title: Direct control of codling moth with pear ester

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Other funding Sources None
Agency Name:
Amount awarded:
Notes:

Total Project Funding: $24,000

Budget History:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007 (extension)
Salaries	0	0	0
Benefits	0	0	0
Wages	8,200	8,200	0
Benefits	1,300	1,300	0
Equipment	0	0	0
Supplies	1,700	1,700	0
Travel	800	800	0
Miscellaneous	0	0	0
Total	**12,000**	**12,000**	**0**

Significant findings

2005

- ❖ Trécé Inc. formulated pear ester as a 5.0% A.I. microencapsulated product (Cidetrak® DA-MEC).
- ❖ Application of the Cidetrak DA-MEC at rates from 0.001 – 1.0% did not effect the distribution of codling moth eggs relative to apple fruit.
- ❖ The distribution of codling moth eggs on shoots treated with 0.001 – 0.01% DA MEC was not affected on four apple cultivars.
- ❖ Spraying apple fruit clusters with a 1.0% Cidetrak DA-MEC solution significantly increased the number of eggs laid near fruit clusters; but concentrations of 0.001 and 0.1% did not.
- ❖ The addition of 0.01 and 0.1% Cidetrak DA-MEC to a seasonal spray program of 0.5 – 1.0 lb Guthion significantly reduced fruit injury 38 – 63% at harvest.
- ❖ Concentrations of Cidetrak DA-MEC as low as 0.00001% on filter paper were attractive to neonate codling moth.
- ❖ Concentrations of Cidetrak DA-MEC $\geq$ 0.1% continued to attract neonate codling moth for at least 7 days, while lower rates were only attractive up to 3 days.
- ❖ A combo 'puzzle-piece' dispenser loaded with sex pheromone and pear ester effectively managed codling moth in its first field test conducted in a California apple orchard..
- ❖ Fruit injury was reduced from 1.4 to 0.3% in apple plots treated with 24 AKISS (**A**ttractive **K**illing **I**nterception **S**ensory **S**tations) per acre baited with a combo pheromone / pear ester lure in a California trial.

2006

- ❖ A combo 'puzzle-piece' dispenser loaded with sex pheromone and pear ester effectively shut-down traps baited with virgin female moths all season and outperformed the Checkmate dispenser late in the season.
- ❖ Traps baited with Cidetrak® CM-DA Combo dispensers did not catch moths and dispensers were seen to significantly reduce close-range moth orientation (< 20 cm).
- ❖ The Pherocon CM-DA and Pherocon CM-DA Combo lures remained effective for at least 7 weeks. The Combo outperformed the Biolure™ 10X lure in orchards treated with 400 Isomate-C PLUS™ dispensers/acre.
- ❖ AKISS performed poorly in 2006 due to short-lived residual toxicity and attractiveness.
- ❖ Moth catches were significantly lower in replicated 5-ac orchard plots treated with either the pheromone-only or combo puzzle piece dispensers than comparable blocks treated with Isomate-C PLUS. No differences in fruit injury were found in orchards treated with any dispenser type.
- ❖ The microencapsulated pear ester formulation (Cidetrak DA-MEC) reduced codling moth injury > 60% when added at rates of 12 – 24 ml/100 gallons to half rates of Imidan.

2007

- ❖ Three experimental 'puzzle piece dispensers' loaded with a blend of sex pheromone and pear ester outperformed a similar dispenser loaded only with sex pheromone and Isomate-C PLUS and in shutting down virgin female-baited traps in a replicated small plot study.
- ❖ The residual content of sex pheromone and pear ester was measured in field-aged commercial lures, Pherocon DA and Pherocon CM-DA COMBO. Both lures remained attractive for > 8 wks.
- ❖ Flight tunnel tests showed that the addition of pear ester expands the dosages of sex pheromone that are attractive to male codling moth.
- ❖ Laboratory tests showed that male codling moths response to sex pheromone can be turned off by pre-exposure to either pear ester or sex pheromone. However, sensory adaptation was not increased by the addition of pear ester to high rates of pheromone.

- ❖ The addition of a microencapsulated formulation of pear ester (Cidetrak DA-MEC) improved most of the nine insecticides tested by decreasing the percentage of codling moth-injured fruit from 25-45% and reduced the incidence of live larvae within fruit up to 80% in field trials.
- ❖ Levels of fruit injury were reduced nearly 70% in small plots where one AKISS was placed on each tree during the first moth generation.

Results and Discussion

The discovery of pear ester as an attractant for adult and larval codling moth has been a tremendous asset for pest management in Washington's orchards. In less than 10 years, a significant proportion of growers are using the Pherocon CM-DA COMBO lure that combines sex pheromone and pear ester to monitor codling moth in sex pheromone-treated orchards. Codling moth male antennae have specific receptors for pear ester and the addition of pear ester improves the orientation of male moths to pheromone-baited traps and increases moth catches. This lure is long-lasting and we have developed the use of action thresholds based on a standardized protocol for its use. The COMBO lure catches a relatively low percentage (5-15%) of female moths and while similar thresholds have also been established for the cumulative catch of female moths, few trap checkers bother to sex the moths. The pear ester lure catches a more equal sex ratio and similar numbers of female moths as the COMBO lure but is not widely used. Hopefully, more growers will adopt the use of these thresholds, in particular, as site-specific spatial pest management becomes more widely practiced. Detection of female codling moth using pear ester-baited traps placed along the borders of orchards will hopefully become a standard program to help growers apply insecticides more judiciously in their orchards.

Codling moth females lay their eggs near or on fruit. Broadcast sprays of pear ester have been shown to cause eggs to be laid on average further from fruit. The increased wandering time/distance contributes to a greater exposure of larvae to insecticide residues. When the microencapsulated formulation of pear ester (Cidetrak DA-MEC) is added to effective insecticides, such as organophosphates and neonicotinyls, their activities can be significantly improved even at reduced rates. Adding pear ester to materials that are only moderately effective for codling moth can also improve these materials. In particular, insecticides that have good activity for leafrollers and some activity for codling moth, such as Success and Intrepid, can be improved. Differences in the levels of improvement that can be achieved by adding pear ester to various insecticide classes have occurred and the potential causes for this need to be better understood. For example, pear ester has strongly improved neonicotinyl insecticides, but not the anthranilic diamides. Cidetrak DA-MEC is exempted from tolerances and can now be tested on up to 100 acres per state. Full registration of this material is expected in 2009.

Cidetrak DA-MEC can also be used by growers to enhance mating disruption. From 2002-2005 Dr. Doug Light evaluated the use of the DA-MEC to enhance sex pheromone in walnuts and applying only 2-3 sprays a year he was able to reduce nut injury in orchards by nearly 50% compared with using pheromone alone. Unfortunately, DA-MEC has not been tested for mating disruption in Washington. However, I conducted a field test in 2007 where Cidetrak DA-MEC was added to several insecticides in an orchard that was also sprayed with Checkmate CM-F sprayable pheromone formulation. Prior to this, all previous tests with DA-MEC for larval activity were conducted in non-disrupted orchards. Despite extremely high moth pressure (170 moths per trap in the 1st flight) levels of fruit injury were ca. 80% lower than expected based on the results from three similar studies conducted at the same time in other orchards where traps had similar moth catches. These data led me to formulate a hypothesis that the pear ester formulation applied as a larval spray interacted with the sex pheromone to enhance the control of codling moth in this orchard. A project has been developed to evaluate the use of pear ester (Cidetrak DA-MEC) to enhance both larval sprays and sex pheromone-based mating disruption. Orchards can be treated with either hand-applied pheromone

dispensers or the sprayable pheromone formulation and pear ester can either be added to each insecticide spray, with applications of other horticultural amendments, or in combination with the sprayable pheromone to maintain a season-long treatment program.

The new Cidetrak 'puzzle-piece' dispenser (Cidetrak CM) developed by Trécé Inc. which releases only sex pheromone has outperformed (shutting down pheromone-baited traps) Isomate-C in field trials conducted over the past two years by both WSU and our laboratory. Similar dispensers formulated with three blends of pear ester and pheromone all outperformed (shutting down virgin female-baited traps) the pheromone-only Cidetrak, Isomate, and Checkmate dispensers in small plot trials. Current studies continue to fine-tune the optimal loading of both pear ester and sex pheromone in this dispenser (Cidetrak CM-DA COMBO) in collaboration with the manufacturer. Hopefully, a combination dispenser will be registered during 2009.

Efforts to develop an effective, easy-to-use, and inexpensive killing station baited with pear ester have been underway since 2001 with support from the WTFRC. The use of mass-trapping with pear ester-baited traps was not effective as we found that female codling moths avoid entering into standard trap designs. Similarly, coating traps with insecticides on both the outside and inside was very effective only during the first moth flight and provide inadequate control during the second moth flight due to the low numbers of female moths that were killed by these traps. Several years were spent evaluating different designs but ultimately these were scrapped as insecticide residues did not last long enough and killing stations had to be frequently serviced and replaced. Future studies will address the use of a new synergized pear ester lure developed by Dr. Peter Landolt and several new designs of the killing station. These studies are primarily focused on developing a useful product for homeowners to manage backyard trees and to develop a tool for growers to supplement control along orchard borders where moth immigration into orchards is concentrated.

FINAL PROJECT REPORT
WTFRC Project #AE-05-503A **WSU Project #13C-3643-4190**

Project Title: Codling moth management with pheromones: key unanswered questions

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Total Project Funding:

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3: 2007**
Salaries [1]	13,0205	112,353	108,305
Benefits [2]	38,081	39,455	37,073
Wages [3]	15,000	19,500	*37,000**
Benefits	1,800	1,950	4,075
Equipment	3,000	0	0
Supplies [4]	7,012	11,700	10,700
Travel [5]	8,100	10,500	9,000
WSU total	83,586	81,393	91,629
MSU total	48,730	53,756	54,915
USDA total	35,082	39,609	40,689
Ag Canada total	35,800	20,700	21,420
Miscellaneous	0	0	0
Total	**203,198**	**195,458**	**206,153**

[1] For WSU part only - salary (1 mo.) for Senior Scientific Assistant; salary (11 mo.) for Research Assoc.

[2] Benefits for Senior Scientific Assistant; 34% for Research Associate.

[3] Hourly help to assist with setting up experimental apparatus, collection and analysis of data.
* *Increase of $20,000 in WSU portion of grant is for Vince Hebert's program to collaborate in assessing release and purity of different pheromone products. Also reflects some reduced allocation to other PIs.*

[4] Supplies will include lures, traps, flagging materials, cell phone charges and fuel.

[5] Travel to experimental plots; pays for one car for 6 months.

Project objectives:

1. Determine the active space of different pheromone sources (females, lures, dispensers, flakes, fibers, etc.) under MD and non-MD situations. (WSU, MSU)
2. Determine where in the tree CM females call. (WSU, USDA, CA)
3. Determine the aggregation of CM in MD and non-MD orchards. (WSU, CA)
4. Determine the impact of pheromone purity, addition of minor pheromone components, and plant volatiles on attraction of CM in MD orchards. (USDA, MSU, CA)
5. Determine the spatial arrangement of pheromone sources that maximizes MD. (WSU, MSU)
6. Define the effect of host plant volatiles on CM pheromone biology. (USDA, CA)
7. Characterize responses of CM from different geographical areas to pheromones and plant volatiles as baselines for future assessments of resistance. (USDA, WSU, MSU)
8. Utilize the information in objectives 1-6 to optimize pheromone delivery technologies for CM control and monitoring. (WSU, MSU)

Project summary:

This project brought together a team of scientists who have been conducting research on tree fruit IPM and behavioral controls of pest for decades. Two post-doctoral students, Lukasz Stelinski and Matthew Grieshop, joined the research team in 2005. Both young scientists contributed to the team and both are now employed as full time scientists at major universities. The research team working on this project met on several occasions over the last three years via conference call and in person in Michigan and Wenatchee to plan and coordinate research. Ideas generated and progress towards objectives was a direct result of the team synergy. This project for the first time developed baseline data on behavior and electrophysiology that can be used to evaluate the development of CM resistance to pheromones. The location of resting CM adults in trees was more evenly distributed than previously thought. The active space of a calling female, female mimics, and other attractive sources, was more clearly identified and generally smaller than expected. The attraction of pheromone components is better understood and confirms that minor components do not seem to add to the behavioral response of males. A combination of kairomones was shown to be more attractive to CM than pear ester. High numbers of mini-dispensers (wax drops, flakes or fibers) distributed throughout the tree canopy had a strong effect on the ability of CM males to located attractive sources. The relationship between the number of pheromone dispensers per area and the release rate per dispensers appears to be the key to making mating disruption more robust. The addition of kairomones to pheromone dispensers does not appear to enhance mating disruption.

Recommendations:

True team projects are expensive to fund but the team assembled to work on this project believe the interaction, synergy and progress towards objectives was greater than would have occurred if individuals were working alone. Lessons learned about CM responses to pheromone and kairomones suggest that research focus should be directed at:

- Defining a pheromone delivery system that optimizes mating reduction in CM;
- Develop effective killing stations for kairomones and pheromones or combinations;
- Determine if combinations of pheromone delivery technologies make a more robust MD system than any technology alone or combinations of MD and lure-&-kill stations;
- Evaluate new kairomones as monitoring systems for CM; and
- Investigate the utility of technologies developed for CM as management tools for leafrollers.

Significant findings – 2007

1. a) Confirming past results, this year's results suggested the *active space of the female* changes with the season, but is *approximately 10m.*

b) Testing of MD and monitoring lures must be done across both generations and under different horticultural planting designs in order to make robust recommendations.

c) Virgin females and 10 mg codlemone lures were similarly attractive to wild BC (30.1 vs. 31.7%) and lab-reared BC males (22.6 vs. 17.6%) but more wild males were recaptured.

2. a) Significant number of moths were found on the ground in screened tents; 21% of females and 34% of males 16 hours after release, and 52% of females and 24% of males after 64 hours.

   b) Using a vacuum method to collect wild CM adults showed that there was a relatively even distribution of male and female moths throughout the tree canopy.

   c) The outcome of an Isomate C Plus treatment was greatly affected by the location of dispensers. Substantial mating high in the canopy occurred when dispensers were placed low, while the greatest mating low in the canopy occurred when dispensers were placed high.

3. a) Laboratory moths released in an orchard were recovered (36% males and 28% females) on ground tarps after a permethrin+PBO as a knockdown agent was applied by airblast-sprayer. Both wild and laboratory moth captures were fairly uniformly distributed across the three rows. In addition to adult moths, several CM larva and a single OBLR were recovered over the course of the experiment.

   b) Fewest moths were captured where both traps and dispensers were placed high (4m) in the tree. However, mating of tethered virgin females was lowest where dispensers were placed at a combination of heights, 2m and 4m.

4. a) In all trapping experiments, traps with acetic acid plus pear ester captured many more male and female codling moths than unbaited traps or traps baited with the individual compounds. Overall, about twice as many males and twice as many females were captured with acetic acid, compared to pear ester, but in two tests this ratio was 5 to 1.

   b) The increased complexity of the female blend (lab females 2006) and wild females (2007) did not appear to significantly affect landing rates in laboratory flight tunnel assays. It therefore seems unlikely lures for MD or monitoring could be improved by adding additional components from female pheromone glands.

5. a) In the first generation CM recapture was suppressed most by the 10-flake dispenser at a rate of 1,500 per acre. There was a gradual reduction in moth recapture when the number of single flakes per acre increased from 500 to 15,000.

   b) In the second generation, CM recapture was suppressed most by single flake dispensers at a rate of 15,000 per acre.

6. None of the plant volatiles, *ethyl (E,Z)-2,4-decadienoate, a linalool, E-3-hexen-1-ol, and farnesol*, tested when added at any ratio with either amount of codlemone, increased male response over codlemone alone in this lab setting.

7. a) In comparative flight tunnel study trials the response of BC and WA moths were statistically indistinguishable and similar across years (2006 and 2007).

   b) Wild moths were significantly less responsive to codlemone or the codlemone + PE combination lure than the lab SIR moths when tested under standardized laboratory conditions.

   c) Laboratory moths are useful for assessing general behavioral responses to various chemical lures in the lab, but can't be compared with wild moths in an absolute sense.

   d) Based on electrophysiological studies there does not appear to be any consistent measurable difference between CM populations in their physiological sensitivity to pheromone or pear ester. Any behavioral differences observed in the field must be the result of differential sensitivity to environmental cues or dispersal propensity, e.g. following diapause compared with non-diapause lab individuals.

8. a) When Hercon micro-flakes were applied to trees at rates of approximately 42, 126 and 420 per tree they disrupted CM at the rate of 83%, 81%, 97% compared to the control. In the same test Isomate C-plus at 400 dispensers per acre disrupted CM attraction to traps by 94%.

   b) A new method of pheromone application was combined with a new pheromone carrier formulation that had a significantly lower moth capture than Isomate C+. The test formulation at

6000 dispensers per acre required approximately 25% less time to apply than the 400 Isomate ropes.

2007 Methods and Results

Objective 1 – Active Space:

Virgin Females - Release Recapture Distances - Grid Releases & Row Recapture

Methods: In 2007, the distance over which wild males emerging from diapause "respond" to calling virgin wild females was tested in the same high-density apple block used in 2005 and 2006. Fifty to 100, two-day-old wild males were released at distances of 5, 10, 20, 40 and 80 m downwind of ten, virgin-female-baited sticky traps. Three releases were made in late spring on May 28, June 4 and June 11 and four releases in summer on August 14, 17, 21 and 24. Catches were recorded daily for three days, but because most moths were recaptured on the first night thus all data presented are limited to catches during the first 24 h only.

Spring Releases. During three spring releases the mean (±SE) percentage of female-baited traps that caught at least one released wild male within the first 24 h was 78 ± 11.1% (7-8 females). Within this same 24 h interval males were caught from all release distances, but generally mean recapture rates declined with distance (5m = 14 ± 4%, 10m = 22.1 ± 3.5%, 20m = 9.1 ± 3.2%, 40m = 5.3 ± 2.3 and 80m = 4 ± 1.5%). As shown previously, maximum catch was from males released 10 m downwind rather than the nearest release distance. This suggests that there is an optimum response distance for a given pheromone source, probably dictated by the horizontal and vertical dimensions of a plume at given downwind distances.

Summer Releases. Males were caught from all release distances within the first 24 h, *but this did not occur in all releases*. Overall, during summer 2007 the mean (±SE) percentage recapture rates within the first 24 h declined with distance, similar to the pattern seen in 2005 & 2006 (5m = 9 ± 4%, 10m = 13.7 ± 3.2%, 20m = 8.5 ± 3.2%, 40m = 2.5 ± 0.3 and 80m = 3 ± 2.3%). Again, maximum recapture was from males released 10 m downwind rather than the nearest release distance. The recapture rates were generally lower in summer 2007 compared with spring 2007, again reflecting the same patterns as seen in 2005 & 2006.

Explanatory Hypotheses: Lack of male recapture from greater distances in most summer releases, and lower overall recapture rates in summer compared with spring in three successive years, can be explained by several hypotheses:

- Hyp. 1) a female plume penetrates a less dense spring canopy easier and over greater distance than it penetrates a more dense summer canopy,
- Hyp. 2) tree canopy is denser in summer therefore summer males are physically impeded and move shorter distances within a summer canopy than they do a spring canopy, or
- Hyp. 3) it is a combination of these two factors.

Practical Significance: 1) These studies suggest the ***active space of the female*** changes with the season, but is ***approximately 10 m***, and 2) the optimal pheromone monitoring lure or disruption dispenser may be different in different seasons, both likely due to crop canopy effects. Hence, testing of MD and monitoring lures must be done across both generations and under different horticultural planting designs in order to make robust recommendations.

Virgin Females *vs.* 10 mg Pheromone lures - Central Point Release & Circular Recapture:

Methods: In 2007, we repeated mark-release-recapture experiments using a single point release of moths and a circular trapping design in an attempt to find a pheromone lure that most adequately mimics a virgin female in terms of its active space, and to determine if the displacement of released males was more or less directional in the presence of different pheromone sources. Marked wild and lab-reared BC moths were released simultaneously from a central release point within a small high-density Gala planting. Traps baited with either a virgin female or a 10 **m**g codlemone lure and deployed in circular patterns around the release point at distances of 10, 20 or 30 m. Releases of 400 -

500, two-day-old males were made May 31 (female traps) and June 14 (codlemone lures). Catches were recorded for three days but only the first 24 h recapture rates are reported.

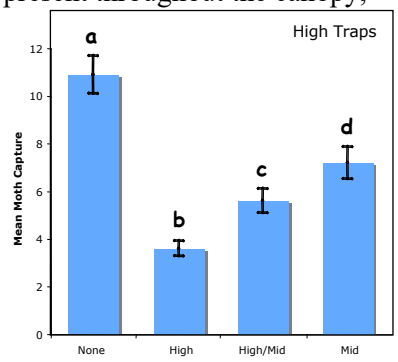
Results. In circular recapture experiments virgin females and 10 mg codlemone lures were similarly attractive in recapturing released wild BC males (30.1 vs. 31.7%), and lab-reared BC males (22.6 vs. 17.6%) but more wild males were recaptured.

Objective 2 – CM Location in the Canopy:

Methods: Two experiments were conducted in 2007. A mark recapture study was conducted in an effort to determine the location of both adult male and female codling moth during daylight hours. Four large tents were erected over single apple trees, extending out over the drive row grass. Releases of moths marked with luminescent powder were made over seventeen dates. On each occasion, twenty male and twenty female moths were released between 18:00-19:00 hours and recaptured using a vacuum method at 16 hours, 40 hours, and 64 hours post release, between the hours 10:00 to 14:00.

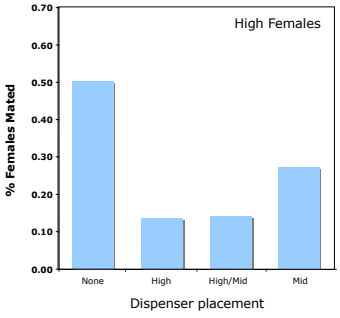
Results and Discussion: Male and female moths were more evenly distributed within the canopy than had previously been reported with equal numbers found in the mid and upper canopy. Moths were also found low in the canopy. In addition, significant numbers of moths were found on the ground in screened tents. The mean temperature in the two microclimates did not vary over a 3-month period, but mean relative humidity was higher in the grass by 13.3% compared to the tree. The higher availability of moisture in the grass could provide an explanation for why moths are found in this habitat during hot, dry summer days.

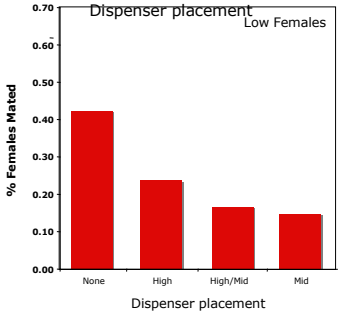
Methods: As a practical follow-up to our finding that moths are present throughout the canopy, an experiment aimed at evaluating the effectiveness of pheromone applied at different heights in the tree canopy was conducted this year at MSU's TNRC. Isomate 'rope' dispensers were placed in the canopy in three ways: all high (upper third), all mid-canopy (*ca* 6-8 ft), or half high and half mid. Capture of males in pheromone traps baited with lures, mating of tethered females and levels of fruit injury were used to evaluate treatment effects in test plots.



Results and Discussion:

- Dispenser placement high in the canopy provided the greatest trap-shutdown;
- Dispenser placement low in the canopy provided the weakest effect;
- All treatments provided equal shutdown of traps placed low in the canopy (data not shown).



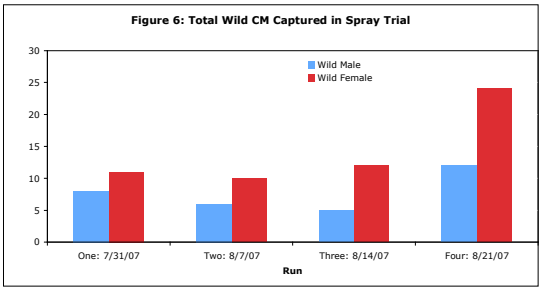


The effect of dispenser placement on CM mating high or low in the canopy.

- Greatest mating high in the canopy occurred when dispensers were placed low;
- Greatest mating low in the canopy occurred when dispensers were placed high.

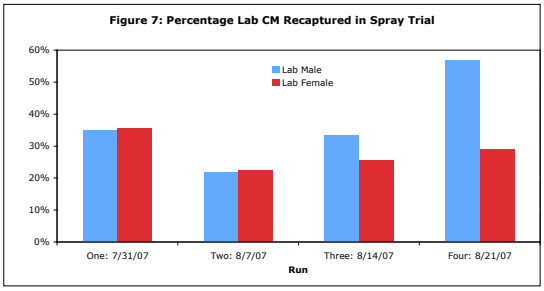
-Objective 3 – Aggregation of CM in Orchards.

Methods: An experiment was conducted to evaluate a method of assessing CM adult distributions in orchards. Four weekly applications of Pyranyl (Pyrethrum + PBO) on three adjacent 200' sections of apple trees. Ground tarps were placed along either side of the treated tree rows.



Applications were made beginning at 8:30 AM on July 31, and 7, 14, and 21 Aug. Immediately prior to each application twenty male and female moths were distributed throughout each of the three 200' tree row stretches. Within 10 minutes of moth placement, Pyranyl was applied using an air blast sprayer at 100 GPA. After 15 minutes the tarps were examined for CM. Moths were collected and brought back to the laboratory and examined for sex and source (marked lab vs. unmarked wild).

Results and Discussion: A weekly average of 7.75 wild males and 14.25 wild females were recovered over the course of the four applications (Figs 6 and 7, at right). Laboratory males and females were recovered at the average rate of 36% and 28%, respectively. Both wild and laboratory moth captures were fairly uniformly distributed across the three rows. The spatial distribution of wild moths was pretty uniform over this relatively small study area. In addition to adult moths several CM larva and a single OBLR were recaptured over the course of the experiment. The pyrethrum sprays could be used to directly sample large contiguous orchard areas. However, initial setup costs are high though spraying and sampling costs are fairly low.



Objective 4 – Pheromone purity, components and plant volatiles.

CM probably uses volatile chemicals from apple and pear fruit in host-finding. These compounds may be useful for monitoring CM or for lure and kill strategies for CM population management. The aim of these studies was to discover kairomones that are chemical attractants for CM under field conditions.

Methods:

Study 1. Combinations of apple and pear odor compounds were evaluated in wing and bucket type traps. Most compounds were formulated in rubber septa. These experiments were conducted in commercial apple orchards.

Study 2. A series of trapping experiments in commercial apple orchards evaluated the attractiveness of apple and pear fruit placed within traps as bait. This included immature apples, infested apples, ripe apples, and ripe pears.

Study 3. The combination of acetic acid (AA) and pear ester (PE) was found to be promising from results of studies 1 & 2, and was further tested in multiple trapping tests. The synergy of the two compounds, when used together as a trap bait, was demonstrated in a series of field experiments that compared the two component blend, each chemical separately, and an unbaited trap. The experiment was conducted near Yakima and Wenatchee, Washington, in Medford, Oregon, in Michigan, in New Zealand, and in Hungary. These tests involved both flights of CM.

Study 4. A series of trapping tests compared different amounts and ratios of AA and PE in traps, when the chemicals were in separate dispensers (bottles and septa), or were mixed in the same dispenser (bottle). The amounts of acetic AA and PE released over time were determined empirically in the laboratory using three methods (gravimetric, SPME, air collections).

Results and Discussion:

Study 1. The combination of AA and PE was attractive in the field, luring significantly more males and females into traps, compared to unbaited traps. This lure was further evaluated to determine if the compounds are synergistic, and if the blend is superior to pear ester in attracting female CM.

Study 2. Both male and female CM moths were consistently captured in apple orchards in traps baited with immature and ripe apples, as well as infested apples and ripe pears. The CM response to ripe pear fruit suggests that moths are attracted to fruit in part to find sugar, and that AA as a sugar fermentation product might enhance moth response to PE (Table 1). These findings also suggest avenues to pursue to seek another attractant based on apple odorants, since apples do not appear to produce PE.

Table 1. # of male and female CM captured/ week baited with apples and pears. Yakima Co. apple orchards.

Sex	Control	Green Apple	Infested green apple	Ripe apple	Ripe pear
Test 1.					
Females	0.0 ± 0.0a	NT	NT	1.9 ± 0.5b	NT
Males	0.0 ± 0.0a	NT	NT	2.0 ± 0.4b	NT
Test 2.					
Females	0.0 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.1ab	0.3 ± 0.3b	NT
Males	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.1a	0.6 ± 0.3b	NT
Test 3.					
Females	0.0 ± 0.0a	NT	NT	0.9 ± 0.4b	4.5 ± 1.0c
Males	0.0 ± 0.0a	NT	NT	0.6 ± 0.3b	7.0 ± 2.0c

Means within a row followed by the same letter are not significantly different at $P < 0.05$; ANOVA followed by Tukey's test. NT is not tested.

Study 3. In all trapping experiments, traps with AA plus PE captured many more male and female CM than unbaited traps or traps baited with the individual compounds. Overall, about twice as many males and twice as many females were captured with AA, compared to PE, but in two tests this ratio was 5 to 1 (Table 2, below).

Table 2. # of male and female codling moths captured per trap-check in traps with acetic acid and pear ester.

Test and Conditions	Control	Acetic Acid	Pear Ester	Combination
1. Yakima, Aug., 2006				
Females	0.0 + 0.0a	0.4 + 0.2a	0.2 + 0.1a	2.2 + 0.7b
Males	0.0 + 0.0a	0.5 + 0.3a	0.3 + 0.2a	2.3 + 0.7b
2. New Zealand, Nov. 2006				
Females	0.0 + 0.0a	0.2 + 0.0a	6.0 + 1.2b	16.9 + 2.2c
Males	0.0 + 0.0a	0.9 + 0.3a	15.5 + 3.2b	32.6 + 3.3c
3. Yakima, 2007 season				
Females	0.0 + 0.0a	0.5 + 0.2b	2.1 + 0.5c	3.6 + 0.6d
Males	0.0 + 0.0a	0.5 + 0.1b	3.4 + 0.6c	6.2 + 0.9d
4. Wenatchee, 2007 season				
Females	0.0 + 0.0a	0.2 + 0.1b	1.7 + 0.3c	2.8 + 0.4d
Males	0.7 + 0.3a	1.6 + 0.4b	2.7 + 0.5c	4.3 + 0.7d
5. Hungary, 2007				
Females	0.0 + 0.0a	0.3 + 0.1	0.7 + 0.2	2.1 + 0.3
Males	0.0 + 0.0a	0.1 + 0.0	0.1 + 0.1	0.3 + 0.1

Means within a row followed by the same letter are not significantly different at $P < 0.05$. ANOVA followed by Tukey's test.

Study 4. With AA dispensed from a bottle and PE dispensed from a septum, greatest captures of CM in traps were with AA in bottles with 5 mm diameter holes in the lid, and septa with a 500 mg load of PE. With this lure, CM catches were similar with UniTraps, Delta traps and Wing traps. When AA and PE were mixed in a bottle with a 3 mm hole in the lid, greatest numbers of CM were captured with 5 to 20% PE in AA. Preliminary laboratory results indicate a stable release of these two compounds from the mixture for at least 2 wks.

Wild Female Gland Extract *vs.* Codlemone in Clean Air

Methods: The role of minor pheromone components found in female pheromone glands as attractants for male codling moth was evaluated in flight-tunnel choice tests. In 2007, we tested synthetic codlemone of the highest purity (99.5%) delivered at a female equivalent rate (10 pg / min) from a microsprayer while paired (10 cm source separation) against a *pheromone gland extract from*

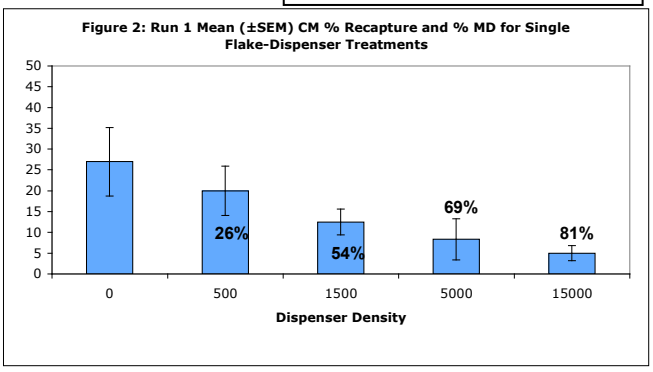
the wild BC moths delivered at an equivalent codlemone rate.

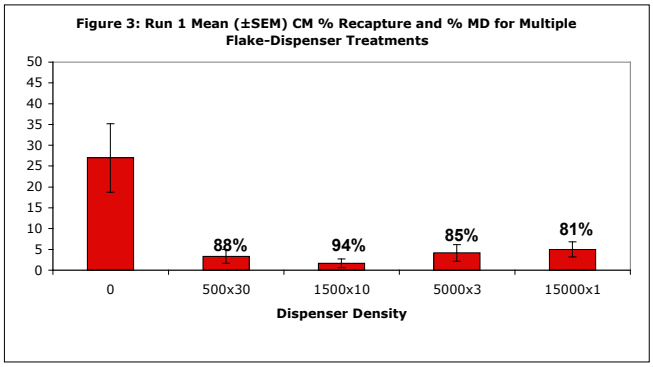
Results: The BC laboratory strain (n = 100 moths flown) and wild BC moths (75 moths flown) landed on each source with equal frequency, indicating wild females and lab females produce an equally attractive source. Overall response among lab males was higher than wild males. The increased complexity of the female blend (lab females 2006) and wild females (2007) did not appear to significantly affect landing rates in laboratory flight tunnel assays. It seems unlikely lures for MD or monitoring could be improved by adding additional components from female pheromone glands. Improved MD will likely come about by modifying the pheromone dispenser release rate.

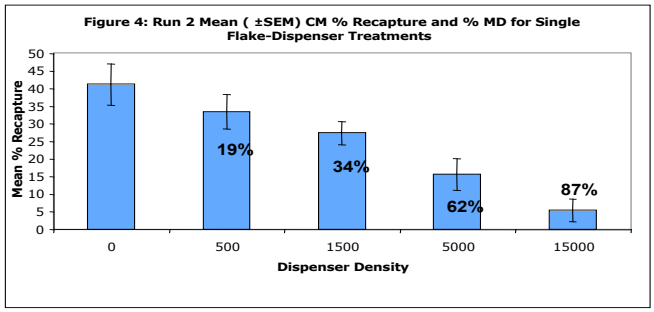
Figure 1: Trees with pheromone

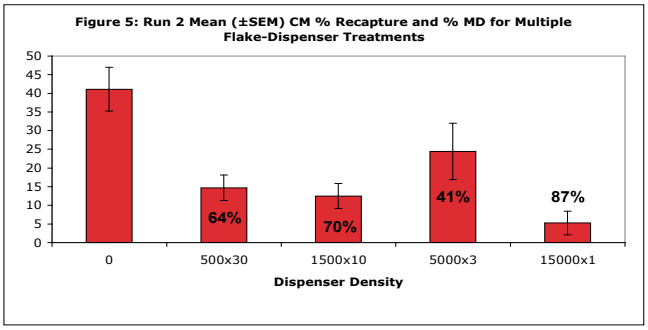
Objective 5 – Spatial arrangement of competing pheromone sources.

Methods: Mark Release and Recapture (MRR) of laboratory reared CM was used to assess eight different hand applied mating disruption treatments utilizing Hercon Flakes. The eight treatments consisted of: 1) an untreated control, 2) 500 single flakes per acre, 3) 1500 single flakes per acre, 4) 5000 single flakes per acre, 5) 15000 single flakes per acre, 6) 500 30-flake clusters per acre, 7) 1500 10-flake cluster per acre, and 8) 5000 3-flake cluster per acre (Figs. 1-3, at right). Treatments 1-5 represented an increase in both the number of pheromone point sources as well as overall active ingredient, while treatments 5-8 represented an increase in pheromone point sources while keeping the amount of active ingredient per area constant. Marked moths were released in the center row of 0.2 acre plots. Two pheromone traps were placed in each plot, within 10m of release point. Marked moths were released during the first (May 28-July 2) and second (July 20-August 12) CM generation. A subset of the dispensers used in the experiment were collected each week and sent to the WSU-FEQL laboratory for volatile collection analysis.









Results and Discussion: Volatile collections from field-aged samples indicated that the release rate of flakes used in this study was greatly reduced after 2-3 weeks, therefore, only the first 2 weeks of data are presented. During the first CM generation recapture declined with increasing number of point sources when single flake clusters were

considered (Fig. 2), however this pattern was not apparent when the total amount of active ingredient was controlled for (Fig. 3). In fact the 1500 10-flake treatment provided numerically superior reduction in moth activity. In the second CM generation the 15000 single-flake treatment provided the most suppression of moth activity (Figs. 4 and 5, at right).

Practical Significance: The treatment providing the most consistent disruption of moth activity was the 15000 single-flake treatment. This lends support to the hypothesis that maximizing low release point sources will enhance mating disruption. However, during the first run total amount of active ingredient seemed to be the determining factor for mating disruption. As moths used during both runs came from the same non-diapausing colony it is possible that some environmental factor may be mediating mating disruption between the first and second CM generation. Potential environmental factors include: temperature, wind conditions, canopy architecture/development, or a combination of factors.

Objective 6 – Effect of host plant volatiles on CM pheromone biology.

Flight tunnel tests of pheromone + plant volatiles - No-Choice tests – clean air

Methods: In 2007, we completed studies started in 2006, where several previously identified plant volatiles (kairomones) known to attract male codling moth to varying degrees were tested as potential synergists of codlemone. Experiments were conducted in a flight tunnel using BC laboratory-reared moths. Plant volatiles (*ethyl (E,Z)-2,4-decadienoate, a linalool, E-3-hexen-1-ol, and farnesol*) added to these septa in serial amounts ranging from 0 - 10,000 mg to produce lures with the following codlemone + kairomone ratios (mg:mg).

Results: In 2007, studies confirmed when using *no-choice tests,* that none of the plant volatiles tested, when added at any ratio with either amount of codlemone, increased male response over codlemone alone in this lab setting. These particular kairomones do not appear to hold promise as improved monitoring tools for CM.

Objective 7 – Baseline characterization of behavior and electrophysiology.

Methods: In 2007, wild CM larvae collected in tree bands from Washington (WA) and British Columbia (BC) in winter 2006, were shipped to Summerland for 2007 -behavioral tests needed to increase sample sizes for those done in 2006. We compared the relative responses of each population to codlemone, pear ester and a natural female pheromone gland extract. Adults from all populations were emerged from diapause larvae and flown (two-day-old) in a flight tunnel.

Results and Discussion: In these flight-tunnel tests, the responses of males from different populations to a 10 mg codlemone lure were numerically different ranging from 42 – 80.4% source contact. The SIR laboratory strain of moths was most responsive (80.4±5.7% in 2007), while wild moths from BC (58.6±5.7%, 2007) and WA (59.2±6.4%, 2007) had near identical intermediate responses and wild MI moths in 2006 showed the lowest and most variable responses to this codlemone lure (42.4±15.6%).

The above procedure was repeated to compare the response of males from different populations to a codlemone + pear ester combination lure (10 mg + 100 mg, respectively). The BC laboratory strain of moths was most responsive to this combination lure (75.8±4.4%), with wild moths from BC (53.9±4.7%) and WA (62.1±10.8%) having similar intermediate responses and wild MI moths (2006) showed the lowest responses to this combination lure (27.1±7.4%).

Practical Significance: The responses of BC and WA moths were statistically indistinguishable and similar across years. Wild moths were significantly less responsive to codlemone or the codlemone + PE combination lure than the lab moths when tested under standardized laboratory conditions. Laboratory moths are useful for assessing general behavioral responses to various chemical lures in the lab, but can't be compared with wild moths in an absolute sense.

Male + Female EAG's

Methods: Physiological responses of over wintered wild male and female CM from different

populations (BC and WA) to codlemone and pear ester delivered individually were measured using electroantennogram techniques (EAG's). Excised antennae from two-day-old moths were challenged with serial dilution series of codlemone and pear ester. The normalized responses of antennae from each population were compared to each other and those of the USDA and SIR laboratory strains. Responses of male and female antennae were compared but analyzed separately.

Males. In 2007, like 2006, there were no significant differences among the wild populations (BC vs. WA) in the EAG responses of males to either codlemone, or pear ester, at any dose tested (n = 30 males / population / chemical). The dose-response regression lines for each chemical were also similar among the wild populations, all increased linearly as a function of log-dose. As expected, male antennae from all wild populations were significantly more responsive to codlemone (ca. 100H) than to pear ester. EAG responses of the BC laboratory strain were significantly more responsive to the three highest concentrations of each stimulus than were any of the wild populations. These differences may reflect a more uniform quality of the non-diapause laboratory-reared insects compared with wild moths emerging from several months in diapause storage.

Females. In 2007, like 2006, females from all wild and laboratory populations exhibited similarly low responses to codlemone and no dose-response relationships were evident in any population over the range of codlemone doses tested. Generally, females within each population had greater antennal response to pear ester than did males from the same population. As expected, females from all populations exhibited significant dose-response relationships to pear ester. Unlike 2006, wild female CM populations from BC and WA exhibited similar EAG responses to pear ester. The response of wild females from BC and WA was also similar to the responses of the BC lab females.

Practical Significance: There does not appear to be any consistent measurable difference between CM populations in their physiological sensitivity to pheromone or pear ester. Any behavioral differences observed in the field must be the result of differential sensitivity to environmental cues or dispersal propensity, e.g. following diapause compared with non-diapause lab individuals.

Objective 8 – Development and optimization of pheromone delivery technology.

Methods: A six-acre apple block (243 trees/acre) was used in the trial. Five 90' by 90' subplots, each consisting of five tree rows were evenly spaced throughout the block and randomly assigned one of five treatments: 0 pheromone, one, three or ten passes with the flake applicator, or Isomate C-plus at 400 dispensers/acre (dpa). Flakes were applied at the rate of 1.0 lb per acre. A deposition test indicated that with each pass approximately 42 flakes were deposited per tree. Marked CM moths were released in the center of each plot once per week for four weeks and traps, two per plot baited with 0.1 mg lure, were checked twice a week and lures were changed weekly. Wild moth captures were recorded in addition to the marked moths. Pheromone treatments were applied on July 23, 2007 with marked CM released on July 25, August 1, 8, 15, and 22.

Results and Discussion: The 1, 3, 10 pass, and Isomate C-plus treatments disrupted CM at the rate of 83%, 81%, 97%, 94% compared to the control (Fig. 1, at right). A slightly different trend was observed for wild moths with 72%, 92%, 90%, and 97% disruption compared to the control for the 1, 3, 10, and Isomate C-plus treatments (Fig. 2, at right). During weeks four and five, an increasing number of CM were recaptured in all three-flake treatments but not in the control or Isomate treatment.

Practical Significance: Results for the 3 and 10 pass flake treatments were similar to the single row treatment experiment conducted in 2006,

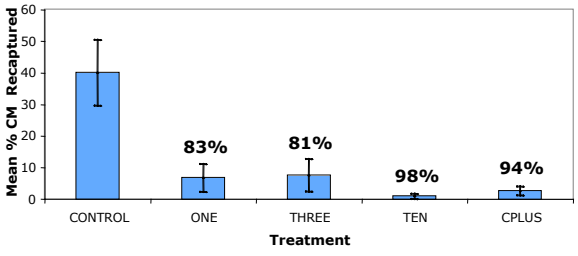


Figure 1: 2007 Mean (±SEM) Percentage Marked Codling Moth Recapture and Percentage Disruption

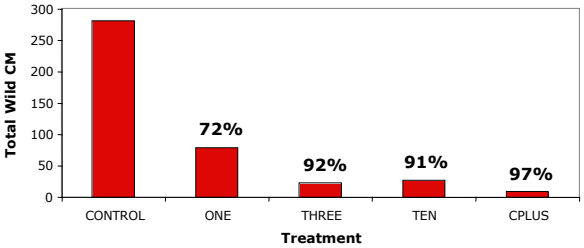


Figure 2: 2007 Wild CM Capture and Percentage Disruption

suggesting that geometric shape of the treated area *did not have* an impact on the flake treatment but had a much greater effect on the Isomate C-plus treatment. This further suggests that MD due to large numbers of relatively low release rate devices, such as the Hercon flake, may be possible in relatively small orchards with higher edge to area ratios. However, it required 10 passes of flakes (ca. 420 per tree) to match the disruption effect provided by Isomate C-plus in rectangular orchard plots. Further research into the spatial scale and application area shapes at which either high density, low release rate (i.e. Hercon Micro-disrupt) and low density, high release rate (i.e. Isomate C plus) pheromone dispensers operate might aid growers in choosing the appropriate technology for their orchard.

Methods: A new method of pheromone application was combined with a new pheromone carrier formulation and tested against Isomate dispensers for control of CM. Tests were performed over the summer of 2007 in various locations throughout southern Michigan. Pheromone dispensers were produced by melting paraffin wax, adding various additives and pheromone. Isomate C+ was applied at 400 dispensers per acre, totaling 73 grams of codlemone per acre. The test formulation was applied at 6,000 dispensers per acre. Three treatments of codlemone concentrations were compared in this test formulation to total amounts of 50, 150 and 500 grams per acre. Five replicates of each treatment and a negative control were compared throughout the second flight of CM.

Results: All pheromone treatments significantly reduced trap catch when compared to the negative control. The test formulation treatment with 150 grams of codlemone per acre had a significantly lower moth capture than Isomate C+. The test formulation at 6,000 dispensers per acre required approximately 25% less time to apply than the 400 Isomate ropes.

FINAL PROJECT REPORT
WTFRC Project Number: AE-05-504 **(WSU Project No. 13C-3643-3190)**

Project Title: Sustainable management of leafrollers in apple orchards

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Organization: WSU-Tree Fruit Research & Extension Center
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Other funding Sources

Agency Name:	**Washington State Commission on Pesticide Registration**
Amount awarded:	**$56,593** (two years funding)
Notes:	These funds were used primarily to support a Ph.D. Student working on resistance issues in leafroller and codling moth.
Agency Name:	**Private chemical companies** (Dow, Valent, DuPont, Syngenta, Cerexagri)
Amount awarded:	**$75,000** (funding over three years)
Notes:	These funds were used to maintain leafroller colonies and conduct field surveys of resistance and establish baseline data for some new products.

Total Project Funding: ***2005:*** 25,898 ***2006:*** 26,922 ***2007:*** 27,631

Budget History

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3:** 2007
Salaries [1] (AR-0.25); (AP-0.083)	7,048 (AR) 4,603 (AP)	7,401 (AR) 4,817 (AP)	7,512 (AR) 4,898 (AP)
Benefits[1] (AR-46%); (AP-34%)	3,242 (AR) 1,565 (AP)	3,626 (AR) 1,638 (AP)	3,456 (AR) 1,665 (AP)
Wages [2]	5,400	5,400	6,000
Benefits	540	540	600
Equipment	0	0	0
Supplies [3]	1,500	1,500	1,500
Travel [4]	2,000	2,000	2,000
Miscellaneous	0	0	0
Total	25,898	26,922	27,631

Footnotes:

[1] **Ph.D. student**. Funding provided by the Washington Commission on Pesticide Registration (WSCPR) was not used because the student did not arrive until January 2006. WSCPR has authorized funds already allocated to be used in 2006 for the student. **Kathleen Pierre (AR Associate in Research)** - rearing and maintenance of leafroller colonies; **Mike Doerr (AP Administrative Professional)** – management of project and bioassay efforts.

[2] Summer labor to assist with rearing of leafrollers.

[3] Leafroller diet components, plastic Petri dishes, glassware. Cell phone charges are allowed under this grant.

[4] Pays for a vehicle for six months used part-time on this project plus fuel and maintenance costs.

Objectives:

1. Develop a dose-mortality bioassay method for insect growth regulators (IGRs) and other new insecticides to establish baseline toxicity data for codling moth and leafroller.
2. Develop discriminating concentrations for key insecticides.
3. Use molecular methods as a tool for early detection of resistance development in leafrollers and codling moth.
4. Evaluate levels of resistance in leafroller populations from orchards suspected of having resistance issues with insecticides.
5. Characterize any cross-resistance in leafrollers between old and new insecticides.
6. Evaluate new insecticides for control of codling moth and leafrollers in field tests.

Significant findings:

1. Baseline susceptibility data for several new insecticides (some not yet registered) have been developed for leafrollers and codling moth (Table 1). These include baselines for insect growth regulators, which do not conform to the typical baseline approaches of measuring mortality.
2. For codling moth we have developed ovicide bioassays. This method allows us to determine if an insecticide has efficacy against the egg stage and which exposure method, topical or residual, is most efficacious. This approach has also been used to develop baseline concentration-response curves on codling moth eggs for new insecticides (Table 1).
3. A new artificial diet has been used to establish dose-response curves using a diet incorporation method. This approach allows evaluation of resistance when fruit or foliage is not available. It also provides a good system for selecting codling moth or leafroller populations by exposing them to insecticides each generation (Table 1). This method can also be used to determine the potential of a pest species to develop resistance to a particular class of insecticide by exposing a population to a diet laced with insecticide that will kill about 70% each generation.
4. Sublethal effects for some insecticides against leafrollers have been characterized. These effects are especially important for insect growth regulators. Understanding the sublethal effects helps in explaining the impact of insecticides that do not have fast killing power, for example, insect growth regulators.
5. In field-aged residue bioassays have been used to estimate the activity periods of different insecticides against leafroller and codling moth (Table 2). This bioassay method has been used to characterize longevity of residues against larvae and egg (of codling moth). These data represent an additional set of baselines against which we can test populations suspected of being resistant. It might be that the LC_{50} values do not shift to a great degree but that larvae are able to overcome residues in a shorter time than susceptible populations.
6. Field trials have demonstrated the efficacy of several new insecticides against codling moth larvae and eggs and leafroller larvae. Field trials are how rates and re-treatment intervals are established for new insecticides. These data form the basis for recommendations WSU publishes in the Crop Protection Guide for Tree Fruits in Washington.

Methods:

Concentration/dose-response bioassays – Apples (CM) or leaves (LR) or artificial diet were dipped into known concentrations/doses of an insecticide and allowed to dry. After an appropriate period (7 to 14 days) the eggs or larvae were assessed for mortality. Data were run through a software program that generated a linear relationship between concentration/dose and mortality providing estimates of LC_{50} values among other useful statistics.

Diet incorporation bioassay – Insecticide was incorporated into a new artificial diet as a water mixture. A small amount of diet was placed into a small cup and newly hatched CM or LR larvae were introduced. After an appropriate period (7 to 14 days) the eggs or larvae were assessed for mortality. Data were run through a software program that generated a linear relationship between concentration/dose and mortality providing estimates of LC_{50} values among other useful statistics.
Survey of field-collected populations - In 2006 only two populations of OBLR larvae were collected. They were reared to the second generation (F2) in the laboratory, and neonate larvae were used in a leaf-disk bioassay to determine their susceptibility to azinphosmethyl (Guthion 50WP, Bayer CropScience) and spinosad (Success 2SC, Dow AgroSciences). Another population was collected from Mattawa where spinosad seemed to fail in summer, but sufficient larvae have not been reared to conduct bioassays.
Reversion of resistance - Field-collected populations that had demonstrated resistance to azinphosmethyl, spinosad and methoxyfenozide were reared through successive generations without selection pressure from insecticides. These populations were periodically evaluated for resistance levels using established bioassay methods to these three insecticides.
Field-aged bioassay – A field-aged bioassay was used to assess the residue longevity of new insecticides. Trees were treated with candidate insecticides and at regular intervals leaves or fruit were collected. Leafroller or codling moth larvae were placed on the leaves or fruit and mortality assessed after appropriate periods to determine the efficacy of aged residues.
Field trials – Field tests were conducted evaluating the efficacy of new insecticides against leafrollers and codling moth. Treatments were applied either by hand-gun or airblast sprayer in replicated deigns. Assessment of leafrollers was made by counting live and dead larvae following treatment and for codling moth the number of injured fruit after each generation.

Results and discussion:

Baseline toxicity data – Over the past several years we have conducted bioassays with new insecticides against codling moth eggs and larvae and leafroller larvae. These bioassays establish levels of susceptibility in laboratory colony populations that serve as baselines for comparing to field collected populations allowing us to assess if populations are developing resistance. These data also provide insights into the inherent toxicity of a new insecticide to a pest.

Table 1. Summary of bioassay results for several new insecticides using susceptible laboratory colonies of codling moth eggs and larvae and leafroller larvae.

Chemical	Year	Source	n	Slope (SE)	LC50-ppm (95% CL)	LC90-ppm (95%CL)
Obliquebanded leafroller larval screening						
emamectin	2005	LAB	350	3.0 (0.5)	0.04 (0.03-0.05)	0.10 (0.08-0.16)
rynaxypyr	2005	LAB	350	1.6 (0.2)	2.9 (1.6-4.4)	18.6 (11.7-38.7)
rynaxypyr	2005	LAB	350	2.0 (0.3)	2.4 (3.5-6.7)	10.2 (6.7-20.0)
novaluron	2005	LAB	350	1.1 (0.2)	27.2 (14.9-44.1)*	438.0 (228.9-1273.9)*
novaluron	2005	LAB	350	1.6 (0.3)	5.8 (2.4-9.7)**	36.7 (22.7-73.2)**
methoxy	2005	LAB	350	2.6 (0.4)	2.9 (2.2-3.6)	8.8 (6.6-13.4)
spinosad	2005	LAB	350	2.8 (0.6)	0.4 (0.2-0.5)	1.0 (0.8-1.7)
azinphos	2005	LAB	350	4.1 (1.1)	11.9 (8.0-14.6)	24.7 (19.5-44.2)
Obliquebanded leafroller larval screening (Diet incorporation)						
spinetoram	2003	LAB	175	1.2 (0.3)	0.2 (0.01-0.39)	
rynaxypyr	2006	LAB	250	1.9 (0.3)	0.31 (0.18-0.44)	1.47 (1.04-2.59)
rynaxypyr	2007	LAB	250	2.6 (0.4)	0.33 (0.24-0.77)	1.03 (0.77-1.60)
rynaxypyr	2007	LAB1	400	5.0 (1.3)	0.09 (0.06-0.11)	0.17 (0.14-0.23)
rynaxypyr	2007	LAB2	400	5.3 (1.8)	0.09 (0.04-0.12)	0.16 (0.13-0.27)

Table 1. Continued.

Chemical	Year	Source	n	Slope (SE)	LC50-ppm (95% CL)	LC90-ppm (95%CL)
Codling moth larval screening – Apple test (larval mortality)						
spinetoram	2003	LAB	700	1.2 (0.4)	0.04 (0.0004-0.13)	
rynaxypyr	2006	LAB	300	2.2 (0.6)	0.8 (0.3-1.3)	3.0 (1.9-6.7)
flubendiamide	2006	LAB	350	2.4 (1.0)	35.0 (7.2-55.8)	117.8 (77.7-324.4)
Exp 1	2007	LAB	300	1.7 (0.3)	3.0 (1.3-4.9)	17.3 (11.2-34.8)
flubendiamide	2007	LAB	300	0.8 (0.2)	72.1 (20.4-190.8)	2577.9 (659.4-1.4x10^5)
Exp 2	2007	LAB	300	1.6 (1.0)	3.4 (no limits)	22.0 (no limits)
Exp 3	2007	LAB	300	0.7 (0.2)	22.7 (2.8-65.1)	1360.1 (348.9-70090.0)
Exp 4	2007	LAB	300	1.7 (0.6)	41.5 (3.8-81.2)	246.4 (132.0-1460.0)
Codling moth larval screening – Diet incorporation (larval mortality)						
rynaxypyr	2006	LAB	350	4.6 (1.3)	0.13 (0.08-0.18)	0.25 (0.18-0.51)
Codling moth ovicidal screening (Topical method)						
Mineral oil	2005	LAB	1350	4.2 (0.2)	3.7 (2.8-4.3)	
flubendiamide	2006	LAB	3207	0.7 (0.04)	519.6 (196.8-4018.5)	38568.0 (4674-1.1*10^9)
rynaxypyr	2003	LAB	1044	0.6 (0.1)	55.2(27.3-116.0)	
emamectin	2006	LAB	2100	No dose response		
Codling moth ovicidal screening (Residual method)						
flubendiamide	2006	LAB	3207	1.8 (0.08)	148.4 (125.7-175.3)	773.2 (573.0-1173.6)
rynaxypyr	2003	LAB	1872	0.6 (0.1)	6.1(0.6-21.3)	
emamectin	2006	LAB	2100	No dose response		

Resistance surveys 2007: Rynaxypyr (Altacor, DuPont) is a new insecticide due to be registered for use on tree fruit in 2008. We have worked with the company to develop methods and baseline data on the susceptibility of codling moth and leafrollers to this product. In 2007 we conducted an extensive study using these methods to assess the susceptibility of codling moth to this new product. Enough larvae were collected and reared to the adult stage to develop complete concentration response curves for six populations allowing the estimation of LC_{50} values (Table 2). There was no indication from these data of resistance in field populations. Because it is difficult to obtain sufficient larvae from commercial orchards to generate complete concentration response curves, two diagnostic concentrations were selected based on susceptibility of laboratory colonies.

Table 2. Results of resistance screening bioassays for different field collected codling moth populations in 2007.

Chemical	Year	Source	n	Slope (SE)	LC50-ppm (95% CL)	RR ratio[1] (95% CL)[2]
Obliquebanded leafroller larval screening						
Altacor	2007	LAB1	400	5.0 (1.3)	0.09 (0.06-0.11)	
Altacor	2007	LAB2	400	5.3 (1.8)	0.09 (0.04-0.12)	1.00 (0.66-1.51)
Altacor	2007	Moxee F1	400	2.2 (0.4)	0.14 (0.04-0.24)	1.54 (0.98-2.41)
Altacor	2007	Donald F1	400	1.4 (0.2)	0.05 (0.02-0.08)*	0.53 (0.31-0.93)
Altacor	2007	NCW Heat F1	300	2.6 (0.7)	0.12 (0.06-0.16)	1.27 (0.78-2.05)
Altacor	2007	Brogan F1	290	3.4 (2.1)	0.10 (no limits)	1.10 (0.47-2.58)
Altacor	2007	Sm. Tract F1	265	1.5 (0.3)	0.09 (0.03-0.16)	0.94 (0.51-1.72)

Field collected populations that did not produce enough individuals to generate complete curves were tested at these diagnostic concentrations to see if there was any difference from the baseline data (susceptible laboratory). Figure 1 shows results from 17 populations collected in Washington and exposed to a 0.2 ppm diagnostic concentration of rynaxypyr. By comparing the percent mortality of the reference population (laboratory colony) to field populations we saw that some has lower percent mortality than would be expected of a susceptible population.

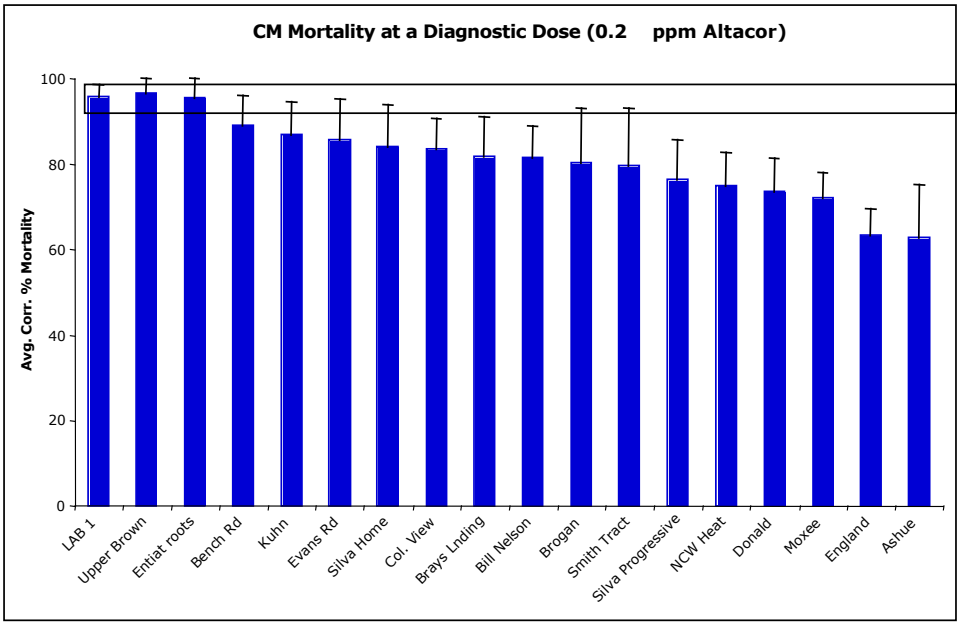
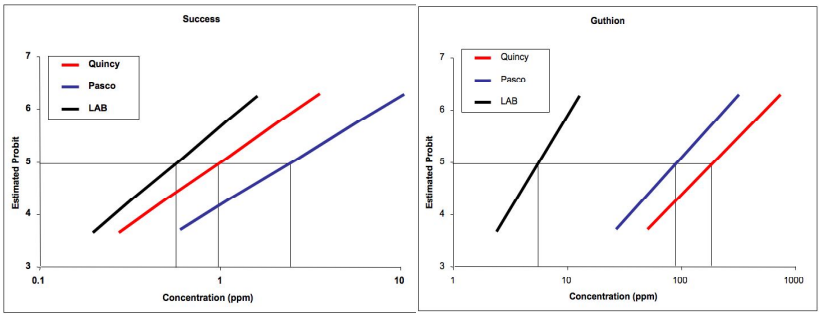


Figure 1. Percent larval mortality of laboratory and field-collected populations of codling moth exposed to a diagnostic concentration (0.2 ppm) of rynaxypyr.

For several years we have been evaluating leafroller populations for resistance to insecticides. We have previously shown that field-collected populations of both leafroller species (OBLR and PLR) were resistant to methoxyfenozide (Intrepid) before it was registered, evidently because of cross-resistance to organophosphate (OP) insecticides. However, these populations were not resistant to spinosad (Success) or emamectin benzoate (Proclaim) indicating no cross-resistance. We have also shown that after only 6 years of use some OBLR populations had developed resistance to spinosad. For example, in 2006 OBLR populations near Quincy and Pasco showed resistance to azinphosmethyl (35.6 and 11.3 times), spinosad (1.9 and 7.2 times) and methoxyfenozide (***Pasco only*** - 10.8 times). The spinosad resistance level is the highest we have detected in Washington but our data suggest that this population should still be controlled if using the high field rate; however, methoxyfenozide would likely not provide sufficient control to be commercially acceptable. While we have not yet completed the tests, it is highly likely that OBLR populations resistant to spinosad will also show resistance to spinetoram (Delegate), a newly registered insecticide in the same class.



Because of our concerns about OP mediated cross-resistance in new insecticides we conducted some preliminary evaluations of OBLR populations susceptibility to rynaxypyr. Table 3 shows bioassay results from six field-collected populations using two different methods (leaf-dip and diet incorporation). Four of the six populations show resistance ratios (roughly the LC_{50} of field population divided by LC_{50} of reference or susceptible population) that indicate a low level of resistance to rynaxypyr and the data further suggest that this is likely due to OP mediated cross

resistance. What this means in terms of the expected level of control of OBLR populations in orchards is difficult to predict at this time but it is certainly not a positive development.

Table 3. Bioassay results for field-collected OBLR populations showing resistance to rynaxypyr, 2006 and 2007.

Chemical	Year	Source	n	Slope (SE)	LC_{50}-ppm (95% CL)	RR ratio[1] (95% CL)[2]
Obliquebanded leafroller larval screening						
Altacor	2006	LAB	250	1.9 (0.3)	0.31 (0.18-0.44)	
Altacor	2006	Mattawa F_3	250	1.5 (0.2)	0.64 (0.32-1.01)	2.0 (1.1-3.9)*
Altacor	2006	Quincy F_3	250	2.0 (0.4)	1.06 (0.62-1.54)	3.4 (1.9-6.2)*
Altacor	2006	Pasco F_9	250	2.2 (0.4)	0.28 (0.16-0.39)	0.9 (0.5-1.6)
Altacor	2007	Lab	250	2.61 (0.39)	0.33 (0.24-0.77)	
Altacor	2007	Plath-F_2	250	3.06 (0.70)	0.39 (0.19-0.57)	1.18 (0.88-1.59)
Altacor	2007	Jarrel-F_2	250	5.12 (0.99)	1.21 (1.01-1.44)	3.07 (2.19-4.29)*
Altacor	2007	Jones-F_2	250	2.9 (0.37)	1.39 (1.02-1.85)	4.18 (2.91-5.99)*

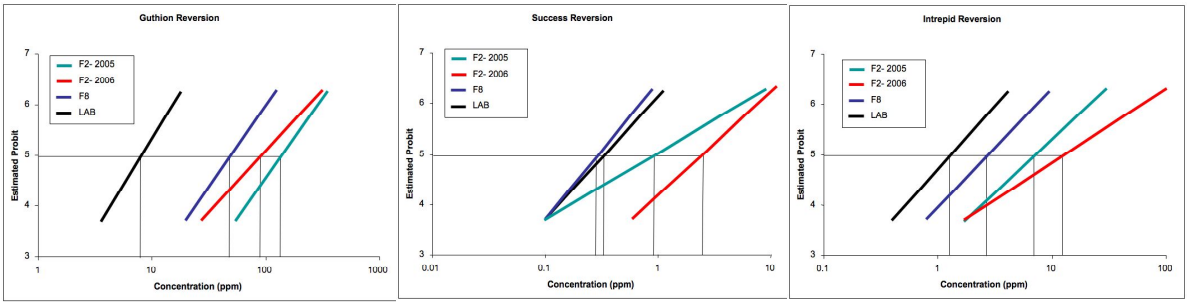
n = number of larvae assayed

[1] Resistance ratio- LC_{50} field population: LC_{50} lab colony

[2] 95% Confidence limits (Lethal Ratio Significance Test, Robertson and Priesler, 1992)

* LC_{50} of field collected population significantly different than the laboratory colony

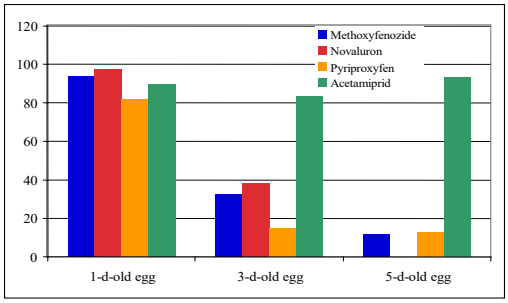
Reversion of resistance - We have shown that at least for one insecticide, spinosad, it is possible that resistance will revert to susceptible levels if selection pressure is eliminated. For example, OBLR populations collected from Pasco in 2005 and reared in the laboratory on artificial diet through eight generations showed a decline in resistance to spinosad to a level the same as the susceptible colony. By contrast these same populations, while showing some decline in resistance to methoxyfenozide and azinphosmethyl, did not show reversion to susceptible levels suggesting their mechanisms of resistance are linked.



Field-aged bioassay – In 2006, field-aged bioassays residues of novaluron (Rimon) were not as long lasting as those of methoxyfenozide for PLR or OBLR. Rynaxypyr (Altacor) and spinetoram (Delegate) showed a long residual activity against OBLR larvae, similar to spinosad (Success) (Table 4). Against CM larvae rynaxypyr (Altacor) showed very good residual activity, lasting as long as azinphosmethyl and longer than spinosad (Success). In an ovicidal bioassay novaluron had slightly better residual activity than methoxyfenozide. In 2007, flubendiamide (Belt) showed long residual activity against OBLR larvae as did to experimental insecticides (Table 4). Against codling moth residues of spinetoram (Delegate) were of similar duration as those of acetamiprid (Assail), while residues of flubendiamide (Belt) and two experimental insecticides were not as effective as acetamiprid. Residues of rynaxypyr were more effective and lasted longer than those of pyriproxyfen against codling moth eggs (Table 4).

Table 4. Corrected percent mortality of leafroller and codling moth exposed to residues of insecticides for different periods, 2006 and 2007.

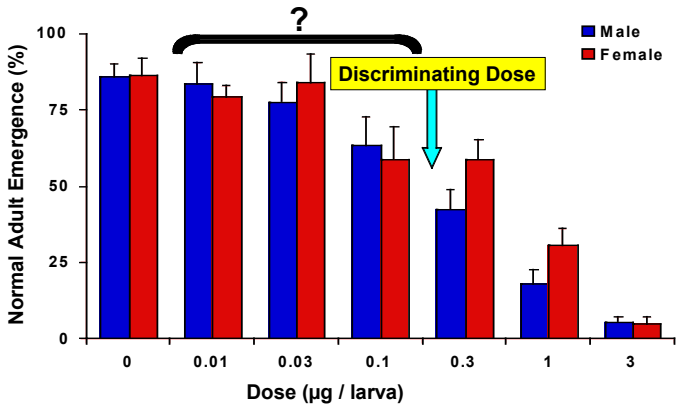
			Corrected percent mortality (DAT)				
Insecticide	Rate/a	Year	1	7	14	21	28
Pandemis leafroller (7-d evaluation)							
novaluron	32 fl oz	2006	90.3	88.6	50.0	72.7	
methoxyfenozide	16 fl oz	2006	100.0	100.0	100.0	100.0	
Obliquebanded leafroller (7-d evaluation)							
novaluron	32 fl oz	2006	91.7	86.1	64.4	62.0	
methoxyfenozide	16 fl oz	2006	100.0	100.0	100.0	100.0	
rynaxypyr	4.0 oz	2006	98.1	100.0	100.0	100.0	100.0
spinosad	6.0 fl oz	2006	100.0	100.0	92.5	95.7	100.0
spinetoram	6.4 oz	2006	100.0	100.0	100.0	100.0	100.0
Codling moth neonates (7-day evaluation)							
rynaxypyr	4.0 oz	2006	100.0	72.2	93.8	91.1	86.2
azinphosmethyl	2.0 lb	2006	100.0	94.4	91.7	82.3	89.6
spinosad	6.0 fl oz	2006	85.0	72.2	33.3	67.7	41.3
spinetoram	6.4 oz	2006	100.0	94.4	41.7	91.1	65.5
Codling moth ovicide (10-day evaluation)							
novaluron	32 fl oz	2006	74.4	87.2	82.3	87.4	
methoxyfenozide	16 fl oz	2006	78.4	76.1	72.2	79.5	
			Corrected percent mortality (DAT)				
Insecticide	Rate/a	Year	1 or 4	7	14	21	28
Obliquebanded leafroller (7-d evaluation)							
Experimental #1	119.2	2007	87.3	79.1	75.4	87.5	55.7
Experimental #2	70.9	2007	100.0	97.0	96.9	97.9	100.0
flubendiamide	70.9	2007	100.0	100.0	100.0	100.0	100.0
Codling moth neonates (apple test live cm)							
acetamiprid	67.5	2007	92.6	100.0	76.7	70.8	86.7
acetamiprid	67.5	2007	88.9	95.5	90.0	91.7	73.3
spinetoram	49.7	2007	100.0	86.4	83.3	91.7	76.7
Experiment #1	119.2	2007	29.7	42.1	48.7	50.0	8.6
Experiment #2	70.9	2007	73.0	81.6	82.1	76.7	60.0
flubendiamide	70.9	2007	59.5	57.9	76.9	80.0	57.1
Codling moth ovicide (apple residue test)							
pyriproxyfen	50.0	2007	47.3	32.7	37.1	32.3	35.7
pyriproxyfen	50.0	2007	47.0	40.6	46.3	37.6	36.8
rynaxypyr	40.0	2007	81.0	76.5	76.4	77.3	78.6



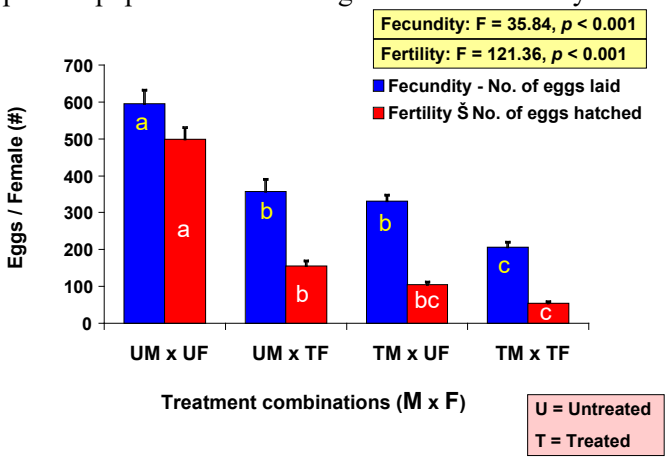
The ovicidal activity of insecticides against codling moth is becoming an important consideration in the management of this pest. We have shown in Table 3 that several insecticides have topic and/or residual effect on codling moth eggs. We have tests of evaluating the residual activity of these insecticides as new eggs are laid on them (Table 4). However, we have not shown previously the impact of egg age on topical applications of insecticides. We developed a method for doing this and the results are of great interest. Some insecticides (methoxyfenozide, novaluron, pyriproxyfen) that have a high effect topically against new eggs had little or no effect against older eggs. Of note was the strong ovicidal effect of acetamiprid against

young and old codling moth eggs. This impact is likely a reason contributing to acetamiprid's efficacy against codling moth.

Lethal and sublethal effects of IGR -
Developing baseline information for insect growth regulators is a challenge because the target insect stage does not always express exposure through mortality. When pyriproxyfen (Esteem) is consumed by a leafroller larvae it lives longer than normal but expresses intoxication by abnormal structures (see photos to the right). To be able to evaluate potential resistance to pyriproxyfen we set out to define a dose that would provide discrimination if susceptible laboratory populations were compared with field-collected



populations. Based on several parameter, like the emergence of adults (Figure below) we believe a discriminating dose is between 0.1 and 0.3 micrograms per larva. From this study, we asked the question about sublethal effects of pyriproxyfen. Larvae that consumed low doses (0.01 and 0.03 micrograms) did not show detrimental effects, at least in external morphology and did not show mortality rates that were different from the untreated controls. We, therefore, exposed larvae to a sublethal dose (0.03 mirograms per larva) and then examined the impact on pupal and adults weight and on the ability of moths to reproduce. The weights of pupae and adult treated with pyriproxyfen were significantly higher than untreated larvae. However, whether male or female destined larvae were treated there was a significant reduction in the number of eggs laid per female, fertility of the eggs (see Figure to right) and the percent of egg hatch. When male larvae were treated and female untreated there was roughly an 80% reduction in egg hatch. When both sexes were exposed to a sublethal dose of pyriproxyfen there was almost a 90% reduction in egg hatch.



Field Trials:
In 2007 we conducted 14 field trials against codling moth and leafrollers involving a total of 53 separate treatments. Over the past 3 years we have conducted 38 separate field trials against codling moth and leafrollers involving a total of 147 separate treatments. Results from these trials form the basis for use recommendations used by the industry for pest control that are published in the Crop Protection Guide for Tree Fruit in Washington (EC-0419).

2007 Leafroller Trials: Acetamiprid (Assail) was evaluated as a leafroller control applied pre-bloom in two field trials. In one trial it was compared with chlorpyrifos (Lorsban) (Table 5) as an industry standard and in another with spinosad (Success) (Table 6). In both cases, Assail failed to provide control while the industry standards provided excellent control.

Table 5. Control of overwintering obliquebanded leafroller larvae, 2007.

Insecticide	Rate (gm AI/a)	Timing	Avg live larvae/ 500 shoots (11 May)
Assail 70WP	67.5	Pink	1.8b
Assail 70WP	67.5	Pink	2.8b
+ HMO	+ 1.0% v:v		
Success 2SC	42.6	Pink	0.0a
Untreated			2.0b

Means in the same column followed by the same letter are not different (P=0.05, Student's t test). Statistics were run on transformed data [Log (y+1)].

Table 6. Control of overwintering obliquebanded leafroller larvae, 2007.

Insecticide	Rate (gm AI/a)	Timing	Avg live larvae/ 500 shoots (24 Apr)
Lorsban 4E	908.0	Pink	0.00a
Assail 70WP	67.5	Pink	2.85b
Untreated			2.90b

Means in the same column followed by the same letter are not different (P=0.05, Student's t test).

2007 Codling Moth Trials: We typically test unregistered insecticides in partnership with private chemical companies to determine their fit into IPM programs. Recently, we have been examining the efficacy of tank mixes of new insecticides applied at a delayed first larvicide timing. This approach seems to work well against high pest pressure situations where good coverage of the target surface is achieved. Two examples of results of field trials are shown below.

In the first test oil or pyriproxyfen (Esteem) was applied at an ovicide timing followed either by tank mixes of acetamiprid (Assail)+pyriproxyfen, rynaxypyr (Altacor)+pyriproxyfen, or with non-tank mix applications of acetamiprid, pyriproxyfen or rynaxypyr. The tank-mix program of Assail + Esteem, preceded by an oil application at 200DD, had significantly more CM injured fruit than the other two tank-mix treatment programs that were included in this trial (Fig. 1). Altacor + Esteem, preceded by oil or Esteem, provided very good CM control through both generations. There did not appear to be a significant advantage to using Esteem instead of oil as a strategy to delay the tank-mix application; however, Esteem could provide the added benefit of leafroller (LR) control, especially when used at the spring application timing. An oil application at 200DD followed by two applications of Altacor or Assail also provided good CM control in this trial. Using an ovicide, like oil or Esteem, to delay the first larvicide application creates the opportunity to reduce the interval between larvicide applications (ca. 17d) and will be a good strategy for using new larvicides, like Assail and Altacor, that have a shorter active residue life than the OP products that they will be replacing. Oil followed by two applications of Esteem did not provide the same level of CM control. In this case, the Esteem applications were likely too late in the generation to have an optimal effect on eggs in the orchard. This illustrates the importance of application timing with new insecticides that effect specific stages of the CM life cycle.

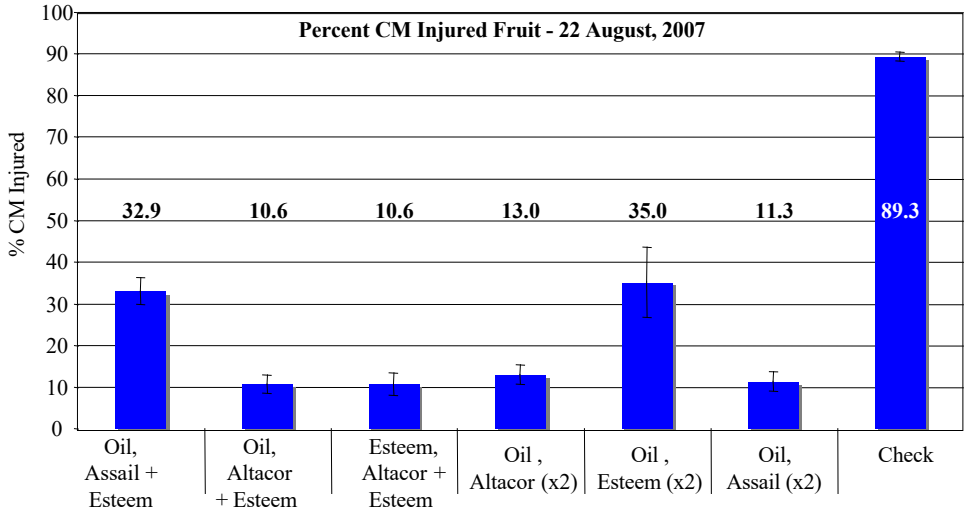


Figure 2. Efficacy of insecticides applied at delayed first hatch timings, either in tank mixes or alone on 17-day re-treatment intervals.

In the second test an experimental insecticide was tested at different rates in comparison to three new insecticides. There was not a significant difference in CM control between the three rates of the Experimental product tested in this trial. Belt provided good CM control after the first CM generation but also had a high number of stings at the time of the final evaluation. Calypso provided CM control that was statistically equivalent to Altacor, which had the smallest percentage of CM injured fruit after each generation.

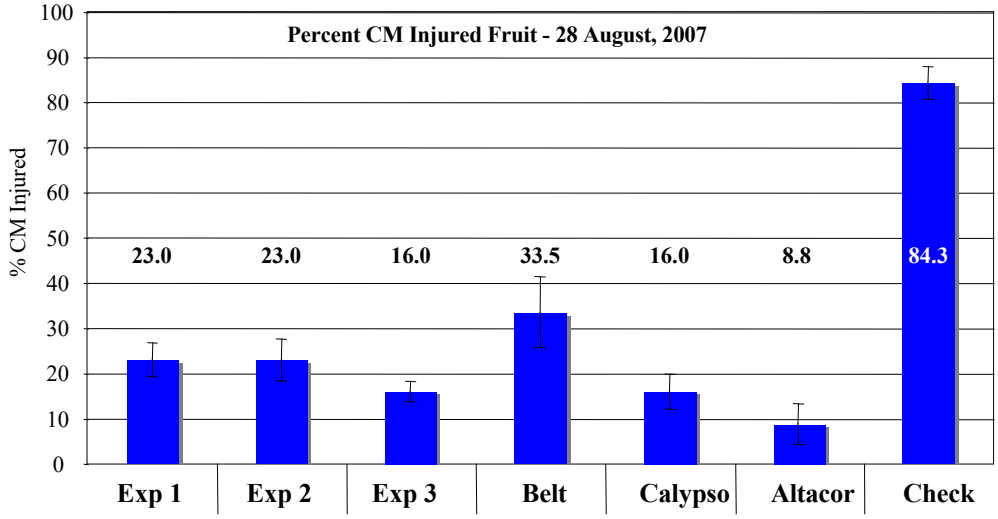


Figure 3. Efficacy of insecticides applied against codling moth at traditional egg hatch timings.

FINAL PROJECT REPORT
WTFRC Project Number: CP 06-601 **WSU Project # 13C-3643-7387**

Project Title: Reinstating integrated mite control in apple orchards

PI: Elizabeth Beers
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Other funding Sources

Agency Name: Washington State Commission on Pesticide Registration
Amount awarded: $12,560 (2006): $12,373 (2007)

Total Project Funding: *Year 2006:* 34,841 *Year 2007:* 36,730

Budget History:

Item	**Year 1:** 2006	**Year 2:** 2007
Salaries	9,374	9,754
Benefits	904	917
Wages	7,800	8,400
Benefits	780	0
Equipment	0	0
Supplies	1,500	1,500
Travel	1,923	2,820
Miscellaneous	0	0
Total	**22,281**	**24,357**

Significant Findings

1. A consistent association was found between the insecticides Assail, Calpyso, and Rimon and increased levels of mites in large scale commercial orchard blocks over three years. The severity and extent of the outbreaks varied widely from year to year.

2. An additive effect in causing mite outbreaks was found between Assail, Calpyso, and Rimon when used in a program with the blossom/fruit thinning materials lime-sulfur and carbaryl; however, there were elevated mite levels in the Imidan+lime-sulfur+carbaryl treatment, as well.

3. Bioassays with spider mites and *T. occidentalis* demonstrated clearly that lime-sulfur and ATS are acutely toxic on contact to all three mite species, while dry flowable sulfur has little or no effect.

4. Lime-sulfur and ATS caused severely reduced prey consumption in *T. occidentalis* when exposed only to residues and contaminated prey; dry flowable sulfur had no effect. The effect of lime-sulfur occurred even at reduced rates, and with or without the addition of petroleum oil. In general, when prey consumption went down, fecundity was also suppressed.

Results and Discussion

Large scale commercial trial. This trial was conducted for three years in commercial orchards from Bridgeport to the Royal Slope. The treatments consisted of three newer codling moth insecticides (Assail, Calypso, and Rimon) compared to an OP standard (either Guthion or Imidan) applied to 1-4 acre blocks. The same treatments were applied to the same blocks in successive years. Mite populations in 2005 (Fig. 1a) were high in only one orchard out of five. The highest levels occurred in the Rimon treatment followed by Assail and Calypso. Very few differences were found in rust mite or predatory mite levels among the various treatments.

In 2006, mite populations were much higher overall in the test orchards (Fig. 1b). Five out of six orchards had high mite populations. Rimon caused an elevated tetranychid mite level in five orchards, Assail in two, and Calypso in three. There was one orchard which had a very high peak of mite in the OP plot. However, this orchard had high mite levels overall in 2006, following high levels in 2005. Trends in predatory mite densities were less clear, although in several blocks the recovery in the population was too late to affect the outbreak of mites during the mid-season. When orchards were used as replicates in ANOVA, there were no statistical differences found among treatments for either tetranychid or predatory mites. Rust mites varied considerably among orchards; there were statistically more rust mites in the Assail treatment, but this was based primarily on two orchards (data not shown).

Mite populations in 2007 were overall much lower than the previous year (Fig. 1c), with peak populations of about six mites/leaf (as opposed eighty mites/leaf in 2006). Only two of five orchards sampled experienced a moderate increase in spider mite levels, with high levels occurring in the Rimon treatment in one orchard, and Assail and Calypso in another. Slightly elevated levels of spider mites also occurred in the OP check in one orchard. Predatory mite levels were higher in two orchards, one apparently in response to increased spider mite densities, and one in response to rust mite densities (data not shown).

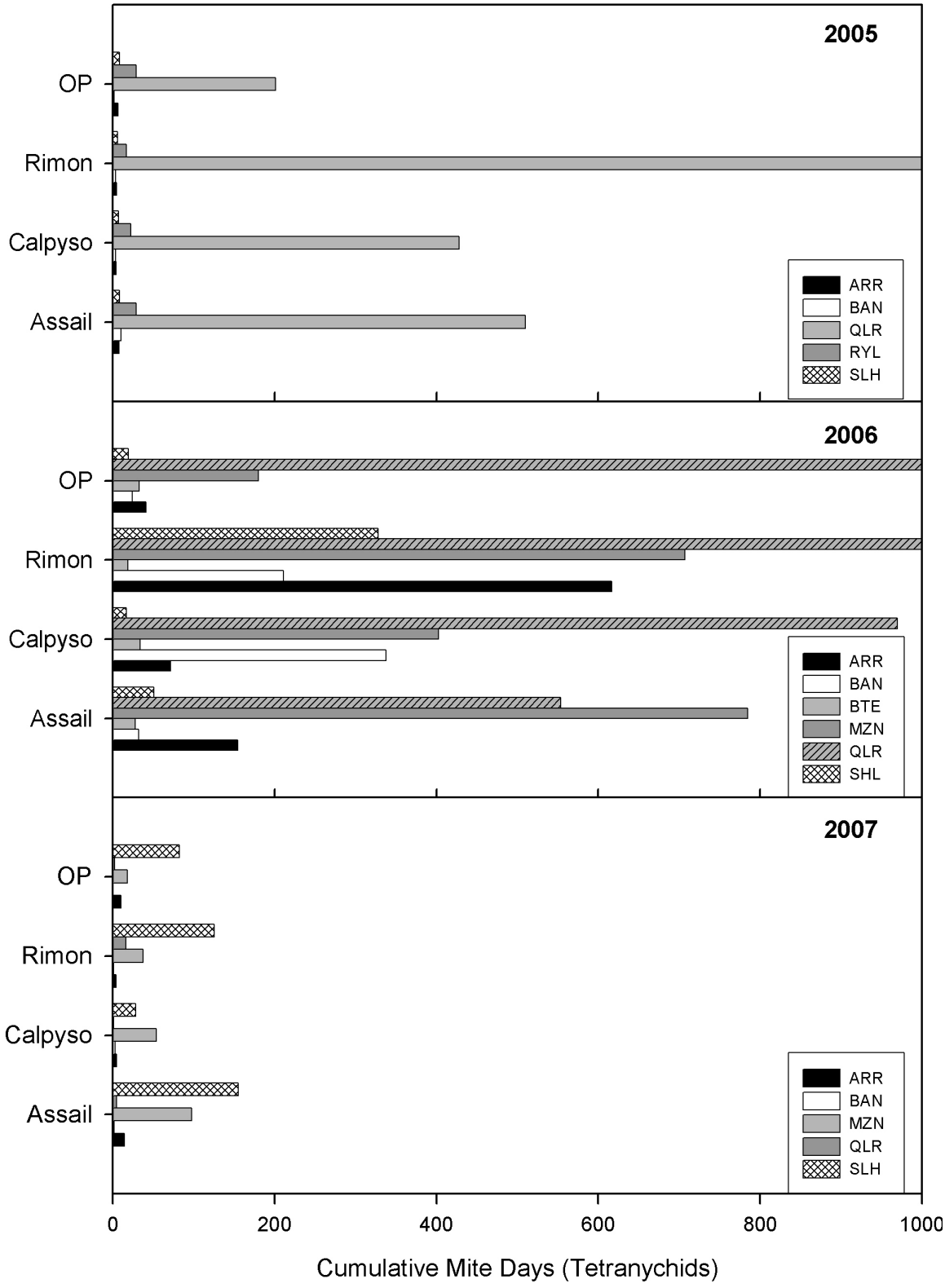

Fig. 1. Tetranychid cumulative mite days resulting from applications of Assail, Calypso, Rimon, and an OP to large commercial orchard blocks, 2005 (1a), 2006 (1b), and 2007 (1c).

Medium plot replicated trials.

Test 1. This experiment examined the additive effects of seasonal programs of two thinning materials, lime-sulfur and Sevin, with four different codling moth materials. The codling moth insecticides (Assail, Calpyso, Rimon and Imidan) were applied either alone, preceded by lime-sulfur, or with lime-sulfur followed by Sevin. All materials were used at typical timings (lime-sulfur at bloom; Sevin at 10 mm fruit; codling moth materials at first and second cover).

The results from this trial were very striking; none of the treatments containing just the codling moth materials caused a mite flare-up; only those incorporating all three groups (lime-sulfur, carbaryl, and the codling moth insecticide) caused a flare-up (Fig. 2). In this test, even Imidan caused a moderate increase in mites. Assail and Rimon (+LS+carbaryl) had the highest peaks, and Calypso+LS+carbaryl had the lowest peak. Predatory mites (data not shown) were highest in the Imidan and Assail treatments, and lowest in the Rimon treatments. Apple rust mite populations were highest in the Assail and Imidan treatments, but Rimon appeared to have a detrimental effect on rust mites. Fruit damage by codling moth was higher in some of the Assail treatments, and lower in the Rimon and Imidan treatments, although most of the damage was in the form of stings. There was also a non-significant trend for woolly apple aphids to be lowest in the Imidan treatments, and highest in the neonicotinyl treatments, with Rimon intermediate.

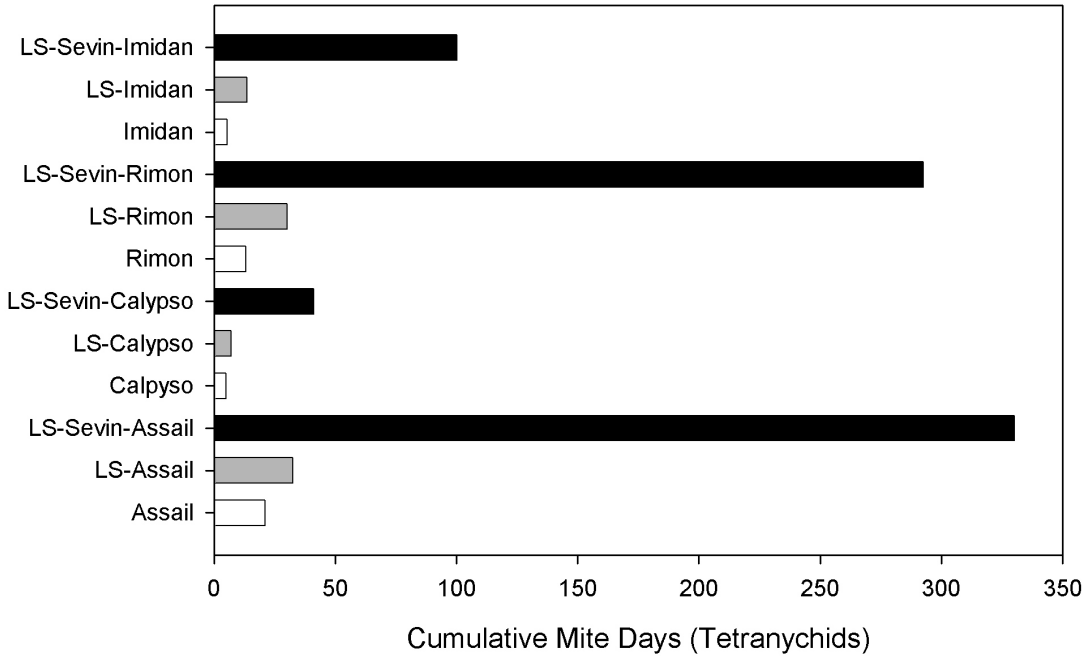

Fig. 2. Additive effect of codling moth programs, lime-sulfur (LS) and carbaryl (Sevin) in an orchard trial.

Test 2. A second test examined the relative effect of ATS vs. lime-sulfur (with or without a Sevin application at 10 mm fruit), in the absence of any of the above-mentioned codling moth materials. Mite populations were low throughout the test, with no significant differences among treatment (data not shown).

Test 3. This test examined the relative effects of one vs. two applications of lime-sulfur and Sevin vs. NAA, all followed by two applications of Assail for the first generation of codling moth, with a

"mite-neutral" check (NAA and Intrepid). In this trial (data not shown) there was a slight trend for higher tetranychid mite populations in treatments containing lime-sulfur, but this occurred only on a few count dates during the peak mite population. There was some increase of mites in the check, also. No significant treatment differences in cumulative mite days for tetranychid or predatory mites.

Test 4. This test looked at the additive effect of Sevin, Assail, and Surround, a kaolin clay material known to flare mite populations. Little treatment effect was seen in this trial; there was a modest mite increase in the plots, but the highest level was in the check (least disruptive) (data not shown).

Test 5. This test targeted the effect of sulfur-containing pesticides on apple rust mite and predatory mites. Either a single or a triple application of lime-sulfur, ATS, or dry flowable sulfur was applied in late June to a high rust mite population, being fed on by low to moderate levels of predatory mites. Lime sulfur had the greatest detrimental effect on rust mites, with flowable sulfur intermediate, and ATS with the least effect (Fig. 3a). All treatments suppressed rust mites in relation to the check. There was no difference between single and triple applications, perhaps because of the population crash due to hot weather. All treatments suppressed predatory mites in relation to the check, and to about the same degree (Fig. 3b). Tetranychid mite populations were low throughout the test, and no stimulation occurred due to the sulfur treatments.

Laboratory bioassays.
A series of laboratory bioassays was done on European red mite, twospotted spider mite, and *Typhlodromus occidentalis*, with emphasis on the latter. The bioassays provided a more detailed examination of the effect of sulfur-containing pesticides than was provided by the field tests.

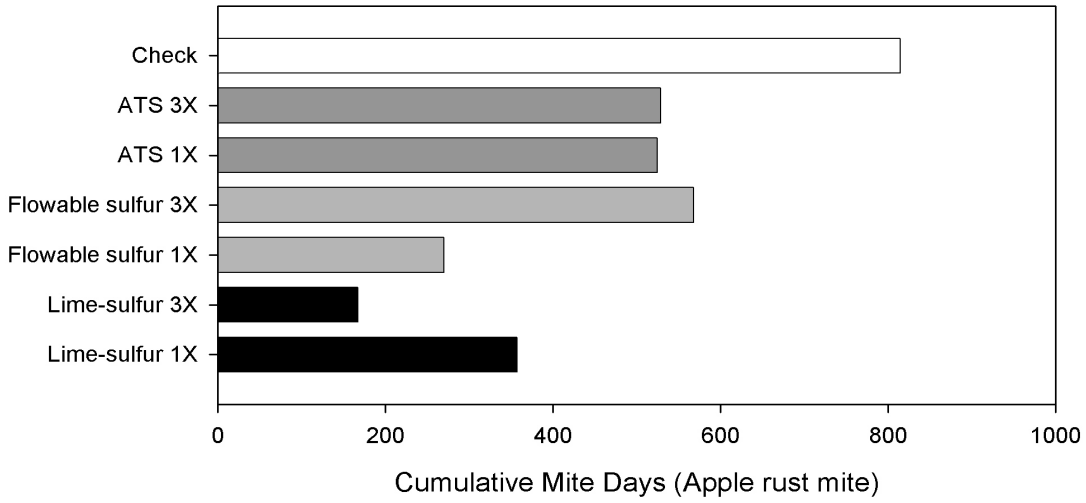

Fig. 3a. Effect of sulfur pesticides on apple rust mite, field experiment.

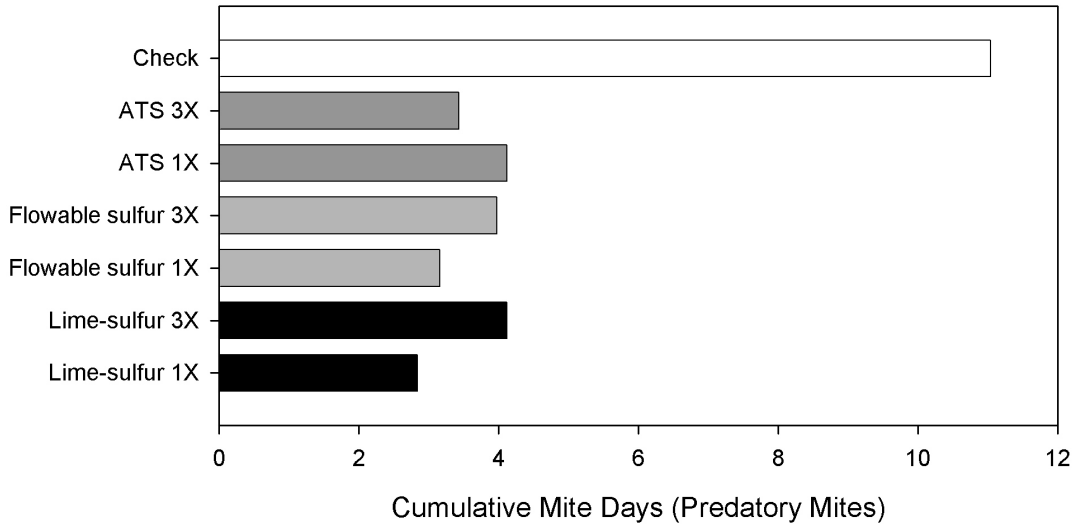

Fig. 3b. Effect of sulfur pesticides on predatory mites, field experiment.

Exposure routes included contact, residual, and contaminated prey; effects measured included direct mortality, and the sublethal effects of prey consumption and fecundity.

Group 1. This series of bioassays examined mortality and fecundity of the three mite species when exposed via contact to lime-sulfur, ATS, and dry flowable sulfur. Two bioassay formats were used, one using synchronous cohorts to provide female mites of the same age, or mites taken at random from the orchard or a laboratory colony. Data were collected daily for 5 d; however, the 48 h data provided the best compromise between check mortality and treatment effect. Lime-sulfur and ATS were acutely toxic to western predatory mite, European red mite and twospotted spider mite, causing high levels of mortality (60-100%) 48 h after treatment (Fig. 4). Dry flowable sulfur showed no acute toxicity with the exception of European red mite, where an intermediate level of toxicity was found. For the most part, sublethal effects on fecundity were not detected; evidently, if the mites survived, they could reproduce normally. The one exception was ATS, which caused a slight reduction in egg production of twospotted spider mite in one bioassay, but no differences occurred in other bioassays. There was no apparent difference in results between bioassays using synchronous cohorts versus those using mites of unknown ages.

Group 2. This series of bioassays (and all subsequent ones) focused on mortality and prey consumption by *T. occidentalis* exposed to contaminated prey and residues on leaf surfaces (residual bioassay). Lime-sulfur at both 8% and 4% caused a significant reduction in prey consumption at 24 and 48 h (Fig. 5); there were no differences in mortality (data not shown). Dry flowable sulfur caused no suppression of prey consumption or mortality. ATS caused a slight increase in mortality (48 h), but there was a measurable decrease in prey consumption at both rates in relation to the check.

Group 3. This bioassay used the three sulfur containing materials at a constant rate of sulfur (the previous tests had rates that were based on field rates). When all three sulfur products were applied at the same rate of S/ml solution (equal to the high rate of dry flowable sulfur), both ATS and lime sulfur caused a reduction in prey consumption at 48 h. In this test, lime-sulfur had a significantly greater effect than ATS. Fecundity was also severely suppressed in these two treatments. There was no difference in mortality at 24 h, which was low in all treatments.

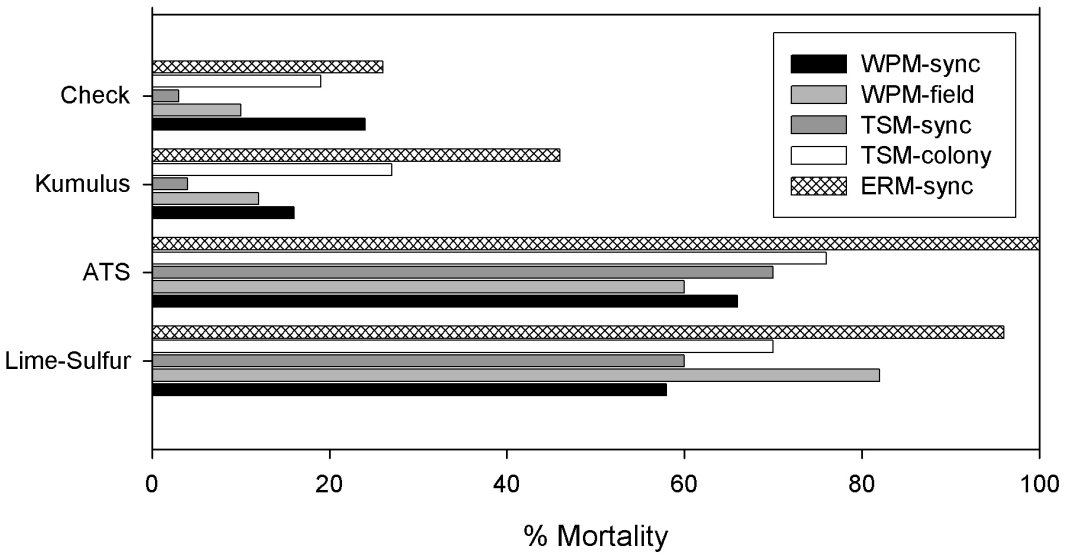

Fig. 4. Percentage mortality after 48 h resulting from contact bioassays with lime-sulfur, ATS, and dry flowable (Kumulus) sulfur (WPM, *T. occidentalis*; TSM, twospotted spider mite; ERM, European red mite).

Group 4. This bioassay looked at the effect of the two rates of lime-sulfur commonly used by the industry (8% and 2%), either alone or in combination with petroleum oil. This is one of the few tests where significant mortality occurred in one or more treatments, specifically, lime-sulfur at 2% (both with and without oil), and lime-sulfur 8% with oil. Prey consumption was dramatically reduced by all lime-sulfur treatments, regardless of rate or addition of oil (Fig. 6). Oil alone caused an intermediate reduction in prey consumption. Fecundity was not different than the checks in any treatment.

Group 5. This bioassay compared the effect of exposure to Sevin either topically (plus residues) or to residues only, in comparison to a water check. Mortality was zero in all treatments. There were no significant treatment differences in prey consumption, fecundity, or viability of *T. occidentalis* eggs.

Discussion

Field Experiments. Given the format of the trial (commercial orchards widely scattered across the state with varying histories), there was a relatively clear and consistent pattern of disruption by all three of the codling moth insecticides used in this trial. The effect of Assail and Calypso was noted in earlier experiments (Beers et al. 2005). No effect of Rimon was noted in small-plot trials, however, this association, first reported by anecdotal evidence, has been confirmed in these experiments. The occasional high population in the OP standard indicates that mite outbreaks can also occur where these compounds are used; it is primarily a question of frequency. OP resistance in *T. occidentalis* from Washington has been documented for decades, with ca. 100-fold resistance levels in comparison to a susceptible population (Croft and Jeppson 1970; Babcock and Tanigoshi 1988).

While the three-year period of this study is insufficient to predict long-term patterns, it seems apparent that year-to-year variation is an important factor. The mechanism underlying this variation is not clear, however, it is likely that temperatures during some key period of development play a role. This variation tends to obscure what are likely real effects of pesticide programs on integrated mite control.

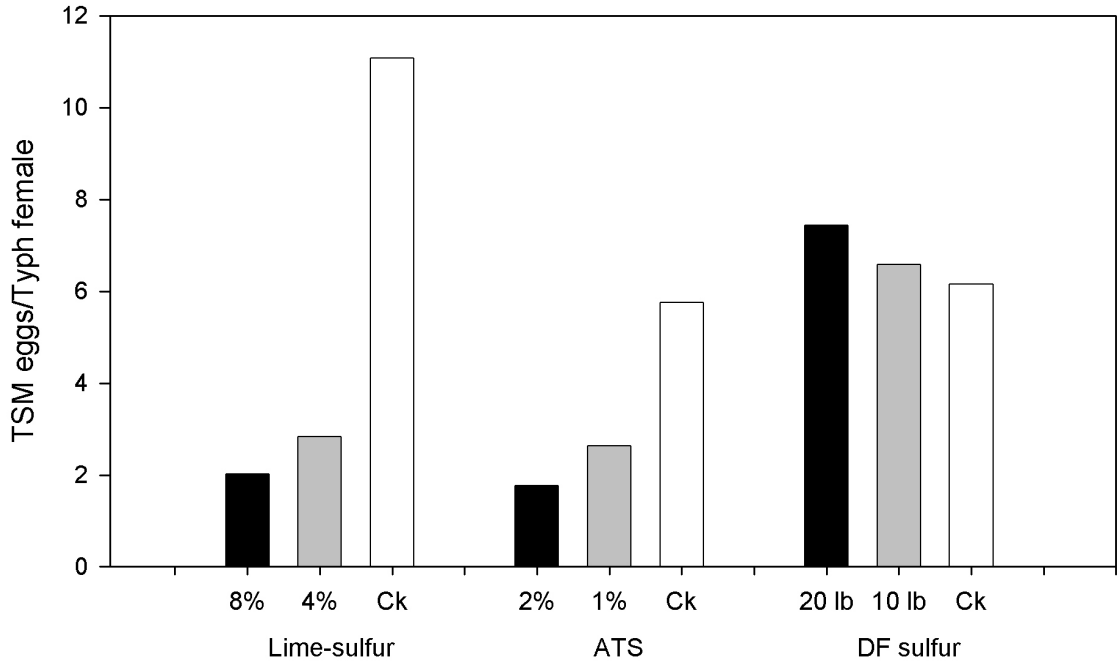

Fig. 5. Sublethal effects of sulfur pesticides on prey consumption by *T. occidentalis* exposed to residues.

The principle of additive effects is demonstrated in the small plot trials, that is, that the probability of a mite outbreak increases with the number of disruptive compounds. Some of the materials currently used for thinning (lime-sulfur, ATS, and carbaryl) have a history of disrupting integrated mite control, mainly through direct toxicity to either *T. occidentalis* or its alternate prey, apple rust mite (Hoyt 1969). These detrimental effects have become codified in the WSU Crop Protection Guide over the years (e.g., Smith et al. 2007), appearing as early as the 1960s (Hoyt 1965). Despite this knowledge, these compounds have been used for decades in Washington orchards, and mostly without causing mite outbreaks. It is likely that increased numbers of applications, coupled with use of more disruptive codling moth programs, has resulted in the higher regional incidence of mite outbreaks.

Bioassays. The bioassays provide confirmatory evidence of toxic effects observed in the field trials. Overall, the residual exposure bioassays confirm the results of the contact/residual bioassays (Group 1); viz., that lime-sulfur and ATS have a significant effect, while dry flowable sulfur has little or none. Lime-sulfur and ATS have significant contact toxicity to all mite species, including *T. occidentalis*, while exposure to residues reduces either prey consumption, fecundity, or both. The net effect of these multiple toxic outcomes is likely to be moderate to severe, at least in the period immediately following application; the longer term impacts are yet to be investigated. In the small-plot field test, flowable sulfur, as well as ATS and lime-sulfur had some effect on *T. occidentalis*, but this may have been mediated through reduction of its prey, apple rust mite. In addition, temperature may have an effect on the toxicity of elemental sulfur, thus dry sulfur may in fact be more toxic at the higher temperatures in the field vs. the laboratory tests. Interestingly, the population of *T. occidentalis* used in this bioassay has developed complete tolerance to carbaryl, which was formerly quite toxic, and considered a major disruptant of integrated mite control (Hoyt 1965, 1969). However, tolerance on the part of *T. occidentalis* has been selected for over time, and was present in Washington *T. occidentalis* populations since the 1960s (Croft and Jeppson 1970, Babcock and Tanigoshi 1988). This underscores the dynamic nature of pesticide susceptibility in arthropod populations. Conversely, we have been using lime-sulfur for over 100 years in Washington, and while we have no baseline data, it is still acutely toxic to mites. There are some compounds that, by

virtue of mode of action, have little chance of causing selection in the target population. The caustic nature or breakdown products of these materials may prove to be an insurmountable barrier for development of a resistance mechanism both in pest and beneficial species.

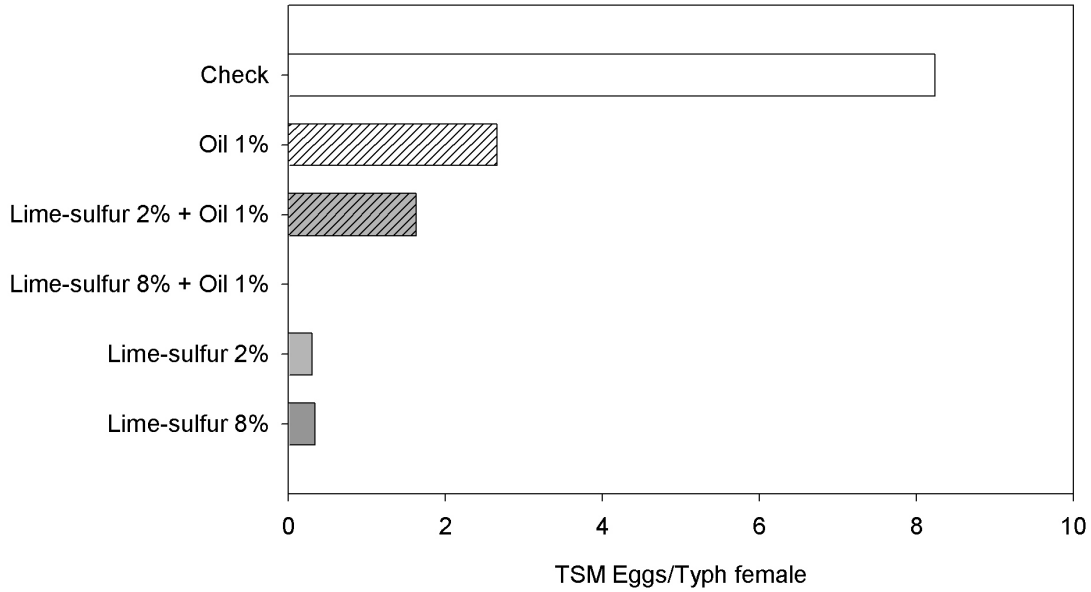

Fig. 6. Sublethal effects of lime sulfur with or without oil on prey consumption by *T. occidentalis* exposed to residues.

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Smith, T. J., J. E. Dunley, E. H. Beers, J. F. Brunner, G. G. Grove, C.-L. Xiao, D. Elfving, F. J. Peryea, R. Parker, M. Bush, C. Daniels, T. Maxwell, S. Foss, and S. Martin. 2007. 2007 Crop protection guide for tree fruits in Washington. Washington State University Cooperative Extension, Pullman, WA.

FINAL PROJECT REPORT
WTFRC Project Number: CP-07-708

Project Title: Improving apple IPM by maximizing opportunities for biological control

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State/Zip	WA 98951	**State/Zip:**	WA 98951

Other funding Sources

Agency Name: N/A
Amount awarded:
Notes:

Total Project Funding: (one year project only)

Budget History:

Item	Year 1:
Salaries	$82,489
Benefits	$20,445
Wages	$3,000
Benefits	$345
Equipment	$0
Supplies	$4,500
Travel	$2,021
Total	**$112,800**

Objectives

1. Determine the effects of thinning sprays on natural enemy abundance, diversity, and phenology.
2. Directly estimate predation intensity on codling moth and leafroller survival in different sprayed environments created in Objective 1 and determine which predators are the key sources of LR and CM biological control.
3. Evaluate the biological capacities of two tachinid species, which are the dominant parasitoids of leafrollers and the influence of habitats, season, and insecticides on their behavior and performance.
4. Develop phenology models for key natural enemies and integrate those data and the ranking of natural enemy importance and seasonality into WSU-DAS management recommendations.

Significant Findings

OBJECTIVE 1: EFFECTS OF THINNING SPRAYS ON NATURAL ENEMIES

- Thinning sprays had apparently only modest effects on tree-dwelling predators, and no noticeable effects on ground-dwelling predators

OBJECTIVE 2: ESTIMATE PREDATION INTENSITY ON CODLING MOTH AND LEAFROLLER

- There were no significant differences observed in predation induced mortality of cocooned codling moth larvae between the two orchard management treatments.
- Several carabid, spider and one "daddy long legs" species had codling moth remains in guts in late-summer and fall at sites having high densities of codling moth. Gut content analyses of mid-summer populations are ongoing and will continue through the winter.
- Three carabid beetle species, two spiders, and one "daddy long legs" species showed no interest in CM larvae in cocoons but they aggressively attacked and ate active larvae in lab studies.
- Free moving leafrollers proved unreliable for estimating predation rates, thus the effort to use sentinel leafrollers in evaluating predation was dropped early in the season.

OBJECTIVE 3: EVALUATE TACHNID SPECIES

- *Nilea erecta and Nemorilla pyste* have a different way of maturing eggs that may dictate their sensitivity to IGR pesticides in the adult stage
- Preliminary studies show damaged leaves + larvae + silk + frass have a greater attraction to *N. pyste* than diet + larvae + silk + frass
- Benzaldehyde added to sticky cards significantly increases trap catch opening the door for monitoring phenology and abundance as well as helping determine movement patterns of both Tachinid species.
- Intrepid and Esteem did not appear to be highly detrimental to immature tachinids, but results reported last year showed that females emerging from treated larvae were sterile. More work is needed to confirm results.

OBJECTIVE 4: DEVELOP PHENOLOGY MODELS FOR KEY NATURAL ENEMIES

- Timing of first appearance in orchards differed substantially among species of predators
- Trees were banded at 13 orchards to collect overwintering predators for emergence study (ongoing)

Results and Discussion

OBJECTIVE 1: EFFECTS OF THINNING SPRAYS

Thinning sprays were applied to 1 acre plots of non-bearing Red Delicious apples located at an orchard in west Yakima. We had four control plots and four treatment plots. Thinning sprays were made to treatment plots on April 28 and May 7 (2% lime sulfur + 2% rocker fish oil), and on May 23 (Sevin 4F; 3 pints per acre with 3 oz. NAA Fruit fix 200 K-salt in 100 ga.). Predatory arthropods were sampled using beat trays (45 per plot) for tree-dwelling predators, and pitfall traps (10 per plot) for ground-dwellers. Samples were taken at scheduled intervals beginning two weeks preceding the first lime sulfur spray, and extending to the last sample in late July. In October, 15 trees per plot were banded to assess overwintering densities of natural enemies.

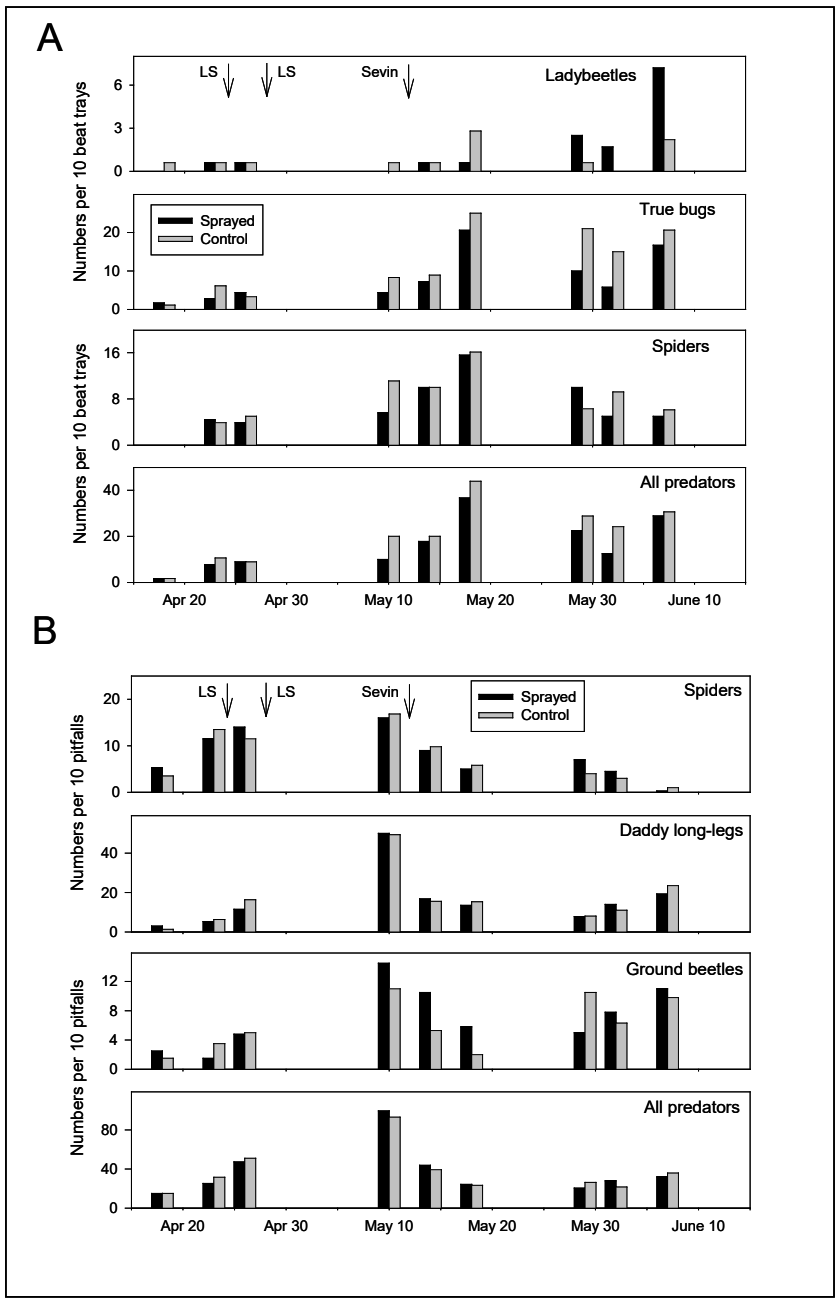


Fig. 1. Effects of lime-sulfur (LS) and Sevin thinning sprays on densities of tree-dwelling (A) or ground-dwelling predators (B).

Tray counts are shown for ladybird beetles, true bug predators, spiders, and all predators (Fig. 1B). The final sample taken on July 27 is not shown, to allow all bars to fit in the figure. There is a weak suggestion for true bugs and all predators that densities declined in the treatment plots (black bars), whereas the opposite was true for the ladybird beetles. If we plot cumulative counts of total predators through time (Fig. 2A), this slight decline in the treatment plots shows up more readily, and appears to coincide with the onset of the thinning sprays. Pitfall samples are shown for spiders, daddy long-legs, ground beetles, and all predators (Fig. 1B). There is no suggestion whatsoever that the thinning sprays affected numbers of these ground-dwelling predators (Fig. 2B). Overwintering predators in bands were dominated by spiders (Fig. 3); early season thinning sprays had no effect on eventual overwintering densities.

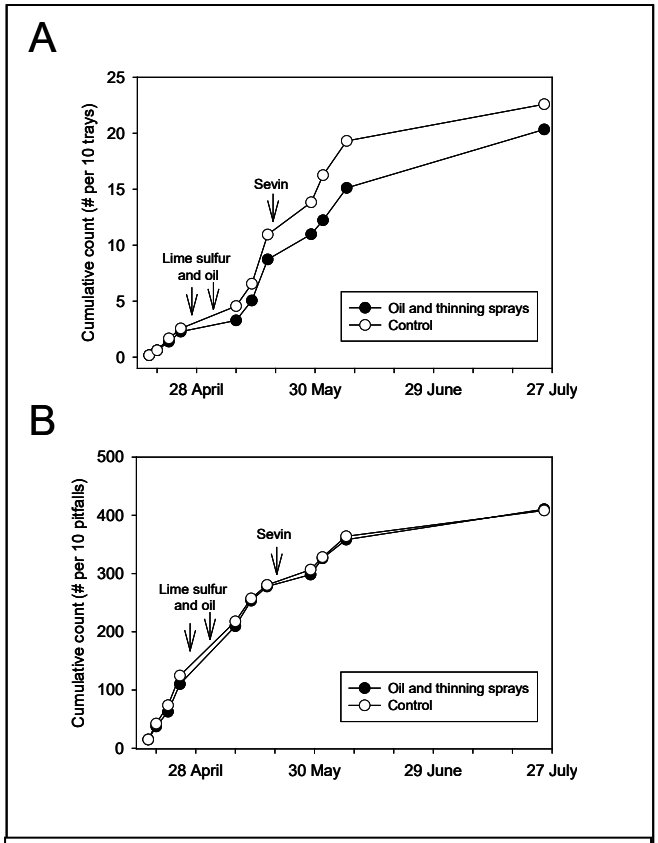


Fig 2. Effects of thinning sprays on cumulative numbers of tree-dwelling (A) and ground-dwelling (B) predators

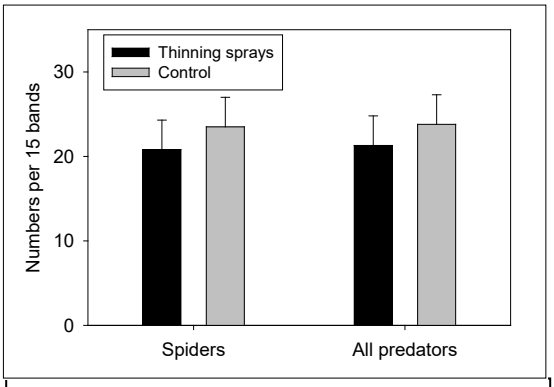


Fig. 3. Effects of thinning sprays on overwintering densities of predators

OBJECTIVE 2: ESTIMATE PREDATION INTENSITY ON CODLING MOTH AND LEAFROLLER

Diapausing male codling moth larvae that had cocooned in small wooden blocks were deployed in each of the eight plots used in the thinning spray experiment, with 18 blocks per plot, at five intervals: mid April until the first thinning spray, mid May, mid June, mid July and mid August, following a spray of Assail (in the thinning plots only) at the end of July. Predation was identified from larval remains showing signs of insect feeding, and holes cut in cocoons that are not characteristic of that caused by codling moth when abandoning the cocoon. Fig. 4 shows clearly that flower and fruit thinning sprays had no effect on codling moth predation. Similarly there was no effect of the late-July Assail spray on predation rates. The most noticeable pattern seen with the sentinel exposures was the appearance and dramatic increase of the category of codling moth disappearance we call 'removed' which was characterized by very clean-cut holes in the cocoons and codling moth larvae missing. Currently, we hypothesize that this removal was done by an as yet unknown spider or by social wasps. We came to this conclusion by providing sentinel blocks to those species of carabids and spiders that were readily collected in the pitfalls, and found that none of these species produced this signature of predation on cocoons. The overall effect of this removal category was to increase mortality from 20% early in the season to some 60-85% later (Fig. 5). Again there was no evidence for an effect of thinning sprays or a single Assail spray on mortality levels. Gut content analyses will be presented at the research review.

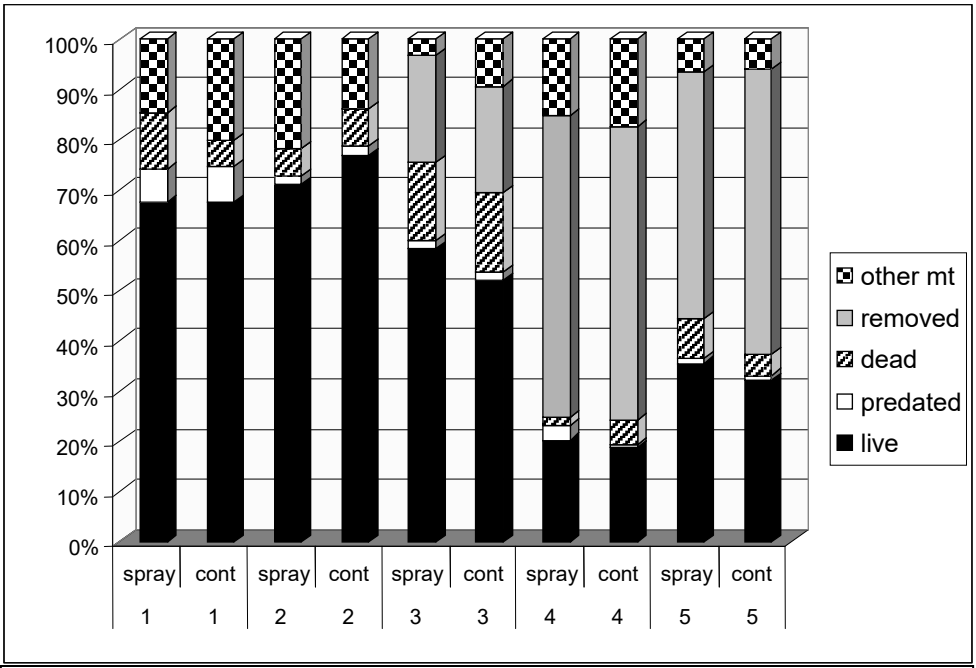


Fig. 4. Effects of lime sulfur spray (between interval 1 and 2), Sevin spray (between interval 2 and 3), and a single spray of Assail (between interval 3 and 4) on the survival and predation of cocooned codling moth larvae deployed in sentinel wooden blocks. The black bars are live larvae, white are dead larvae showing clear predation marks, dashed bars are larvae dead from unknown causes, gray bars are "removed" larvae accompanied by a cleanly cut hole in cocoon; other "mt" appears to be codling moth that left cocoon naturally.

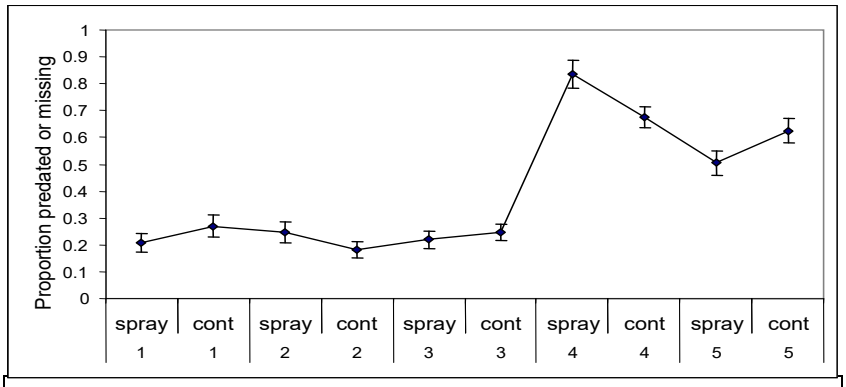


Fig. 5. Pattern of mortality of cocooned codling moth in sprayed and unsprayed plots during the season. Removal of codling moth through a characteristic hole in the coocons accounts for the upswing in mortality in period 4 and 5 (Jul, Aug).

OBJECTIVE 3: EVALUATE TACHNID SPECIES

Potential to limit OBLR population growth. We examined the development and incubation of eggs in the adult parasitoid because the rate at which parasitoids can produce eggs has implications to the amount of time needed for a parasitoid population to respond to an increasing or decreasing pest population. We found that *N. erecta* females emerge with approximately 500 eggs in each ovary (Fig. 6), but, *N. pyste* ovaries contain only 3-5 eggs at emergence (Fig. 7). Initial tests of reproductive rates with *N. pyste* have measured up to 16 eggs deposited in a 24 hr period, so the maturation of an egg from the basal cell must be relatively rapid. The differential ways that eggs mature are also potentially a key factor in susceptibility to IGR insecticides. For example, it is possible that *N. pyste* adults exposed to IGR's will not be able to mature eggs until residues decline. Conversely, it is also possible that *N. erecta* emerging from an intoxicated leafroller may all be sterile. Further work to answer these questions is required.

In parasitoids with similar modes of egg development, adult feeding is critical to egg production. Tachinid flies are primarily nectar feeders, so it is possible that relative distance to nectar sources could affect parasitism by this species in orchards.

Experiments are underway to determine how parasitoid egg production is affected by size and age of flies, and exposure to different host densities. This will provide the information necessary to understand the value of these parasitoids as mortality factors of OBLR in orchards in terms of parasitism rates, total reproductive potential, and ability to respond to OBLR outbreaks.

We also have begun to examine the modes of attraction of the flies into orchards. In general, flies are more visually oriented than other parasitoids, but their olfactory capabilities are also important. Among the key question we hope to answer is how the flies find their hosts in the environment. One possibility is that the flies key in on volatile compounds given off by larvae, larval products such as silk or frass, or damaged foliage.

Our approach is to begin evaluating the suite of potentially important odors simultaneously, and then remove individual components to try to isolate particular sources of attraction. These studies are performed in Y-tubes under wind current. One side of the tube has an odor of possible interest at the source, and the other side has a control odor. Initial trials were performed to try to work out reasonable timeframes for response and sources of flies that respond reliably. Responses can be affected by the intrinsic state of the insect, so age, mating status, and number of eggs can affect the results.

Fig. 6. The ovary of a 3-day-old virgin female *N. erecta* contains ~ 500 eggs, each measuring approx. 0.2 mm. One ovary is pictured.

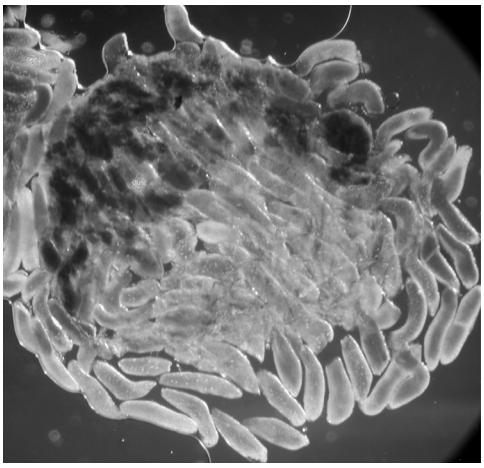
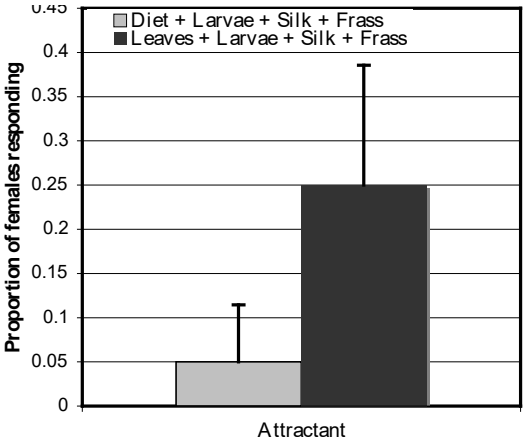


Fig. 7. The ovary of a 3-day-old virgin female *N. pyste* contains 3-5 eggs (arrow), each measuring approx. 0.7 mm. Both ovaries are pictured.

Fig. 8. Results of preliminary trials evaluating the attraction to hosts, host products and their associated odors to *N. pyste* females.



Our initial trials suggest that despite being reared from OBLR on artificial diet, *N. pyste* females are more responsive to the odors produced from the more natural association of damaged leaves, larvae, silk, and frass, compared to larvae, silk, and frass on artificial diet (Fig. 8). Response levels were relatively low, which raises the possibility that some aspects of these trials are suboptimal. However, *N. erecta* showed a response rate of 42 % to damaged leaves, frass, silk and larvae. This apparent disparity in the response rate of the two species is congruent with differences in the mode of attack of the two species. Because *N. erecta* oviposits on foliage near hosts, one might expect that this species is more adapted to detect host-associated odors. By contrast, *N. erecta* attacks hosts directly, and may be more visually attracted to hosts. Future work will address these issues.

Detecting Adults in the Field. The ability to detect parasitoids with a trap would provide an easier way to estimate their population levels, phenology, and potentially a way to detect parasitoids in non-orchard habitats to help determine which habitats are utilized. The literature provides several examples of potential plant volatiles that are released when insects feed on foliage. We tested squalene, which occurs in golden delicious foliage that has been damaged by leafminer, but does not occur when foliage is damaged mechanically. This suggests that squalene may act as a kairomone to attract natural enemies. Another compound, which has been reported by David James at WSU-Prosser to specifically attract tachinids in a different crop system, is benzaldehyde. This is a common aromatic compound that is associated with fruit and flowers.

Traps consisted of 2 ml of squalene, benzaldehyde. or water (for the controls) in glass vials attached to sticky cards which were hung from foliage. There were five traps representing each compound in a block with known tachinid population. Traps were refilled frequently and were rotated to avoid spatial bias. Benzaldehyde proved more attractive than either squalene or the control traps (Fig. 9), capturing specimens of both *N. pyste* and *N. erecta*. Squalene did attract significant numbers of green lacewings in this test,

Fig. 9. The number of tachinids attracted to sticky cards baited with benzaldehyde, squalene, and water.

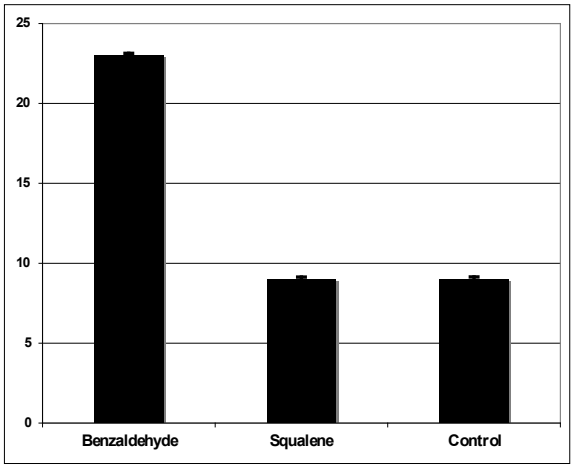


Fig. 10. Parasitism success of *N. pyste* attacking larvae in the three treatments.

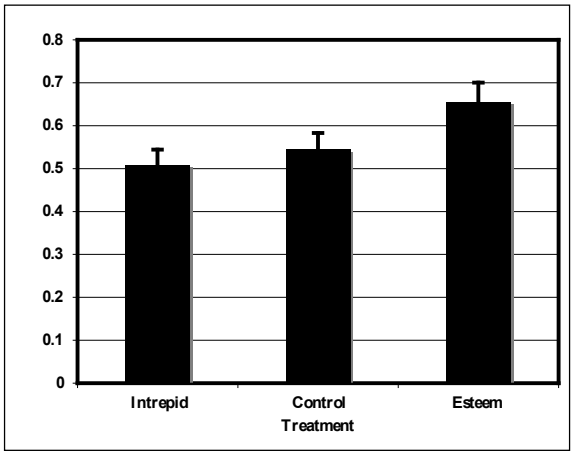
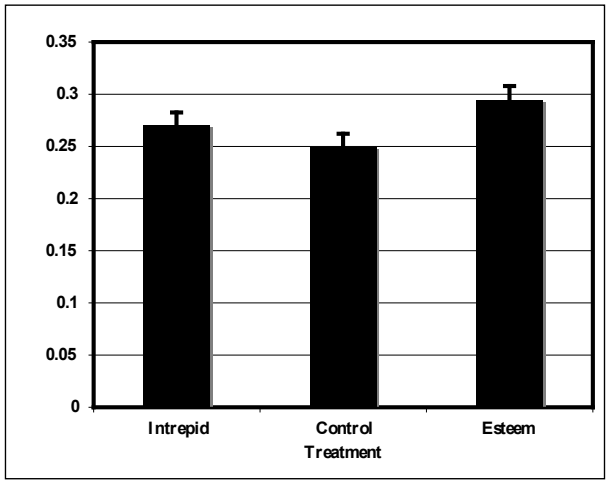


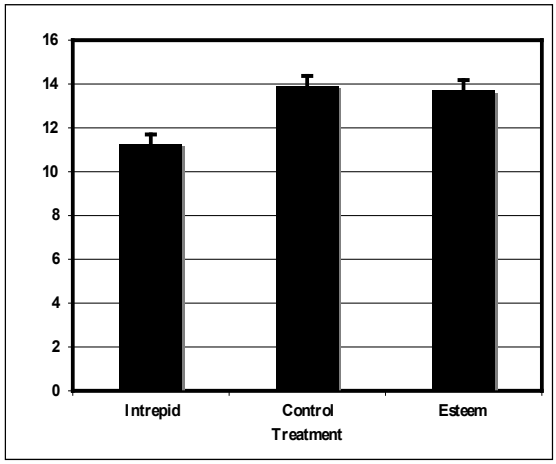
Fig. 11. Mass of *N. pyste* pupae reared from treated and control host larvae.



suggesting that it may be useful in monitoring lacewing populations.

Effects of IGR insecticides on Tachinid Parasitism. Late applications of IGR insecticides for leafroller coincide with the time when tachinids are most common. To provide an estimate of the impact, OBLR larvae from the WSU colony were force-fed on leaf discs treated with low doses of Esteem, Intrepid, or water. These larvae were then exposed to adult *N. pyste* for parasitism and their development tracked until the emergence of the adult fly. Last year we reported primarily on the effects of Esteem; this year we are able to provide data for Intrepid, and thus a more comprehensive comparison.

Fig. 12. The rate of development of the endoparasitic stages of *N. pyste* in treated hosts.



In terms of parasitism success, the maggots inhabiting OBLR that had been treated with a sublethal dose of Esteem were more likely to survive to the adult stage than were their counterparts in control or Intrepid-treated larvae (Fig. 10). This suggests that treatment of host larvae with Esteem actually made these larvae more favorable hosts for the flies. One potential explanation is that the host was weakened by intoxication, compromising the immunological response against the parasitoid, or the flies may have reacted favorably to the hormone analog itself. The flies reared from Esteem and Intrepid treated hosts were also significantly larger than control flies (Fig. 11). In terms of development time within the host (endoparasitic stages), the maggots that were in hosts treated with Intrepid took longer than those in control or Esteem-treated hosts to reach the pupal stage (Fig. 12), suggesting that this compound had a negative effect on their development. The development of adult structures took significantly longer when parasitoids were reared from Esteem-treated OBLR, but flies reared from Intrepid-treated hosts took roughly the same amount of time to mature as flies reared from untreated hosts.

While our experiments demonstrate that exposure to low doses of Esteem and Intrepid in the host are not very harmful to the immature stages of *N. pyste*, the adults may be very susceptible. Hormones are important regulators of the development of sexual function in insects, so it is not surprising that exposure to compounds such as Esteem and Intrepid, which are synthetic hormone analogues, might interfere with sexual development. Fertility effects of insect growth regulators have been demonstrated in a variety of insects, and *our research report last year suggested that exposure to Esteem in the host had a negative impact on the fertility of adult flies*. Effects of Intrepid exposure on adult fertility are currently being run.

OBJECTIVE 4: DEVELOP PHENOLOGY MODELS FOR KEY NATURAL ENEMIES

Two methods are being used to obtain quantitative information on early-season predator phenology, suitable for inclusion in the WSU decision aid system: (1) weekly tray samples in orchards; (2) monitoring emergence of overwintering predators. From the tray samples, there were distinct species' differences in timing of first appearance in orchards (Fig. 13). For instance, early arriving species included some ladybird beetles (*Microwesia*, *Stethorus*, *Hippodamia*), true bugs (*Deraeocoris*), and spiders (*Philodromus*, *Sassacus*). Later-arriving species included some ladybird beetles (*Coccinella septempunctata, Harmonia axyridis*, *Coccinella transversoguttata*), several true bugs (*Anthocoris* spp., *Orius tristicolor*, *Campylomma verbasci*), and *Chrysoperla plorabunda* (a green lacewing).

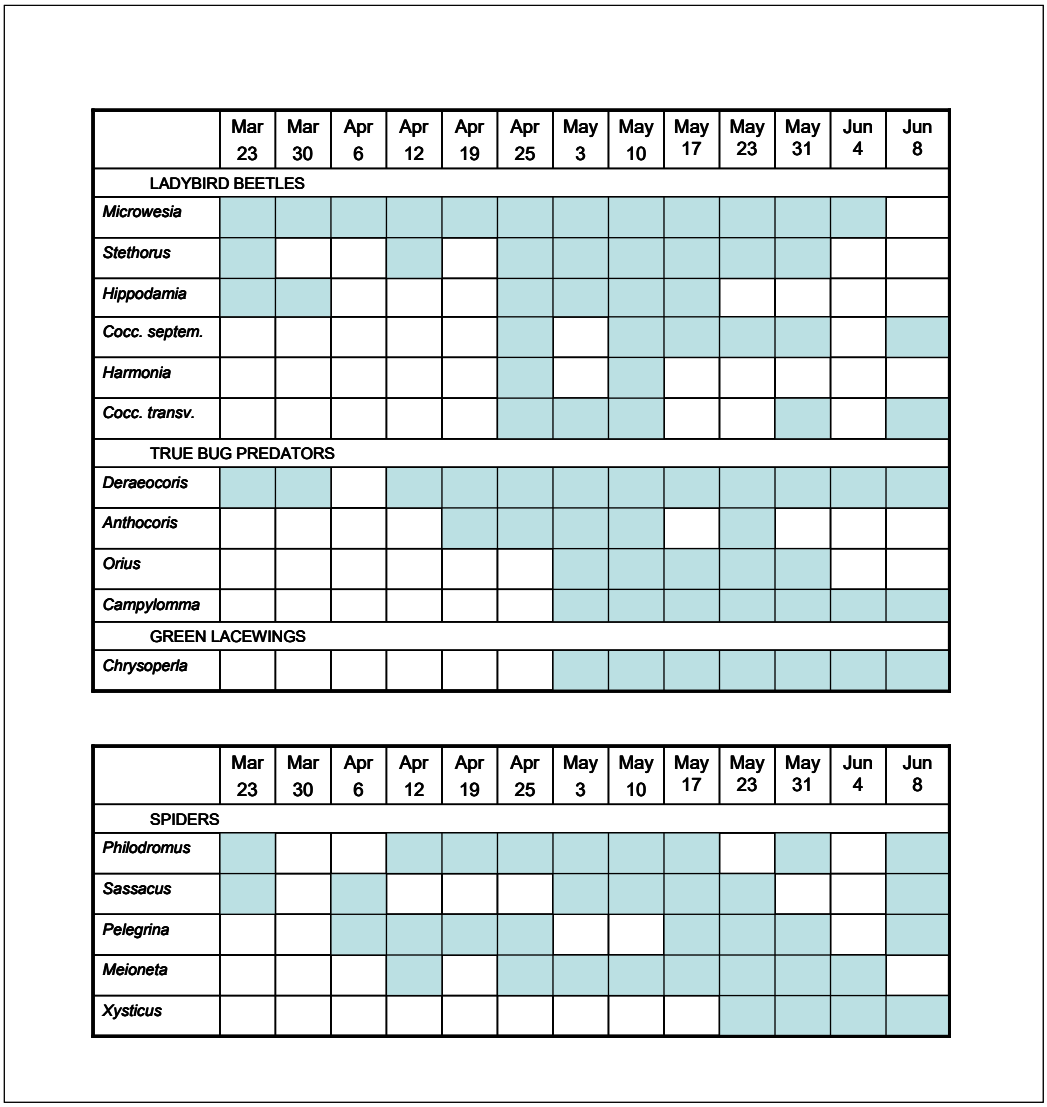


Fig. 13. Presence (indicated by filled squares) of predators in two apple orchards as determined by beating tray samples.

For the emergence study, we have banded trees at orchards in the Wenatchee area and the Yakima area. At the Wenatchee sites, we have set up eight different sites with 40 bands per site:

1. TFREC 5 (untreated)
2. TFREC 24 (untreated areas banded)
3. Columbia View 18 (mostly commercial sprays)
4. WSU-Sunrise (Organic Reds)
5. Wenatchee Valley College orchard (organic w/virus)
6. Brewster reds (organic w/virus)
7. Orondo Goldens (Commercial)
8. Mattawa Organic Galas (Organic w/virus)

At the Yakima sites, we have banded trees at 5 orchards, with 50-100 bands per site:

1. Mike Young goldens (organic)
2. Borton reds (soft, non-bearing)
3. Moxee mix of varieties (untreated)
4. Leach golden/red (organic)
5. Brooke reds (organic)

We will collect these bands before first snowfall and hold them in screened outdoor lean-to's. In late January, we will evaluate natural enemy emergence from the bands by checking them every 2-3 days. We have a data logger recording temperature in the containers so that we can determine emergence on a degree-day scale. The beating tray data (to be continued in 2008) will be used to confirm degree day models estimated from the emergence data.

At the Wenatchee sites, we also banded 25 trees in three orchards from late-June to the end of October at weekly intervals. We recorded the number of codling moth larvae, earwigs, lacewings, spiders, ants, and other insects. Besides codling moth, earwigs were the most common in all three plots and averaged just shy of 3 earwigs per codling moth collected over all three plots throughout the season. Spiders were the next most common, but were found at roughly 1/3 the number of earwigs and lacewings were roughly 18 times less common than earwigs. These data and others suggest that earwigs need to be more seriously evaluated for their role in BC. The amount of predation on CM may be high simply because there are so many earwigs that are found in similar locations to where CM larvae spin-up, but the numbers may be inflated because earwigs have an aggregation pheromone. This would tend to make the ratio of codling moth to earwigs a misleading statistic that would overestimate the importance of earwigs.

FINAL PROJECT REPORT **YEAR**: 1 of 1 (final report delayed 1 year)

Project Title: CSI in the orchard: finding the killers of 4 key apple pests

Co-PI:	T Unruh	**Co-PI(2):**	D Horton
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Cooperators: Eugene Miliczky, Nina Barcenas, Pablo Palmandez

Budget 1:
Organization Name: USDA-ARS
Contract Administrator: Bobbie Bobango
Telephone: 509-454-6575
Email: bobbie.bobango@ars.usda.gov

Item	Year 1: 2006
Salaries	$13825
Benefits	$ 7122
Wages	$0
Benefits	$0
Equipment	$0
Supplies	$0
Travel	$0
Miscellaneous	
Total	**$20947**

Footnotes: Supported Eugene Miliczky

Budget 2:
Organization Name: WSU-Entomology
Contract Administrator: Barb Smith
Telephone: 509-335-5504
Email: niehoff@wsu.edu

Item	Year 1: 2006
Salaries	$21243
Benefits	$ 2897
Wages	$0
Benefits	$0
Equipment	$0
Supplies	$0
Travel	$0
Miscellaneous	
Total	**$24140**

Footnotes: Supported Pablo Palmandez

Understanding the causes of pest insect mortality is critical to discovery of the best bio-rational methods to control these pests. Unlike death from disease or parasitoids, unwitnessed predation cannot be measured with confidence - the predator eats the evidence. The most unbiased measure of predation is direct observations of predators eating the prey (pest) of concern or direct measurement of a predator's consumption history based on physical or biochemical gut content analysis. This study was designed to perfect methods to determine key predators of four key pests of apples by detection of prey remains in the form of PCR of prey DNA in the predator's guts: codling moth, OBLR, woolly and rosy apple aphids. After a season of sampling the gut content analysis of the aphids was eliminated because the predators of these species were readily "caught in the act" through the season. The project has been plagued by difficulties in DNA extraction and contamination of reagents with amplification products. This has prompted plans to develop an antibody for codling moth. Several PCR primers were designed and optimized for the detection of codling moth and OBLR.

Objectives:

- **Design multiplex PCR methods to specifically detect DNA of codling moth, OBLR, woolly and rosy apple aphids in predator guts**
- **Collect predators throughout the season and the day/night cycle and use the method to estimate predation frequency and to rank predator importance**
- **Conduct laboratory feeding studies to help interpret data collected in the field**

Significant findings:

1. Primers designed for the CO-1 gene proved non-specific in the case of beetle predators of codling moth and OBLR. Additional primers were designed for the ITS-1and ITS-2 regions of codling moth and OBLR. These functioned well in multiplex format. Primers were not designed for the aphids.
2. A salt-based extraction protocol was developed to work with all predator species but was especially useful for large carabids, earwigs, and spiders where multiple expensive extraction columns would be required
3. DNA extraction and PCR proved to be too expensive($7/specimen) for high-throughput sample analysis. Progress was further hindered by sample contamination with PCR products. These two factors (expense and contamination by PCR product) has lead to the development of a LAMP (loop-mediated amplification) procedure which is still being optimized.
4. About 2,500 predators were collected from 6 orchard sites using pitfall traps, beat tray sampling and by direct collection. Only 10% of these were analyzed by PCR.
5. Feeding and digestion trials were conducted for a large, common carabid species *Pterostichus* sp. and the spiders *Cheiracanthium mildei* and *Holoena sp*. Prey signal retention exceeded 2 days in these species.

Results and discussion (by objective):

Objective 1*:* PCR primers were originally developed using the CO-1 gene based on comparative moth, beetle, lacewing, and bug sequences found in Genbank and DNA sequences for codling moth and OBLR developed in the lab. These provided high activity but many spurious bands in the carabid beetle predators. Thus primers were redesigned for internal transcribed spacer genes of the rDNA gene cluster (ITS-1 and ITS-2) based on DNA sequences for codling moth and OBLR developed in the lab. These eliminated cross reactivity problems with similar sensitivity.

Multiplex PCR methods were developed for codling moth and OBLR using ITS-2 primers. This allowed both species to be detected simultaneously in a single PCR amplification of a specimen, reducing by half the PCR portion of the expense of predator gut analysis. We also developed a rapid salt alcohol DNA extraction protocol but we still must dissect the gut from very large predators (large carabid beetles and spiders). For the large predators, the calculated expense for PCR of a single specimen was in excess of $7, $6 of which was labor. This expense prevented the analysis of the majority of collected specimens.

Objective 2*:* Predators were collected from 6 orchards using 3 major collection methods: beat tray samples, 24 hr pitfall trapping, and by visual search and collect. Six orchards were sampled and over 2,500 predators were collected. DNA analysis has just begun and is not reported here. The following narrative describes the orchard and provides a summary of predator abundance by predator type and by orchard Table 1).

Orchards:

1. Moxee fujis was a mixed variety block consisting primarily of Fujis in which various experimental insecticide treatments were applied in addition to herbicides. Our samples were taken in untreated parts of the orchard. Uncultivated land with mixed native and introduced plants surrounded this orchard on all sides. This block was heavily infested with Codling moth and we released OBLR larvae on sample trees on 2 dates.
2. Moxee small block consisted of 19 apple trees of various varieties bordered by pears and soft fruits on 3 sides and uncultivated land on the fourth. No insecticide treatments applied to the trees but early in the season the ground cover was sprayed with diazinon for ant control. and herbicides were used. This block was heavily infested with Codling moth in the second generation and we released OBLR larvae on 2 dates.
3. Mike Young's was a commercial 3 acre organic block of large golden delicious trees mostly bordered by other orchards with some exposure to uncultivated land. This orchard was very heavily infested with Rosy apple aphid but incidence of Codling moth was low due to mating disruption and repeated granulovirus applications.
4. Scott Leach's was a commercial organic block of red and golden delicious trees bordered by other orchard and an irrigation canal. The orchard had a moderate infestation of Rosy apple aphid and a low to moderate infestation of Wooly apple aphid, noticeable mostly on crown suckers. Codling moth incidence was low.
5. Wallace block was a nearly abandoned block of red and golden delicious trees bordered by other orchards and a highway. This orchard had received very little management for a number of years. The irrigation system was poorly maintained and the trees had not been pruned. Also, the orchard had not been mowed for more than a year and small trees of several species had established themselves in the understory. Codling moth and San Jose scale infestations were both ~100% on the fruit and there was a low to moderate infestation of Wooly apple aphid. Experimental trapping/monitoring of codling moth was being conducted in this orchard.
6. The Garza Office block is a former commercial organic orchard consisting of red and golden delicious trees now being used for experimental codling moth treatments. It was surrounded by other orchards and weedy, uncultivated land. We sampled in control plots where coding moth

levels were as high as 50% fruit damage. There was also a low level of Wooly apple aphid, primarily around the bases of the trees.

Table 1. Summary of 2006 predator collection data for CSI project. Abbreviations for expected prey: CM = codling moth; LR = leafroller; RAA = Rosy apple aphid; WAA = Wooly apple aphid. Abbreviations for type of sample: PT = pitfall trap; BT = beat tray; HC = hand collection; CB = cardboard band/bundle; SN = sweep net. Relative abundance of predators indicated by: - absent; + low abundance; ++moderate abundance; +++ high .

Orchard Number						
Parameter	1	2	3	4	5	6
Expected Prey	CM, LR	CM, LR	RAA	RAA, WAA	CM, WAA	CM, WAA(?)
Types of Sample	PT, BT, HC,CB	PT, BT, HC, CB	PT, BT, HC, SN	BT, HC	PT, BT, HC, CB	PT, BT, HC
Sampling period	June - August	June - October	June – July	May - July	July - October	August – October
Predator abundance	Moderate	Moderate to high	High	High	High	Moderate
# predators taken	100 – 200	> 500	> 500	300 -400	> 500	300 – 400
	Relative abundance of predator taxa collected					
Lacewings	+	+	+++	+++	++	-
Predatory bugs	+	++	+++	+++	+++	-
Lady beetles	+	+	++	++	++	-
Syrphid flies	-	-	++	++	+	-
Earwigs	+	+++	++	-	+	-
Ground beetles	++	+++	+++	-	+++	++
Rove beetles	+	++	+	-	+	+
Ants	+	+	-	++	-	-
Spiders	++	+++	++	++	++	++
Daddy-longlegs	-	+	+	-	+	+

Note on revised scope of objective 2 . Collections of predators in trees infested with Rosy apple aphid showed a very strong bias toward three predator groups in this order of abundance: lacewings>ladybeetles>syrphid flies> all others (mostly predatory bugs). Observations make it clear that these species are in curled leaves eating the aphids arguing against expensive assays of these predators. No primers were designed for the two aphids species and no PCR studies were conducted.

Objective 3. Evidence of predator feeding on our key pests must be interpreted based on knowledge of feeding frequency and digestion rates. We completed 2 laboratory feeding and digestion studies , a large carabid beetle, Pterostichus species, and one spider, Chieracanthium mildei. Predators were fed a single large codling moth larvae and allowed to digest up to 48 hr (beetle) or 96 hr (spider). Results were unambiguous, both these predators digest very slowly and prey signal is largely retained after 2 to 4 days (See Figure 1).

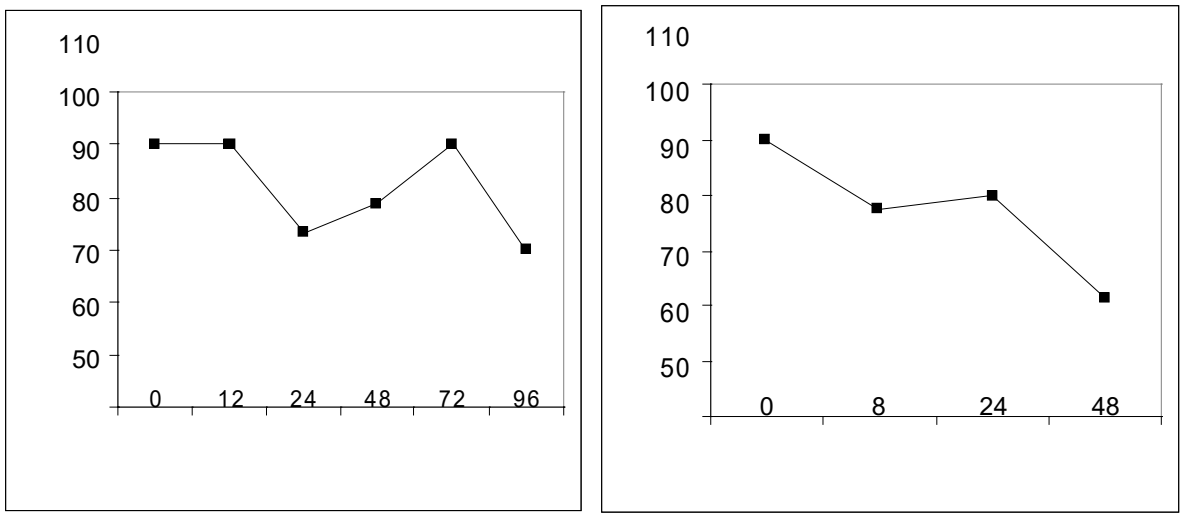


Fig. 1. Digestion pattern of a spider (C*heiracanthium milde*i) and a large carabid beetle (*Pterostichus* sp.) following feeding on a single large codling moth larvae. No signal was observed in controls.

Studies of insects in the field showed a strong association of positives with time of the season. Carabids collected early in summer showed no codling moth in their guts while those from late summer into fall, when codling moth are wandering, showed a high percentage. However, this percentage fell off dramatically later in fall. This is depicted in figure 2.

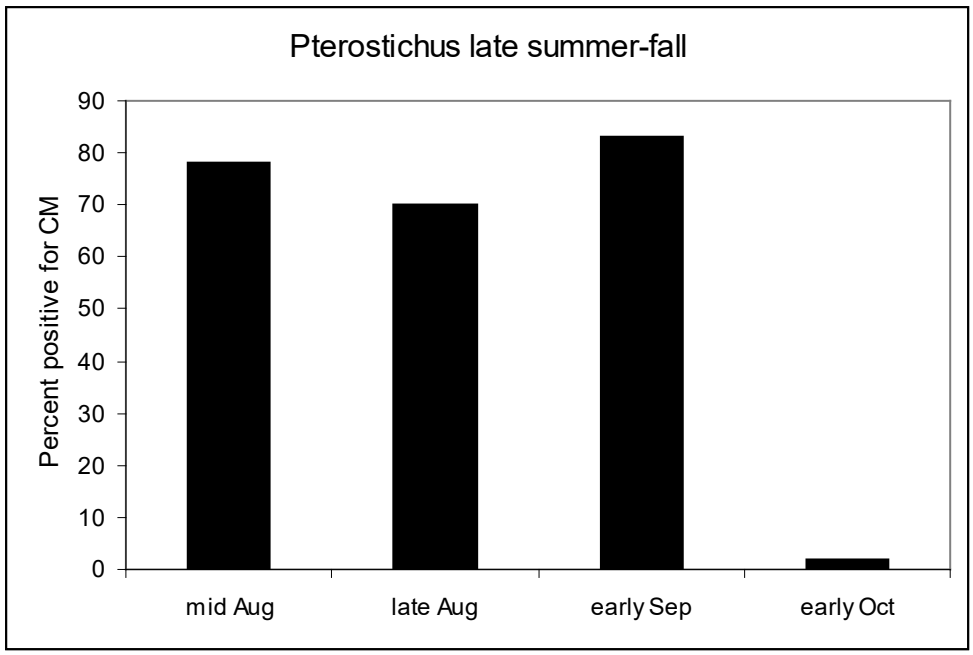


Fig.2. Late summer pattern gut contents of the carabid, Pterostichus, assayed for codling moth. A sharp decline is evident in late fall.

A similar pattern may be present with spiders and other predator groups but we have collected less data on these species. However, from individuals collected from Moxee and Wallace (high CM sites) during late August to early September, positives for codling moth in guts were high. Figure 3 summarizes data from over 50 predators (3 spiders types and earwigs).

Overall, our results suggest significant predation by both carabids (only the large carabids) and selected spiders and our data remains largely limited to the late summer early fall period when codling moth larvae are exiting fruit and seeking cocooning sites. See discussion of predator behavior below.

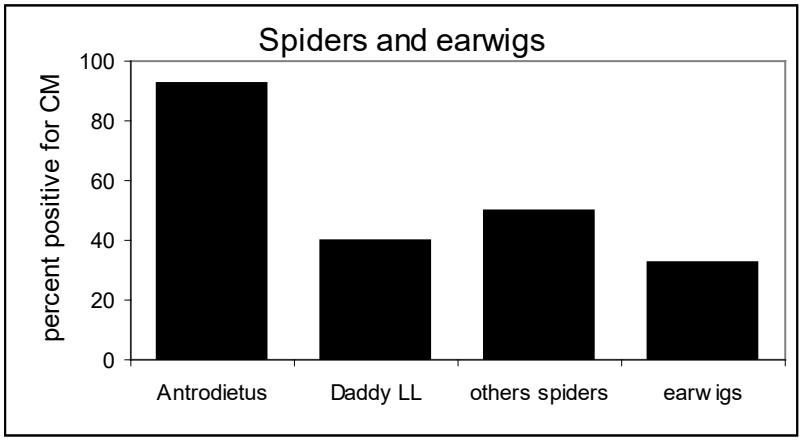


Fig.3 Gut content summary for the spider Antrodietus, the Opilionids (daddy long legs), 3 other spiders combined, and earwigs. (n>10 for each bar).

The future of predator gut-content analysis: methods and sampling issues

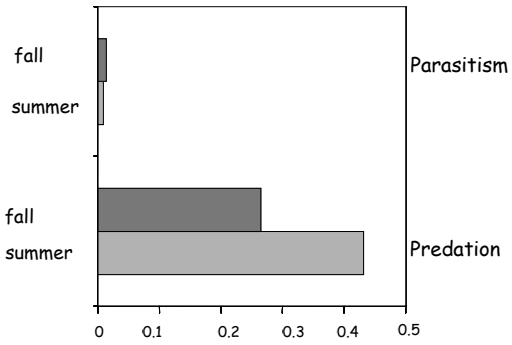
Recent studies in press suggest that PCR for gut analysis suffers several problems not found with the use of monoclonal antibodies (Fournier et al., submitted Oecologia). These are the high expense for both materials and labor for DNA extraction and the PCR assay itself and problems with repeatability of the PCR and cross contamination in labs doing high throughput PCR by PCR product. These problems do not exist with ELISA, but ELISA using monoclonal antibodies suffers from the high cost and time associated with developing the monoclonal(s). There are two alternatives to these problems which we are explorint

First, polyclonal antibodies need to be reconsidered. The original ecological applications of insect predator gut content analysis employed polyclonal, not monoclonal, antibodies whose non-specific fraction was removed by cross precipitation with non-target antigen (predator and non-target prey proteins). Since that time molecular biologists have helped develop and use polyclonal antibody produced against small antigens (short polypeptides) which can show high tissue and species specificity. This approach offers the potential to have high antibody specificity without investment of the time and money to develop monoclonals. Insect hemolymph contains several proteins (hexamerins, arylphorins, tyrosine oxidases) which are highly abundant and display regions of high divergence providing target domains for developing polyclonal antibodies.

A second approach is to detect DNA in predator guts using recently developed non-PCR methods. Of particular interest is the isothermal method called LAMP (loop mediated amplification procedure) which employs a polymerase enzyme that self-cycles at 60 C, and a chemical reaction that produces a visible precipitate (or fluorescent dye) allowing the determination of positive detection without the need to run a gel or visualize with a real-time instrument. Proponents of the method claim it is much more tolerant of dirty samples (potentially dramatically reducing extraction costs) and is equal to or more sensitive than PCR. This last point raises again the concern for cross contamination, but because no gels are run and the results can be seen through the closed tube, cross contamination and lab-wide contamination should be dramatically reduced. We have already developed two primer sets for the LAMP amplification of codling moth ITS-2. A third advantage of LAMP is that instead of using 40 base pairs of primer sequence the method employs four complex primers that cover 120-150 bases of the target sequence dramatically reducing the likelihood of cross reactivity with non-target sequences.

The final concern of gut content analysis is the issue of sampling the predator community. Recent studies in the laboratory have show that the carabid predators *Pterostichus* and *Harpalus*, the spider predators *Holoena,* and 3 other spider species captured in pitfall traps have shown that these predators do not consume codling moth larvae in their cocoons during exposures of 5 or more days. However, upon presentation of live, active codling moth larvae, all of these species showed aggressive and rapid attack and consumption of the larvae. These results together with evidence of predation of codling moth cocoons in the field in other studies (Figure 4), suggest that our pitfall trap captures may not be representative of key predators of codling moth.

Fig.4 Predation & parasitism of cocooned codling moth larvae in two commercial orchards



Analysis of gut contents of the predators collected herein will be expanded under the apple IPM project (Jones, Horton and Unruh) and will include evaluation of the peptide-polyclonal and LAMP approaches. Improved understanding of predators that attack codling moth in their cocoons will also be evaluated in the IPM project using video surveillance of sentinel larvae.

FINAL REPORT **DURATION**: 1 YEAR

Project Title: Apple maggot host attractants

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Budget 1:

Organization: USDA-ARS		**Contract Administrator:** Bobbie Bobango	
Telephone: 509-454-6575		**Email:** Bobbie.Bobango@ARS.USDA.GOV	
Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries	0		
Benefits	0		
Wages	$11,000[1]		
Benefits	$1,100		
Equipment	0		
Supplies	$1,500[2]		
Travel	$1,400[3]		
Miscellaneous			
Total	**$15,000**		

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

Budget note: $26,130 was also approved from the Washington State Commission on Pesticide Registration to support this work.

Budget 2:

Organization: Cornell University		**Contract Administrator: Donna Loeb**	
Telephone: 315-787-2325		**Email: drr2@cornell.edu**	
Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries	$9,916		
Benefits	$5,084		
Supplies			
Travel			
Miscellaneous			
Total	**$15,000**		

Objectives: The project objectives were to develop an effective lure for Washington apple maggot flies based on discrimination of host fruit odors.

1. We will collect flies for flight tunnel tests and will use traps baited with hawthorn and apple volatile blends in central and western Washington.
2. We will do a preliminary comparison of the behavioral responses, in a flight tunnel, of apple maggot from Washington and New York to odorants already identified from apple and eastern hawthorn fruits.

Proposed Schedule of Accomplishments

Time Line	Objective 1: Field tests of odorants	Objective 2: Wind tunnel tests
2007	Test identified eastern fruit volatiles in western and central WA	Preliminary tests of eastern fruit volatiles with WA flies

Significant Findings:

- In the field, apple fruit volatiles were attractive to Washington *Rhagoletis* flies, although traps baited with ammonia overall caught slightly more flies than those baited with apple fruit volatiles.

- In the field, eastern hawthorn fruit volatiles were usually not attractive to Washington *Rhagoletis*, but occasionally were attractive to flies in apple trees.

- Effects of fruit volatiles appeared to depend on whether the host tree was apple or hawthorn, with overall responses to the apple volatiles higher in apple than ornamental hawthorn trees.

- In flight tunnel tests, apple origin flies from Washington were more attracted to apple than eastern hawthorn volatiles.

- In flight tunnel tests, black hawthorn origin flies from Washington were highly active, but did not show high responses to either apple or eastern hawthorn volatiles.

- In flight tunnel tests, ornamental hawthorn origin flies from Washington also did not show high responses to either apple or eastern hawthorn volatiles.

Results and Discussion:

1. We will collect flies for flight tunnel tests and will use traps baited with hawthorn and apple volatile blends in central and western Washington.

Flies for future flight tunnel tests were collected in central Washington and western Washington in 2007. A total of 58 pupae was collected from black hawthorn fruit in central Washington, and a total of >2,000 pupae was collected from apple and a similar number from black hawthorn and ornamental hawthorn in western Washington. Sticky red sphere traps baited with apple volatiles, eastern hawthorn volatiles, and ammonium carbonate were deployed in central and western Washington to capture apple maggots. The following areas and host trees were trapped using these treatments:

Central Washington: Wenas Wildlife Area, Yakima County

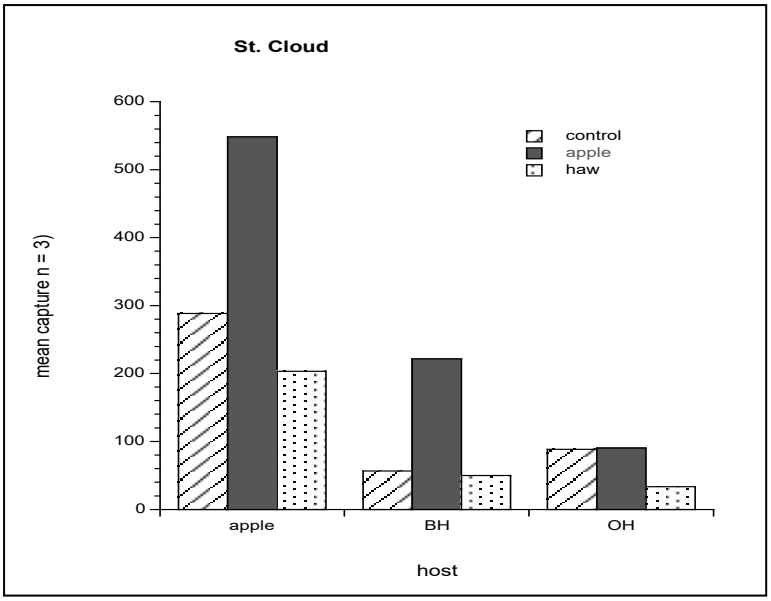
1) Host: Black Hawthorn (BH)

Western Washington: Saint Cloud Ranch, Skamania County
1) Host: Apple
2) Host: Black Hawthorn (BH)
3) Host: Ornamental Hawthorn (OH)
Western Washington: Washington State University (WSU), Clark County
1) Host: Apple
2) Host: Black Hawthorn (BH)
3) Host: Ornamental Hawthorn (OH)

Western Washington: Puyallup, Pierce County
1) Host: Apple, site 1 (Fruitland orchard)
2) Host: Apple, site 2 (Fourth street)
3) Host: Ornamental Hawthorn (OH)

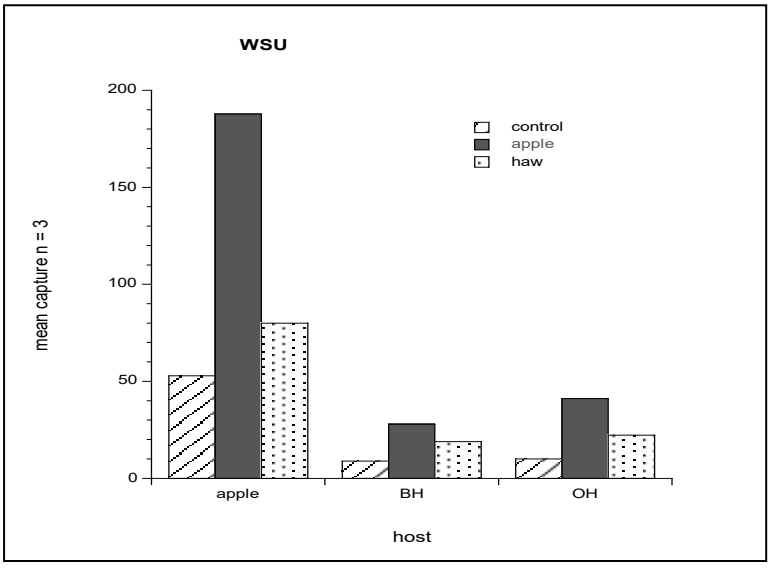
For each of the 10 site/host tree combinations, there were 3 or 5 replicates, arranged in a randomized block design. Traps were rotated among trees once or twice a week over 2 to 3 months. The numbers of flies caught on traps when grouped by host trees on which traps were hung are shown in Figs. 1-3.

Fig. 1. Numbers of apple maggot flies caught on red spheres baited with fruit volatiles at St. Cloud Ranch, WA, 2007



At St. Cloud (Fig, 1), the apple volatile-baited treatment caught more flies than the control on apple, but not statistically different numbers on black and ornamental hawthorn trees. The hawthorn volatile-baited treatment did not catch more flies than in the control.

Fig. 2. Numbers of apple maggot flies caught on red spheres baited with fruit volatiles at WSU, 2007.



At WSU (Fig. 2), the apple volatile-baited treatment caught more flies than the control on apple and on black hawthorn, but not statisticallydifferent numbers on ornamental hawthorn.

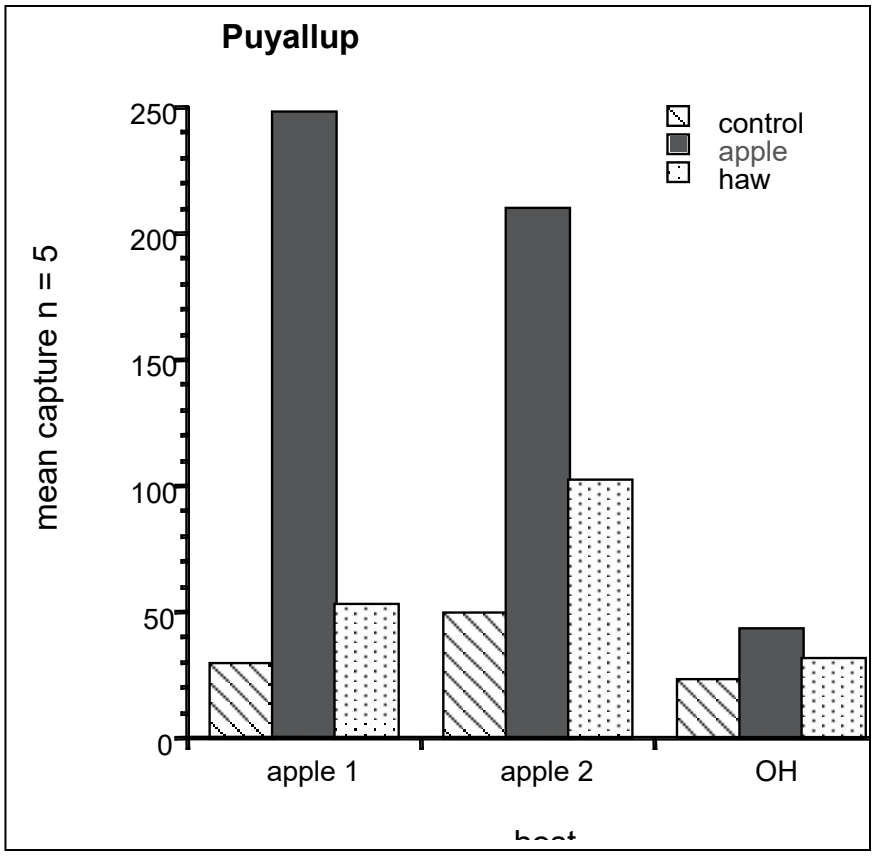


Fig. 3. Numbers of apple maggot flies caught on red spheres baited with fruit volatiles in Puyallup, WA, 2007.

In Puyallup (Fig. 3), the apple volatile-baited treatment caught more flies than the control on apple, although not on ornamental hawthorn. The hawthorn volatile-baited treatment caught more flies than the control on apple, but not on ornamental hawthorn. The apple volatile-baited treatment caught significantly more flies than the hawthorn volatile-baited treatment on apple.

Overall, the ammonia treatment caught more flies than the control at 3 of 4 sites, and more than the apple lure treatment at 1 of the sites. The apple lure treatment also caught more flies than the control at 3 of 4 sites, and more than the ammonia treatment at 1 site. The hawthorn lure treatment caught more flies than the control at 2 of 4 sites (both in Puyallup), but not more than the ammonia and apple lure treatments at any site.

2. We will do a preliminary comparison of the behavioral responses, in a flight tunnel, of apple maggot from Washington and New York to odorants already identified from apple and eastern hawthorn fruits.

We conducted wind tunnel tests in 2007 using flies that were reared from apple, black hawthorn, and ornamental hawthorn (apple, black hawthorn, and ornamental hawthorn origin flies) in 2006 or from the field in early summer 2007 (these were not the flies noted above that were collected in 2007 for future flight tunnel tests). Apple origin flies (Fig. 4) took flight more frequently in the presence of the apple than haw lure, and landed more frequently on apple- than haw-baited spheres, at about 20-40% versus about 0-20%. Black hawthorn origin flies (Fig. 5) took flight at high frequencies in the presence of either haw or apple lures, but the percent upwind flights over a 1 m distance and landing on the source by these flies on haw and apple lure-baited spheres was lower, at <15%. The percentages that took flight were higher than in apple origin flies. Ornamental hawthorn origin flies took flight at similar frequencies in the presence of the haw or apple lure, and the percent landings on haw and apple lure-baited spheres were low, at <20% (Fig. 6).

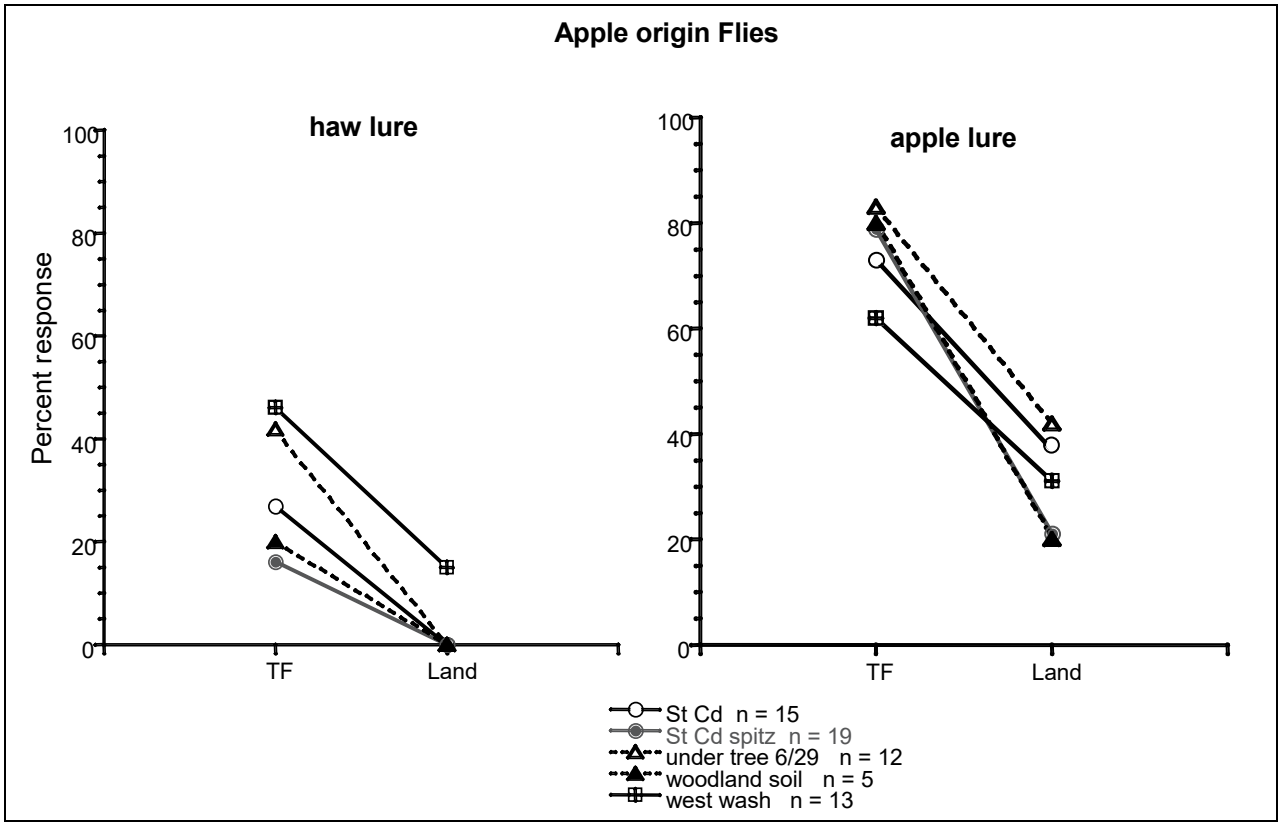


Fig. 4. Responses of apple origin flies collected in Washington to haw and apple lures on red spheres inside a flight tunnel. TF = take flight. Land = land on baited red sphere.

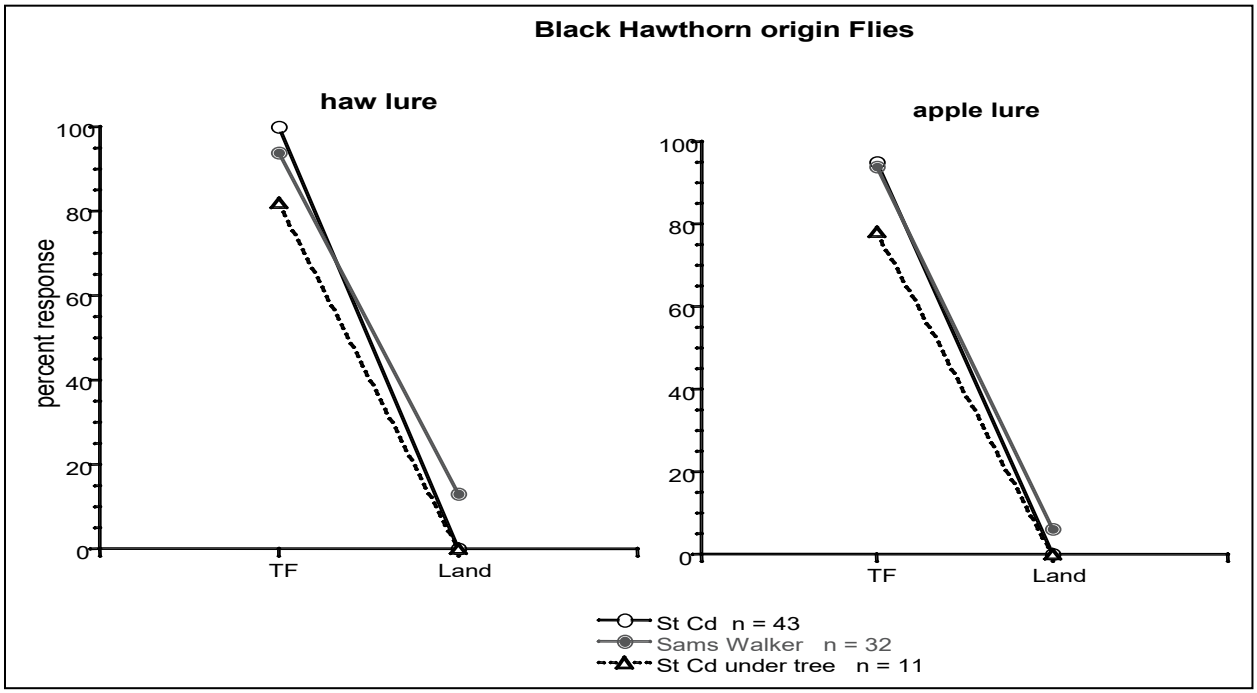


Fig. 5. Responses of black hawthorn origin flies collected in Washington to haw and apple lures on red spheres inside a flight tunnel. TF = take flight. Land = land on baited red sphere.

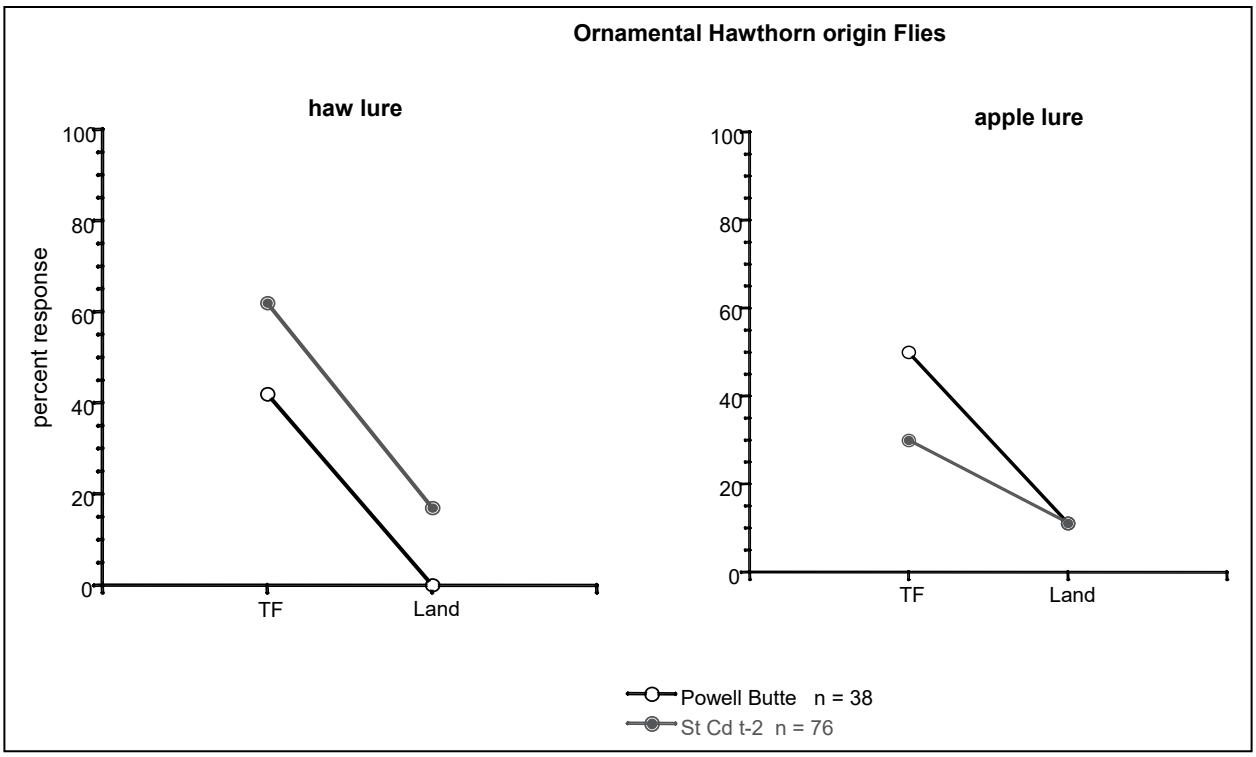


Fig. 6. Responses of ornamental hawthorn origin flies collected in Washington to haw and apple lures on red spheres inside a flight tunnel. TF = take flight. Land = land on baited red sphere.

Discussion

1. We will collect flies for flight tunnel tests and will use traps baited with hawthorn and apple volatile blends in central and western Washington.

Our results show that fruit volatile blends are attractive to *Rhagoletis* flies in Washington state. In particular, the apple volatiles attracted nearly as many flies as the ammonium carbonate lure. The eastern hawthorn volatile lure was usually not attractive, except in one instance where it was more attractive than controls on apple in Puyallup.

In the apple maggot survey and detection program conducted by the Washington State Department of Agriculture (WSDA), traps are placed in non-commercial apple, black hawthorn, and ornamental hawthorn trees for monitoring apple maggot, so our results have relevance to the program. In particular, we found that apple volatiles appeared to work best in apple trees. However, whether this was because apple volatiles in hawthorn trees resulted in apple-hawthorn volatile antagonism is unclear, because hawthorn volatiles in apple trees in Puyallup also were somewhat attractive.

In Wenas, in central Washington, numbers of flies caught on traps baited with apple lures in black hawthorn did not catch significantly more flies than the control or hawthorn lure. Whether a lure containing components of black and not eastern hawthorn odor would have attracted more flies in these hawthorn trees needs to be examined. We need to survey these low population areas and develop a black hawthorn specific lure to compare with apple and ammonia.

Host effects may also be caused by different seasonal phenologies of the hosts' fruit development and the interaction of their odors when the plants are near one another. Apples and black hawthorn fruit generally ripened earlier than ornamental hawthorn fruit, but different apple varieties used in tests ripened at different times and there appeared to be much overlap in ripening times of apple and black hawthorn. Possibly the mix of odors from different hosts when hosts occurred together or the lack of some odors when hosts were more isolated affected the attractiveness of the apple or hawthorn lure.

A major benefit in using fruit volatiles rather than ammonia is that snowberry maggot flies were not attracted to the apple fruit volatiles, whereas they were attracted to ammonia. Snowberry maggot flies look nearly identical to apple maggot flies and are abundant in central Washington. They were commonly caught on red spheres baited with ammonia, but not with apple volatiles, hung in hawthorn trees. Because the two flies are so similar in appearance, in regulatory work it is necessary to look at each fly under a microscope to examine it for morphological differences. This is a time consuming process. The ideal attractant blend for apple maggot, therefore, is one that is highly attractive but yet does not attract too many non-target flies and snowberry maggot flies. One possibility is an attractant blend based on volatiles from black and not eastern hawthorn fruit.

Our results with the eastern hawthorn fruit volatile lure showed it was not or only slightly attractive in some cases to Washington apple maggot flies. Why they were only occasionally attractive is unknown. Possibly this was because flies in particular areas were broadly responsive. In the eastern U.S., the hawthorn volatiles tested attracted many flies on hawthorn, suggesting there are genetic differences between western flies on apple and hawthorn and eastern fly populations on hawthorn.

2. We will do a preliminary comparison of the behavioral responses, in a flight tunnel, of apple maggot from Washington and New York to odorants already identified from apple and eastern hawthorn fruits.

The behavioral responses of flies reared from apple and from black and ornamental hawthorn toward haw and apple lure odors differed. Apple origin flies (Fig. 4) responded more to apple than haw lures, consistent with field trapping results showing there are attractive components in the apple lure. However, while high percentages of black hawthorn origin flies took flight and were therefore very active flies, few of these flies were attracted to either haw or apple lures (Fig. 5). Taken together with results with apple origin flies, it appears there might be a host effect on responses to fruit odors. Although the percentages of ornamental hawthorn origin flies that took flight were lower than of black hawthorn origin flies, these flies also did not appear to respond to haw and apple odors (Fig. 6).

Future work should (1) confirm if there are true differential responses of flies to the apple lure among apple and different hawthorn trees; determine (2) whether the apple lure is effective with traps in central and western Washington if traps are replaced only once or twice during the season; (3) whether volatiles from black hawthorn in Washington are attractive to flies reared from black hawthorn; and (4) whether a lure with volatiles from black hawthorn fruit would be effective and more selective for apple maggots in Washington than the apple lure, when used in apple and black and ornamental hawthorn trees.

FINAL REPORT **DURATION**: 1 year

Project Title: DNA and morphometric diagnosis for apple and snowberry flies

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Cooperators: None

Organization: USDA-ARS		**Contract Administrator:** Bobbie Bobango	
Telephone: 509-454-6575		**Email:** Bobbie.Bobango@ARS.USDA.GOV	
Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries			
Benefits			
Wages	12,700		
Benefits	1,270		
Equipment			
Supplies	800		
Travel	230		
Miscellaneous			
Total	**$15,000**		

Budget 2:

Organization: University of Notre Dame		**Contract Administrator:** Rick Hilliard	
Telephone: 574-631-5386		**Email:** Hilliard.1@nd.edu	
Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	9,000		
Travel	1,000		
Miscellaneous			
Total	**$10,000**		

Objectives:
Our objectives are to discover diagnostic morphological and molecular differences between apple and snowberry flies and to measure genetic differences among Washington populations of the apple maggot fly to detect possible hawthorn and apple host races and to support quarantine and remediation programs in Washington.

1. Re-evaluate morphometric variation between apple and snowberry maggot
2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS
3. Determine if host races of apple maggot exist in Washington using microsatellite variation.

Proposed Schedule of Accomplishments:

Objective	2007
1. Morphometrics	Rear flies from apple and snowberry; shape measures determined
2. ISSRs/RAPDS	Primers screened on 10 flies of each species
3. Microsatellites	Initial genotyping of flies from WA and eastern U.S.

Significant Findings:

● Various body structures of apple and snowberry maggot flies, including the ovipositors, generally were larger in apple maggot flies, but there was overlap in all of measures.

● Body measures within apple maggot flies depended on the location or host origin.

● Wing shape analysis was a highly promising method to separate apple maggot from snowberry maggot flies; wings of the two have different shapes based on principal components and canonical variates analyses.

● Twenty four RAPD primers were surveyed on composite and individual insects and of these five show promise for diagnostic gene regions. Additional primers are being analyzed.

● Use of microsatellite genetic markers suggests significant differences exist between apple maggot flies reared from apple and hawthorns in Washington.

● 60 PCR primers developed to amplify microsatellite loci for eastern populations of apple maggot fly also worked for western flies from Washington State.

● Six of the eight loci (all except p71 and p18) displayed significant allele frequency differences among the apple, black hawthorn, and ornamental hawthorn populations ranging on the order of from 10 to 25%. These data are consistent with the existence of apple, black hawthorn, and ornamental hawthorn host races in Washington state.

Results and Discussion:

Results
1. Re-evaluate morphometric variation between apple and snowberry maggot flies.
Body Measure Analyses. Apple maggot and snowberry maggot flies reared from different hosts and from different areas in Washington differed in body measurements (Table 1). Apple maggot flies from apple from western Washington (Vancouver and Skamania) did not differ, except in wing length, and usually did not differ from apple maggot flies from ornamental hawthorn from Puyallup.

Apple maggots from all sources, however, were in general larger based in body measurements than snowberry maggots. Snowberry maggots from central Washington generally had larger body measurements than snowberry maggots from western Washington (Table 1). Although apple maggots were larger than snowberry maggots, there was considerable overlap in body measurements, for example, in wing lengths (data not shown). There was also overlap in ovipositor lengths (Fig. 1). Principal component analyses (PCA) using the 9 body measurements resulted in significant species effects: comparisons of means of principal component 1 show that apple maggots differed from snowberry maggots. Apple maggots from apple from western Washington (Vancouver and Skamania), from apple from Puyallup, and from ornamental hawthorn from Puyallup did not differ, and differed from apple maggots from ornamental hawthorn and from black hawthorn in western Washington. Snowberry maggots from central and western Washington also differed in means of principal component 1.

Wing Shape Analyses. Inspection of representative wings (Fig. 2) suggests the apple maggot wing is narrower and less rounded apically than the snowberry maggot wing. PCA using the 14 landmark data resulted in significant species effects for principal component 2 * principal component 1 (Fig. 3), showing that the wings of apple and snowberry maggots differ significantly in shape. Canonical variates analysis (CVA) for variate 2 * variate 1 using the landmark data (Fig. 4) resulted in even clearer separation of differences in the wing shapes of snowberry and apple maggot flies.

2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS.
We screened 24 RAPD primers with Qiagen extracted DNA from Snowberry (SB) and Western Apple Maggot (WAM). To screen the primers a male and female from Snowberry and Western Apple Maggot were selected for PCR. If there was a difference in banding pattern then that primer was used again for three males and three females from SB and WAM. Of the 24 RAPD primers we found 6 to be too light or negative, 13 with the same banding pattern across species, and 5 that look promising by showing unique bands in one or more individuals of one of the two species. We are currently continuing to evaluate those five primers with additional individuals of each species to clarify the results. No additional funding will be required for the evaluation of the RAPD variation; however, during 2008 we will examine additional RAPD primers and several ISSR primers**.** Apparently diagnostic bands will be TA cloned, sequenced, and primers designed and tested**.**

3. Determine if host races of apple maggot exist in Washington using microsatellite variation.
One goal of the study was to score apple maggot flies collected from apple and hawthorn in Washington for microsatellite genetic markers to assess where host races of the fly exist in the state. The one year of funding allowed us to determine that all of the 60 pairs of PCR primers developed to amplify variable microsatellite loci for eastern populations of apple maggot, also worked for western flies from Washington State. A subset of eight loci were scored for approximately 25 flies each collected from apple, black hawthorn, and an ornamental hawthorn host in Washington (These eight loci are designated p71, p17, p11, p80, p39, p25, p18, and p13). Six of the eight loci (all except p71 and p18) displayed significant allele frequency differences among the apple, black hawthorn, and ornamental hawthorn populations ranging on the order of from 10 to 25%.

Discussion

1. Re-evaluate morphometric variation between apple and snowberry maggot.
Body Measure Analyses. Results in this study indicate that apple maggots are larger than snowberry maggots in Washington in all body measures and that there may be differences even within the species depending on the host and region in which fruit are collected. Size measures alone can separate the species. Some characters such as the head width and ovipositor lengths seem the least

variable of the structures measured, but graphical analyses indicate that there is considerable overlap in lengths of structures, including the ovipositor. Wasbauer (1963) and Wescott (1982) found that ovipositor lengths were longer in apple maggot than in snowberry maggot female flies. Wescott (1982) stated that there was a very small overlap in ovipositor lengths of flies from Oregon, with only five flies in the 'problem area' of 0.88-0.98 mm. Our data show that such overlap is not uncommon.

Wing Shape Analyses. Our results represent the first demonstration of differences in wing shape between apple and snowberry maggots. In particular, the apple maggot wing appears longer and narrower than that of the snowberry maggot. In cases where body measurements do not separate the species, wing shape may be used to separate them. An advantage of using wing shape over body measures may be that possible fruit quality effects on body size are reduced or eliminated; fruit quality unlikely affects wing shape. Free computer software from the State University of New York (SUNY) Stony Brook is available that can be used for wing shape analysis (website: http://life.bio.sunysb.edu/morph). In particular, the videodigitizing program TPSDig and the program CVAGen can be used for this purpose. CVA is a method that can be used to find axes along which fly groups are best discriminated. Scores for individuals can be used to assign unknown species to groups. Scores are then plotted to depict the distribution of specimens along axes. Use of such a technique may help solve the problem of difficult to identify flies that could affect quarantine decisions in commercial apple growing areas. Further work will be conducted to determine if wing shape analysis can identify flies that have body measurements that fall in overlap regions of frequency distributions. Also, the results using PCA and CVA need to be hold up to larger sample sizes. Inclusions of more samples in analyses are planned for the upcoming year.

2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS. Previous studies (Barcenas, Unruh, Yee) that examined sequence differences in a region of the mitochondrial gene CO-1 and in intron 1 of the nuclear gene, elongation factor alpha, show the potential to provide 95% or greater classification of snowberry maggot versus Western apple maggot. However, 95% is not diagnostic. A similar level of classification capacity has been recently reported by Jim Smith (Michigan State University), Mike Klaus (WSDA) and colleagues using amplified fragment length polymorphisms. We believe it is possible to discover a gene region or regions that show greater classification capacity and may be diagnostic in the absence of ongoing or a recent history of cross mating between these species. Further work will be conducted with RAPD and ISSR markers to discover such a marker. This will entail analyzing the five promising RAPD primers with larger numbers of flies collected from throughout the state and testing 6 ISSR primers. This work will be supported on base funding.

3. Determine if host races of apple maggot exist in Washington using microsatellite variation. Microsatellite data are promising and consistent with the existence of apple, black hawthorn, and ornamental hawthorn host races of apple maggot in the state. However, further work is needed to increase the numbers of sample sites surveyed, the numbers of flies genetically scored, and the number of microsatellite loci analyzed at sites to confirm the findings suggesting apple and hawthorn host races in Washington state and to evaluate their source of origin. In particular, we now must concentrate on collecting and genetically scoring a series of sympatric sites from across Washington in which flies attacking apples, native, and ornamental hawthorns co-occur in close proximity. If these populations display the same pattern and degree of genetic differentiation displayed in the preliminary analysis, then the host race hypothesis will be verified. In addition, the success of the markers in potentially resolving frequency differences in what should be closely genetically related races of apple and hawthorn populations of apple maggot, provides a basis for expanding the analysis to snowberry maggot to determine whether the microsatellite loci can help distinguish these two taxa from each other.

Table 1. Mean measurements (± SE) (mm) of various structures of female apple maggot fly and snowberry maggot fly from different hosts and sites in Washington

	Host, AREA	Head Width	Inter-eye width	Wing Lngth	Wing Band 2	Wing Band 3	Hind Femur	Hind Tibia	Hind Tarsus	Ovipositor Length
AM	Apple,	1.70	0.66	4.38	0.82	0.40	1.45	1.29	1.33	1.03
(n = 40)	Vancouver WA	(0.01)a	(0.01)a	(0.04)b	(0.01)b	(0.01)a	(0.01)a	(0.01)a	(0.02)a	(0.02)a
AM	Apple,	1.73	0.64	4.50	0.84	0.40	1.45	1.31	1.36	1.03
(n = 58)	Puyallup	(0.004)a	(0.004)ab	(0.01)a	(0.006)ab	(0.002)a	(0.005)a	(0.005)a	(0.005)a	(0.004)a
AM	Ornmtl. Haw.	1.52	0.56	3.93	0.70	0.35	1.26	1.10	1.18	0.99
(n = 50)	Vancouver	(0.02)c	(0.008)d	(0.05)d	(0.01)d	(0.008)b	(0.02)c	(0.02)b	(0.02)b	(0.01)b
AM	Ornmtl. Haw.	1.70	0.63	4.44	0.86	0.40	1.42	1.28	1.35	1.02
(n = 40)	Puyallup	(0.01)a	(0.006)bc	(0.03)ab	(0.01)a	(0.008)a	(0.01)a	(0.01)a	(0.01)a	(0.01)ab
AM	Black Haw	1.61	0.60	4.20	0.76	0.33	1.32	1.15	1.21	1.05
(n = 10)	Vancouver	(0.03)b	(0.02)c	(0.08)c	(0.03)c	(0.02)b	(0.03)b	(0.03)b	(0.02)b	(0.02)a
SB	Snowberry	1.38	0.51	3.62	0.74	0.36	1.17	1.04	1.12	0.79
(n = 66)	C. WA	(0.01)d	(0.004)e	(0.03)e	(0.01)cd	(0.01)b	(0.01)d	(0.01)c	(0.01)c	(0.01)c
SB	Snowberry	1.32	0.50	3.53	0.68	0.35	1.12	0.98	1.08	0.74
(n = 28)	Vancouver	(0.02)e	(0.01)e	(0.05)e	(0.01)d	(0.01)b	(0.02)e	(0.01)c	(0.01)c	(0.01)d
One-way ANOVA[a] *F*		135.1	72.0	107.7	37.0	9.2	103.1	90.2	68.4	128.3
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

AM, apple maggot; SB, snowberry maggot;
[a]d.f. = 6, 285.
Means followed by same letters are not significantly different (LSD test, $P > 0.05$).

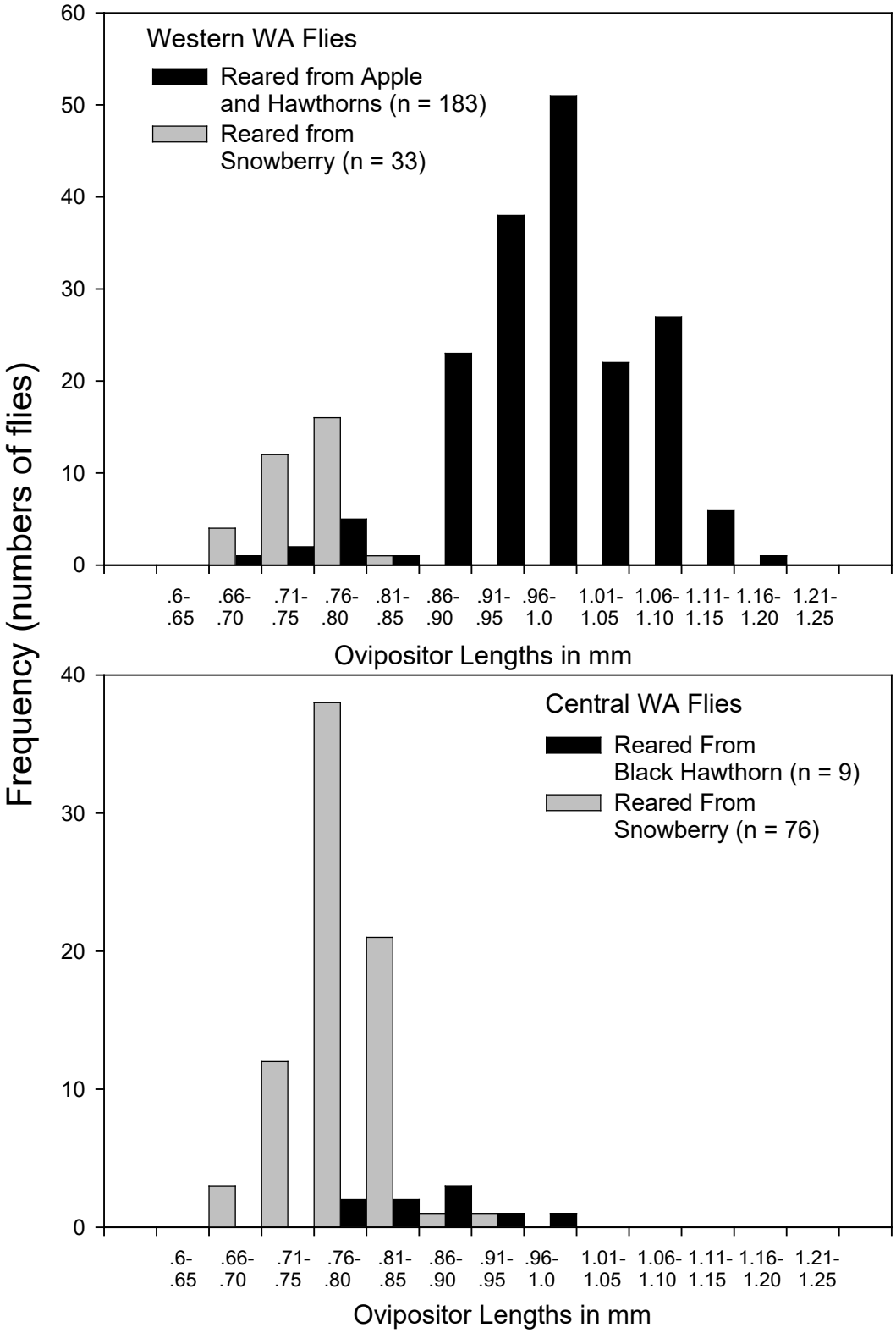
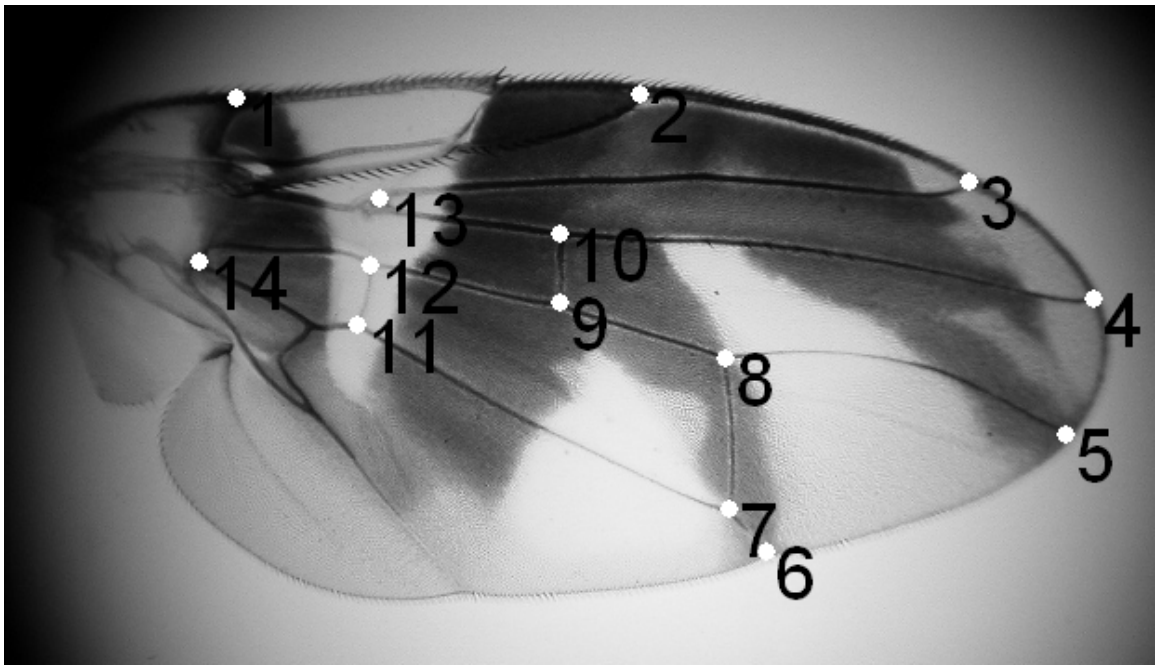


Fig. 1. Distributions of ovipositor lengths of snowberry and apple maggot flies.

A



B

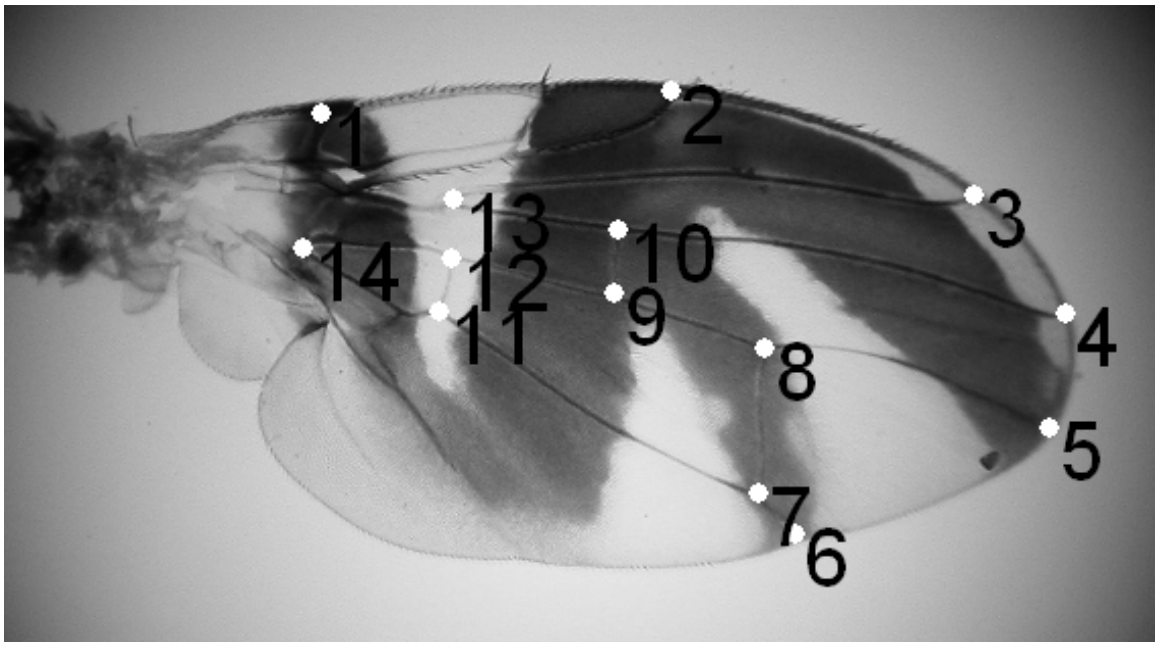


Fig. 2. Right wings of female (A) apple maggot (from western Washington) and (B) snowberry maggot (from central Washington), showing the 14 landmarks used for analyses.

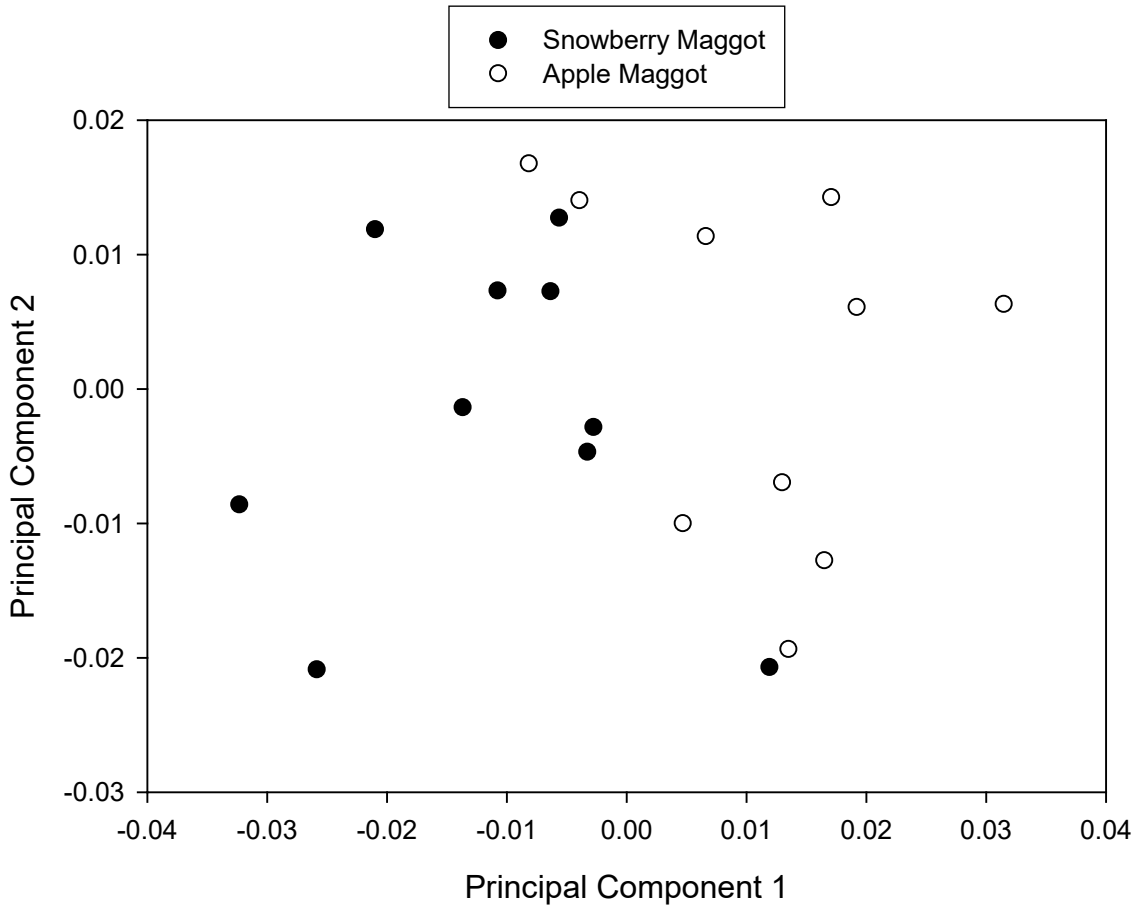


Fig. 3. Principal components analysis (PCA) of fly wing shapes: Plot of principal component 2 * principal component 1. Each X_1, X_2 value is from one fly.

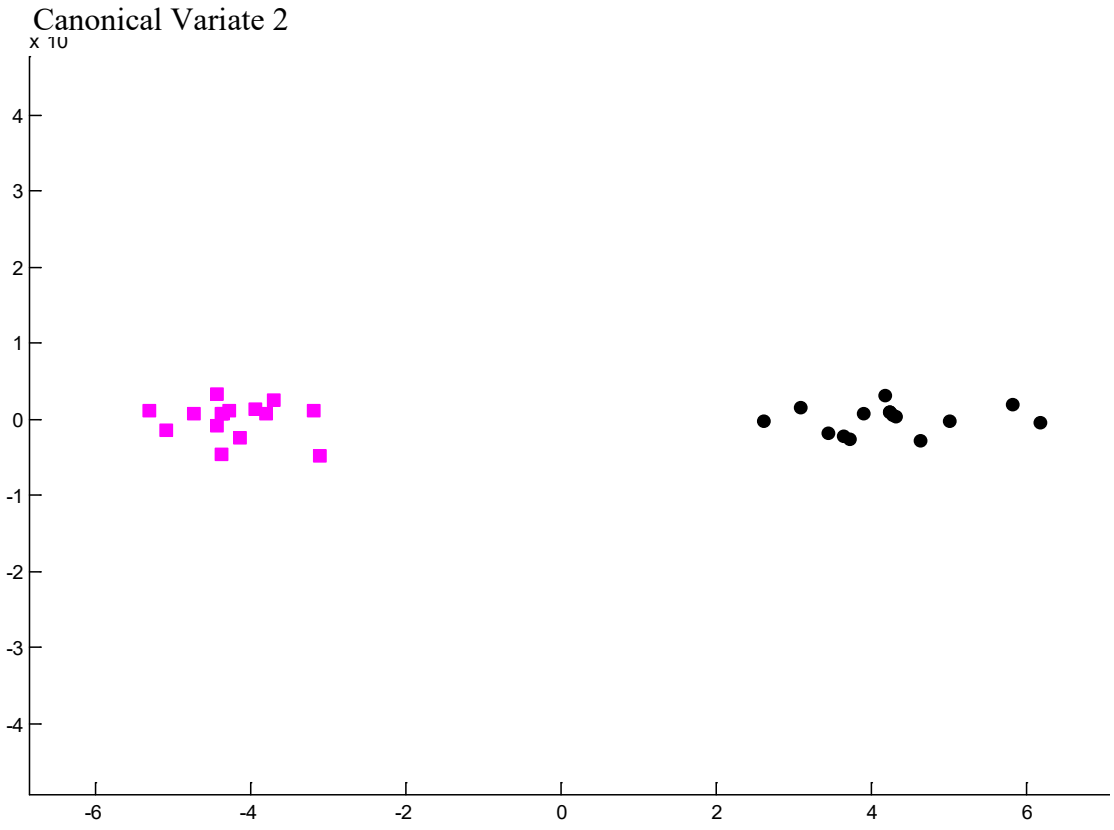


Fig. 4. Canonical variates analysis (CVA) of fly wing shapes: Plot of canonical variate 2 * canonical variate 1 of 30 fly wings. Each X_1, X_2 value is from one fly. Squares = apple maggot fly; circles = snowberry maggot fly.

CONTINUING PROJECT REPORT **YEAR**: 1 YEAR (with extension)
WTFRC Project Number: CP-07-700 **WSU Project #13C-3661-6366**

Project Title: Decay control and management of fungicide resistance

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

Cooperators: Selected packinghouses across the state

Total project funding request **Year 1***:* $90,000

Other funding Sources: NONE

WTFRC Collaborative expenses:

Item	2007	2008
Stemilt RCA room rental	7,323.68	
Crew labor	0	
Shipping	0	
Supplies	0	
Travel	0	
Miscellaneous	0	
Total	7,323.68	0

Footnotes: The RCA room rental cost was based on the 46-bin space used for 2007 research.

Budget 1: (no new funding is being requested)
Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker and Kevin Larson
Telephone: 509-335-7667; 509-663-8181 X221 **Email address:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2007	2008
Salaries	50,000	
Benefits	15,850	
Wages	10,000	
Benefits	1,150	
Equipment	0	
Supplies	10,000	
Travel	3,000	
Miscellaneous	0	
Total	90,000	0

Note: No additional funding being requested for 2008.

Objectives:

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

Significant findings:

This project dealt with postharvest diseases. We have set up all experiments that we proposed to do on the 2007 crops. At the time of writing this report, these experiments are still in progress. The fruit are still in cold storage for either future treatments or decay development. Most experiments will have results in spring 2008.

A one year extension of this project has been approved by the WTFRC.

Methods:

In 2007, we set up a few experiments to evaluate various pre- and postharvest integrated programs for control of storage diseases in Red Delicious and Fuji apples before packing as well as decay after packing. Selected fungicides will be applied within 2 weeks before harvest. Pre- and postharvest drench treatments have also been applied to the fruit. The fruit are currently in cold storage. Various fungicides and biocontrol treatments will be applied to the fruit 5 and 7 months after harvest. Fruit will be evaluated for decay development.

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

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

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

An experiment was conducted to evaluate thermofogging fungicides for control of postharvest diseases. Commercially harvested fruit were used for this experiment. Both fludioxonil and pyrimethanil as thermofogging treatments were tested. The fruit will be evaluated for decay in April 2008.

In collaboration with Bruce Campbell, an experiment was conducted to evaluate 2,5-dihydroxybenzoic acid and 2,3-dihydroxybenzaldehyde for management of fludioxonil-resistant strains of *Penicillium expansum* and for decay control. Results are expected to be available in mid-February 2008.

Results and discussions:

Because this project deals with postharvest diseases, all experiments are still in progress at the time of writing this report. The fruit from these experiments are currently in cold storage either for future treatments or for decay development. Most experiments will not have the results until spring 2008.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-07-701

Project Title: Augmenting fungal control in apples with natural compounds

PI:	Dr. Bruce C. Campbell	**Co-PI(2):**	Dr. Jong H. Kim
Organization:	Plant Mycotoxin Research Unit (PMR)	**Organization:**	same as PI
Telephone/email:	510-559-5846/bcc@pw.usda.gov	**Telephone/email:**	510-559-5841 jhkim@pw.usda.gov
Address:	USDA, ARS, WRRC 800 Buchanan St.	**Address:**	same as PI
City:	Albany	**City:**	
State/Province/Zip	CA 94710	**State/Province/Zip:**	

Cooperators: Drs. Russell Molyneux and John Beck (chemists), Plant Mycotoxin Research Unit, Western Regional Research Center, USDA-ARS, Albany, CA 94710.

Total project funding request: Year 1 (07): $32,421 **Year 2 (08):** $33,555 **Year 3 (09):** $34,730

Other funding Sources

Agency Name:
Amount requested or awarded:
Notes:

Budget 1: (*Required information – please complete all information***)**
Organization Name: WRRC, USDA-ARS **Contract Administrator:** Gwyn Watson
Telephone: 510.559.6029 **Email address:** gwatson@pw.usda.gov

Item	2007	2008	2009
Salaries	$20,276	$21,880	$23,924
Benefits			
Wages			
Benefits			
Equipment	$7,146	$4,491	$4,449
Supplies	$3,857	$5,987	$6,357
Travel	$1,142	$1,197	
Miscellaneous			
Total	**$32,421**	**$33,555**	**$34,730**

Footnotes:

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

1. **Determine an effective method for delivery of newly discovered natural compounds, leading to a target-specific strategy for a safe and economic approach to fungal pathogen control in the field or during processing and storage.**
2. **Identify the most efficient molecular targets [*e.g.*, mitochondrial superoxide dismutase (Mn-SOD)] for newly discovered compounds using functional genomics approaches.**
3. **Augment antifungal activity of natural compounds, through synthesis of structural derivatives, based on structure-activity relationships of analogs of identified antifungal natural compounds.**

Significant Findings (Year 1):

- Activity of conventional antifungal agents, fludioxonil, strobilurin and antimycinA, which target the oxidative and osmotic stress response systems, was elevated by co-application of certain benzo analogs (aldehydes and acids).
- Fungal tolerance to 2,3-dihydroxybenzaldehyde or 2,3-dihydroxybenzoic acid was found to rely upon mitochondrial superoxide dismutase (*SOD2*) or glutathione reductase (*GLR1*), genes regulated by the *HOG1* signaling pathway, respectively. Thus, certain benzo analogs can be effective at targeting cellular oxidative stress response systems.
- The *SLT2* and *BCK1* genes, encoding mitogen-activated protein kinase (MAPK) and MAPK kinase kinase (MAPKKK) in cell wall integrity pathway, respectively, were essential for fungal tolerance to thymol, *o*-coumaric acid, 2,3-dihydroxybenzaldehyde and berberine hemisulfate.
- The activity of conventional antifungal compounds interfering with cell wall integrity, *i.e.*, Congo red or calcofluor white, was elevated by coapplication with thymol or 2,3-dihydroxybenzaldehyde, demonstrating the chemosensitizing capacity of the identified compounds on fungal growth.
- Our data proved the effectiveness of targeting cellular stress response system such as oxidative stress response or cell wall construction/integrity pathway for control of fungi. Results also show certain natural compounds are effective synergists to commercial fungicides and can be used for improving control of food-contaminating pathogens. Use of such compounds for fungal control reduces environmental and health risks associated with commercial fungicides, and lowers cost for control and the probability for development of resistance to these fungicides.

METHODS:

Chemicals. Antifungal compounds fludioxonil, kresoxim-methyl, antimycin A, alkaloid, cell wall interfering agents (Congo red, calcofluor white), and benzaldehyde analogs were purchased from Sigma Co. (St. Louis, MO). Each compound was dissolved in dimethylsulfoxide (DMSO; absolute amount <20 $\mu L\ mL^{-1}$ media) before use.

Microorganisms and culture condition. *Saccharomyces cerevisiae* wild type BY4741 (*Mat* a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and selected deletion mutants lacking genes in (a) antioxidative stress response/multidrug resistance systems or (b) cell wall construction/integrity system were obtained from Invitrogen (Carlsbad, CA) and Open Biosystems (Huntsville, AL), as follows (See also Kim *et al*., 2005, 2007): Gene regulation mutants: *yap1*Δ, *msn2*Δ, *msn4*Δ, *hot1*Δ, *sko1*Δ, *rim101*Δ; Transporter/assembly protein mutants: *flr1*Δ, *yor1*Δ, *pdr5*Δ, *vph2*Δ, *tfp1*Δ/*vma1*Δ; Signal transduction mutants: *sho1*Δ, *sln1*Δ, *ste50*Δ, *ste20*Δ, *ypd1*Δ, *ssk1*Δ, *ptp2*Δ, *ptp3*Δ, *hog1*Δ, *hog4*Δ, *ssk22*Δ, *ssk2*Δ, *ste11*Δ; Antioxidation mutants: *ctt1*Δ, *cta1*Δ, *osr1*Δ, *trr1*Δ, *trr2*Δ, *tsa1*Δ, *grx1*Δ, *grx2*Δ, *trx1*Δ, *trx2*Δ, *glr1*Δ, *gsh1*Δ, *gsh2*Δ, *sod1*Δ, *sod2*Δ, *ahp1*Δ; DNA damage control/energy metabolism mutants: *rad54*Δ, *sgs1*Δ, *acc1*Δ, *gpd1*Δ, *hor2*Δ;Osmoregulation mutant: *hog1*Δ (MAPK), *hog4*Δ (MAPK kinase; MAPKK; Scaffold activity),

*ssk22*Δ (MAPKK kinase; MAPKKK), *ssk2*Δ (MAPKKK), **ste11*Δ (MAPKKK); Cell wall construction/integrity mutant: *slt2*Δ (MAPK), *mkk1*Δ (MAPKK), *mkk2*Δ (MAPKK), *bck1*Δ (MAPKKK), *wsc1*Δ (Sensor-transducer), *mid2*Δ (Sensor for cell wall integrity signaling), **kss1*Δ (MAPK), **ste7*Δ (MAPKK), **ste11*Δ (MAPKKK); Morphological switch mutant: **kss1*Δ (MAPK), **ste7*Δ (MAPKK), **ste11*Δ (MAPKKK); Mating response mutant: *fus3*Δ (MAPK), **ste7*Δ (MAPKK), **ste11*Δ (MAPKKK), *ste5*Δ (Scaffold protein), *ste2*Δ (Receptor for "alpha" factor pheromone), *ste3*Δ (Receptor for "a" factor pheromone); Sporulation mutant: *smk1*Δ (MAPK); PKC-signaling pathway mutant: *rom2*Δ (GDP/GTP exchange protein), *fks1*Δ (β-1,3-D-glucan synthase), *fks2*Δ (β-1,3-D-glucan synthase), *rlm1*Δ (Mcm1p-Agamous-Deficiens-Serum Response Factor <MADS>-box transcription factor), *swi4*Δ (Transcription factor), *pkc1*Δ (Protein serine/threonine kinase; dipoid), where the asterisk (*) indicates overlapping biological roles of the marked gene in more than one pathway (Reference for the description of each deletion mutant: www.yeastgenome.org, accessed on October 3, 2007). Yeast strains were grown on YPD (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) or SG (0.67% Yeast nitrogen base w/o amino acids, 2% glucose with appropriate supplements: 180 μM uracil, 200 μM amino acids) medium at 30 °C (5 to 7 days). *Penicillium expansum* NRRL974 and *Aspergillus flavus* NRRL3357 (obtained from National Center for Agricultural Utilization and Research, USDA, Peoria, IL) were cultured at 28 °C on potato dextrose agar (PDA) (5 to 7 days). *A. fumigatus* AF293, wild type, and *A. fumigatus* mitogen-activated protein kinase (MAPK) deletion mutants *sakA*Δ and *mpkC*Δ (Xue *et al*., 2004; Reyes *et al*., 2006) were grown at 37 °C on PDA medium (5 to 7 days).

Antifungal bioassays. Yeast cell dilution bioassays were performed on SG agar media to monitor activity of and gene deletion mutant hypersensitivity to antifungal compounds, as follows (See Kim *et al.*, 2005, 2007): $1x10^6$ cells of the wild type or respective deletion mutants of *S. cerevisiae*, cultured on YPD medium, were serially diluted 10-fold in SG liquid medium supplemented with amino acids and uracil five times to yield cell dilution cohorts of 10^6, 10^5, 10^4, 10^3, 100 and 10 cells. Cells from each dilution of respective yeast strains were spotted adjacently on SG agar medium incorporated with individual benzo analogs to be tested or antifungal reagents and incubated at 30 °C. Results were recorded based on a designated value of the highest dilution where a colony became visible after 5 to 7 days of incubation, as follows: Score "0"- no colonies were visible from any of the dilutions, Score "6"- colonies were visible from all dilutions, Score "1"- only a colony from the undiluted cells (10^6 cells), "2" only colonies from the undiluted and 10^5 cells were visible, *etc*. Thus each unit of numerical difference was equivalent to a 10-fold difference in the sensitivity of the yeast strain to the treatment. Sensitivities of filamentous fungi were measured based on percent radial growth of treated fungal colonies compared to control colonies, receiving only DMSO [Vincent equation: % inhibition = 100 (C-T)/C, C: diameter of fungi on control plate; T: diameter of fungi on the test plate; Vincent, 1947]. Fungi (~200 spores) were diluted in phosphate buffered saline (PBS) and spotted on the center of PDA plates with or without antifungal compounds. Growth was observed for 5 to 7 days. For testing the potential of chemo-sensitization by test benzo analogs, compounds were added to the growth media together with fludioxonil, strobilurin (kresoxim-methyl) or antimycin A. Radial growth was recorded as described above. Minimum inhibitory concentrations (MICs) of antifungal compounds in *S. cerevisiae* or filamentous fungi were based on the lowest concentrations of compounds where no visible growth of cells were observed either on SG or PDA plates, respectively.

Results and discussion:

Fungal tolerance to benzo analogs depends on cellular Mn-SOD. *Based on yeast cell dilution bioassays, 2,3-dihydroxybenzaldehyde had the highest antifungal activity,* i.e.*, no visible growth of wild type* S. cerevisiae *at ≥ 80 μM, among eight benzo analogs tested. Highest to lowest antimicrobial activity was, as follows: 2,3-dihydroxybenzaldehyde > 2,5-dihydroxybenzaldehyde > 2,4-dihydroxybenzaldehyde > 3-hydroxybenzaldehyde > vanillin, 4-hydroxybenzaldehyde, veratraldehyde > benzaldehyde. An*

almost identical relationship in the relative antifungal activities of the analogs was observed among the various fungi, i.e.*,* Penicillium *and aspergilli tested.*

Among forty-five mutants of *S. cerevisiae* examined, where genes in oxidative stress response/multidrug resistance systems were individually deleted, the *sod2*Δ [mitochondrial superoxide dismutase (Mn-SOD) deletion] mutant showed hypersensitivity to 2,3-dihydroxybenzaldehyde (at 10 μM) compared to the wild type strain. This greater sensitivity strongly indicated Mn-SOD activity is crucial for fungal response/tolerance against toxicity of benzaldehyde derivatives. It appears this gene is a promising candidate as a potential target for fungal control.

The *sakA*Δ mutant of *A. fumigatus* is hypersensitive to benzaldehyde derivtives. *Aspergillus* can be used as a model filamentous fungal pathogen to validate the target or mode of action of natural compounds identified through yeast screening in view that the entire genome of several species of *Aspergillus* (*i.e*., *A. nidulans*, *A. fumigatus*, *A. flavus*) have been sequenced and annotated to a great extent, and manipulation of its genome has been well established, allowing the studies of functional genomic responses to treatments. Responses of *sakA*Δ and *mpkC*Δ mutants derived from *A. fumigatus* AF293, to the benzo analogs was also examined. The MAPKs SakA and MpkC are orthologous proteins to Hog1p of *S. cerevisiae* (Xue *et al*., 2004; Reyes *et al*., 2006). Growth of AF293 and *mpkC*Δ was inhibited by 32 to 72% with 0.2 to 0.25 mM 2,3-dihydroxybenzaldehyde, respectively. The *sakA*Δ strain was more sensitive to these treatments, showing 56 to 100% reduction, respectively, in radial growth. Like Hog1p in *S. cerevisiae*, SakA may play a role in regulating Mn-SOD activity and, thus, tolerance to 2,3-dihydroxybenzaldehyde. The same trends were observed with 2,5-dihydroxybenzaldehyde, but higher concentrations (0.8 to 1 mM) were needed to achieve similar levels of growth inhibition.

2,3-Dihydroxybenzoic acid inhibits fungal growth by disrupting cellular glutathione homeostasis. The acid derivative of 2,3-dihydroxybenzaldehyde, 2,3-dihydroxybenzoic acid, was also examined in order to investigate structure-activity relationships with regard to acid or aldehyde moieties. The 2,3-dihydroxybenzoic acid inhibited growth of *S. cerevisiae* (MIC in wild type ≥ 7 mM). Also, growth of a number of *S. cerevisiae* deletion mutants was inhibited by 2,3-dihydroxybenzoic acid at 4 mM, including *glr1*Δ, *gsh1*Δ, *gsh2*Δ, *vph2*Δ (vacuolar ATPase assembly protein deletion), *vma1*Δ (vacuolar ATPase deletion). Also, like in treatments with 2,5-dihydroxybenzoic acid in our prior study (Kim *et al*., 2007), exogenously supplemented GSH resulted in a strong recovery of growth of these *S. cerevisiae* strains. These findings suggest the mechanism of antifungal activity of 2,3-dihydroxybenzoic acid is, as with the 2,5- analog, disruption of cellular GSH homeostasis. Thus, the GSH reductase gene (*GLR1*), another gene relatively downstream within the *HOG1* signaling pathway, may play an important role for fungal tolerance to this, or related, compounds. The sensitivities of the *vph2*Δ and *vma1*Δ mutants may result from disruption of the normal ability for transportation, sequestration and detoxification of toxic compounds in vacuoles.

The concordance of these results demonstrates there is a structure-activity relationship between the acid and aldehyde moieties in that they affect different target genes in the *HOG1*-signaling pathway. The 2,3- and 2,5- dihydroxybenzaldehydes targeted *SOD2*. Whereas, 2,3- and 2,5- dihydroxybenzoic acids targeted *GLR1*, disrupting glutathione homeostasis.

Chemosensitization to conventional fungicides by 2,3-dihydroxy benzaldehyde and benzoic acid derivatives: targeting cellular signal transduction/oxidative stress response systems.

The dihydroxybenzo analogs, 2,3-dihydroxybenzaldehyde and 2,3-dihydroxybenzoic acid, were examined as potential chemosensitizing agents for target-gene based control of fungi. Some fungi having mutations in certain MAPK genes, involved in signal transduction of oxidative stress responses, can escape toxicity of phenylpyrrole fungicides, such as fludioxonil (Kojima *et al*., 2004). In this regard, we found MAPK mutants, *sakA*Δ and *mpkC*Δ, of *A. fumigatus* were tolerant to fludioxonil toxicity. However, co-

application of 2,3-dihydroxybenzaldehyde (at 0.2 mM) or 2,3-dihydroxybenzoic acid (at 11 mM) with fludioxonil effectively prevented these mutants from developing this tolerance to fludioxonil. This prevention of tolerance by co-application of either of these compounds may result from the disruption of genes downstream in this MAPK pathway. In particular, based on the results with the deletion mutants of *S. cerevisiae* it is likely that these aldehyde and acid analogs target the antioxidative gene *sod2* and the glutathione homeostasis genes.

The potential chemosensitizing effect of 2,3-dihydroxybenzaldehyde was also tested on the activity of kresoxim-methyl, a strobilurin fungicide, and antimycin A.

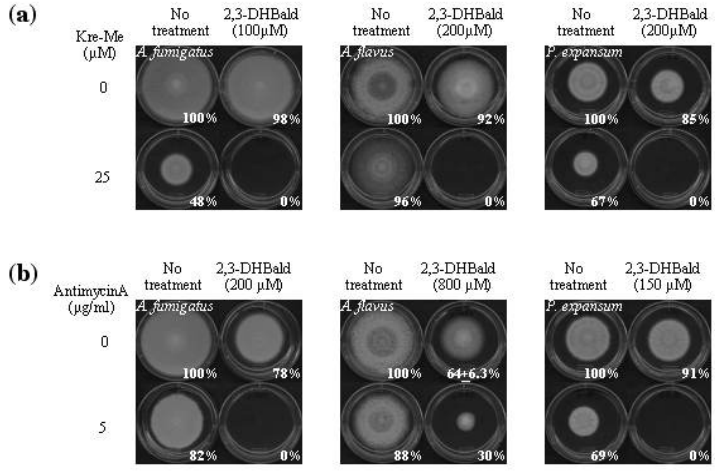


Figure 1. Targeting the mitochondrial antioxidative stress system with 2,3-dihydroxybenzaldehyde in combination with (**a**) strobilurin (kresoxim-methyl) or (**b**) antimycin A had an enhanced antifungal effect against the filamentous fungi, *A. fumigatus* AF293, *A. flavus* NRRL 3357 or *P. expansum* NRRL 974. Standard deviation: <5%, except where noted.

Both of these fungicides disrupt complex III of the mitochondrial respiratory chain. This disruption eventually results in cellular oxidative stress caused by abnormal release of electrons from the respiratory chain and the production of toxic superoxides. Mn-SOD plays an important role in detoxifying these oxidative stressors. Co-application of 2,3-dihydroxybenzaldehyde enhanced the antifungal activity of both fungicides against the filamentous fungi examined, *A. fumigatus, A. flavus* and *P. expansum*. Co-application of 100 or 200 µM 2,3-dihydroxybenzaldehyde to kresoxim-methyl (25 µM; **Figure 1a**) or antimycin A (5 µg mL^{-1}; **Figure 1b**) resulted in complete (100%) inhibition of fungal growth, except *A. flavus* (70% inhibition). Whereas, if any of these compounds are applied alone at these rates fungal growth is only slightly inhibited.

Natural compounds to which *slt2*Δ/*bck1*Δ mutants of *S. cerevisiae* showed sensitivity: chemo-sensitization of cell wall-interfering agents.

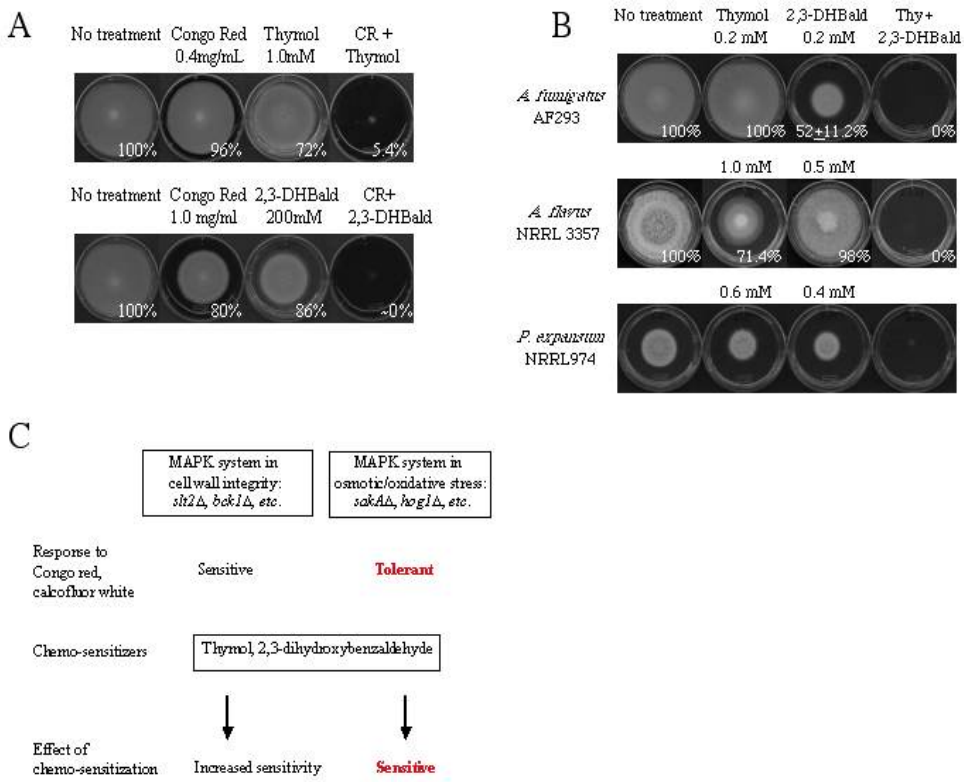


Figure 2. (A) Co-application of Congo red (0.4 to 1.0 mg/ml) with thymol (1.0 mM) or 2,3-dihydroxybenzaldehyde (0.2 mM) enhanced its antifungal activity against *A. fumigatus* (*i.e.*, ~95% to 100% growth inhibition), (B) Co-application of thymol and 2,3-dihydroxybenzaldehyde achieved 100% growth inhibition in *A. fumigatus* AF293, *A. flavus* 3357 and *Penicillium expansum* 974, indicating these two compounds affect common cellular target. (C) Diagram summarizing the chemosensitizing effect of thymol and 2,3-dihydroxybenzaldehyde on fungi.

We then tested the antifungal effects of natural compounds by using *slt2*Δ, *bck1*Δ, *wsc1*Δ and *swi4*Δ mutants (See Methods) of *S. cerevisiae*. We found that *slt2*Δ and *bck1*Δ mutants showed the highest sensitivity (10^3 to 10^4 times) to thymol, 2,3-dihydroxybenzaldehyde, *o*-coumaric acid and berberine comparing to the wild type or *wsc1*Δ/*swi4*Δ strains.

In the bioassay using *S. cerevisiae*, thymol showed chemo-sensitizing effect to Congo red, where co-application of thymol (0.5 mM) and Congo red (100 and 500 μg/ml) resulted in ~10 to 10^4 times higher sensitivity of yeast cells (wild type, *slt2*Δ and *bck1*Δ mutants) comparing to the independent treatment of each compound. Results also showed that co-application of Congo red (0.4 to 1.0 mg/ml) with thymol (1.0 mM) or 2,3-dihydroxybenzaldehyde (0.2 mM) enhanced its antifungal activity against *A. fumigatus* (*i.e.*, ~95% to 100% growth inhibition; **Figure 2A**), while no chemo-sensitizing effect was observed with *o*-coumaric acid or berberine (data not shown), indicating thymol or 2,3-dihydroxybenzaldehyde may affect common cellular target of cell wall-interfering agents. We found that 100% of growth inhibition can be achieved in *A. fumigatus* AF293, *A. flavus* 3357 and *P. expansum* 974 (**Figure 2B**) by co-application of thymol and 2,3-dihydroxybenzaldehyde, strongly indicating these two compounds affect common cellular target.

Summary. During this first-year cycle we identified a potentially effective approach to fungal control using newly discovered natural compounds that have a target-specific basis of activity. Antioxidative stress response and cell wall integrity systems of fungi can be an efficient molecular target of phenolics for pathogen control. We proved positive interaction between phenolics and conventional fungicides significantly augment the fungicidal effects of commercial fungicides by reducing the costs of

application, development of resistance, or contamination of the environment. We conclude natural compounds such as phenolic agents that do not have any significant medical or environmental shortcomings could be useful in control programs involving conventional antifungal or antimycotoxigenic agents.

References:

Kim JH, Campbell BC, Yu J, Mahoney N, Chan KL, Molyneux RJ, Bhatnagar D & Cleveland TE (2005) Examination of fungal stress response genes using *Saccharomyces cerevisiae* as a model system: targeting genes affecting aflatoxin biosynthesis by *Aspergillus flavus* Link. *Appl Microbiol Biotechnol* **67:** 807–815.

Kim JH, Campbell BC, Mahoney N, Chan KL, Molyneux RJ & May SG. (2007) Enhancement of fludioxonil fungicidal activity by disrupting cellular glutathione homeostasis with 2,5-dihydroxybenzoic acid. ***FEMS Microbiol Lett*** 207: **284-290.**

Kojima K, Takano Y, Yoshimi A, Tanaka C, Kikuchi T & Okuno T (2004) Fungicide activity through activation of a fungal signalling pathway. *Mol Microbiol* **53:** 1785–1796.

Reyes G, Romans A, Nguyen CK & May GS (2006) Novel mitogen-activated protein kinase MpkC of *Aspergillus fumigatus* is required for utilization of polyalcohol sugars. *Eukaryot Cell* **5:** 1934-1940.

Vincent, J.M. (1947) ***Nature*** 189: **850.**

Xue T, Nguyen CK, Romans A & May GS (2004) A mitogen-activated protein kinase that senses nitrogen regulates conidial germination and growth in *Aspergillus fumigatus*. *Eukaryot Cell* **3:** 557–560.

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

WTFRC Project Number: CP-07-708 **(WSU Project #13C-3643-5368)**

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

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Total project funding request: ***Year 1*:** $60,112 ***Year 2*:** $62,452 ***Year 3*:** $65,060

Other Funding Sources: NONE

Budget 1:

Organization: WSU-TFREC **Contract Administrator:** ML Bricker; Kevin Larson

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Item	Year 1: 2007	Year 2: 2008	Year 3: 2009
Salaries[1]	$28,332	**$28,332**	$29,465
Benefits	$10,200	**$11,049**	$11,491
Wages	$12,000	**$12,480**	$12,979
Benefits	$1,380	**$1,959**	$2,038
Equipment	$0	**$0**	$0
Supplies[2]	$6,000	**$6,300**	$6,615
Travel[3]	$2,200	**$2,332**	$2,472
Total	$60,112	**$62,452**	$65,060

Footnotes:

[1] Tawnee Wilburn 0.75 FTE

[2] Supplies include general lab supplies and ELISA-specific supplies, field supplies including traps, lures, markers, cell phone charges

[3]Travel costs are within-state travel to field sites and vehicle costs

Objectives:

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

Significant Findings:

- CM age grading allowed us to separate moths on traps into young, middle aged and old individuals.
- CM male mating status can be assessed but only for 2 to 3 days post-mating. After that point, mated males are distinguishable from virgin moths.
- We caught few young individuals during the second and third flights, probably because the temperatures are high during those periods, resulting in accelerated moth aging.
- OBLR age grading was more difficult; only two categories could be developed. Male mating status was not problematical.
- In our marking studies, we found that older CM were recaptured at significantly shorter distances compared to middle-aged individuals.
- Mated CM also appeared to fly significantly further than unmated moths.

Significant Progress:

Objective 1. Methods: We examined male and female CM and OBLR moths for a suite of characters to help determine moth age. The characters were:

6. Amount of fat body in the abdomen
7. Size of fat body cells
8. Color of the fat body
9. Color of the reproductive system
10. General condition of the reproductive system
11. Size of the abdomen
12. Condition of the scales on the moths

For each character, we assign a numerical value, either 0, 1, or 2, with 0 assigned to the younger trait (e.g., white would be 0, cream 1, and yellow 3 for characters #3 and #4). We found that virtually all of the variability in codling moth could be explained by just using the first three characters, with #2 and #3 accounting for 78% of the variance.

We also examined the mating status of the moths. For females, we look for spermatophores; for males we examine a specific segment of the seminal duct that harbors the semen and record the color (clear in mated individuals, opaque in virgin individuals). For OBLR, Evenden and Judd (2003) showed this coloration remains for long periods so that you can determine male mating status. We found that the same color pattern exists in codling moth; however, it reverts more quickly so that we can only tell whether they have mated within the last 2 to 3 days. During the summer, when males die quickly, this is not a problem, but in the spring and late fall when males live longer, we can only detect recent matings. We will be performing lab studies to see if we can quantify the reversion on a degree-day scale.

Results: We trapped CM and OBLR in two locations over the entire season, dissecting all moths captured. At the TFREC site we captured 552 CM (270, 218, and 64 in flights 1-3). During the first flight, we found that young individuals comprised only 15.2% of the total captures, with 50% of the

individuals being middle aged and 34.8% being old individuals. The percentage of young individuals caught decreased steadily throughout the season, with only 6% and 1.6% caught in the second and third flights (fig. 1). The old class increased dramatically during the second flight and declined slightly during the third. These trends are likely a result of a combination of factors. First, it is possible that the lures used (Combo DA) may have had some age bias where younger individuals were less attracted. However, the differences between generations are likely the result of the fact that longevity runs on a degree-day basis, which changes considerably over the season. Previous studies funded by the commission in my lab have allowed us to quantify CM and OBLR adult mortality over time; with CM 50% mortality occurs by 102 DD after emergence. Using the temperature records from TFREC and Columbia View, we can show that longevity varies from 14 to 5 days in the first flight, is roughly 4 days throughout the entire second flight, and is low initially but increases throughout the third flight (fig 2). The short longevity during the second flight and the first part of the third flight make it much less likely that young individuals are captured on the traps (because they age so rapidly). In addition, if the trap checking interval is greater than 3 days, trap captures appear to be primarily middle or old individuals because unless they die, moths continue to age on the trap. In our studies, traps were checked at mostly 1 day intervals throughout the year. However, during the second generation. when we were focusing on Objective 3, intervals increased up to 2 to 3 days, which probably skewed our results towards older individuals.

Fig. 1. Percentage age distribution of CM trap captures per flight at TFREC 2007.

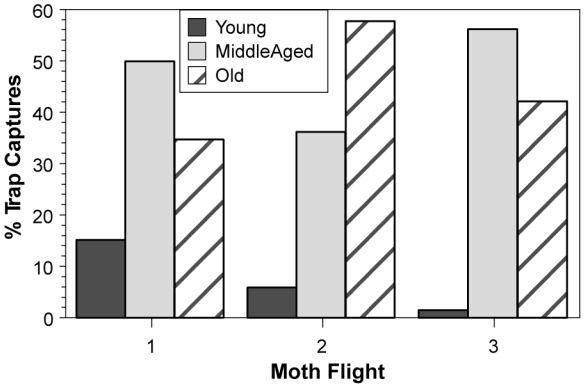


Fig. 2. Time to 50% adult male CM mortality at TFREC 2007

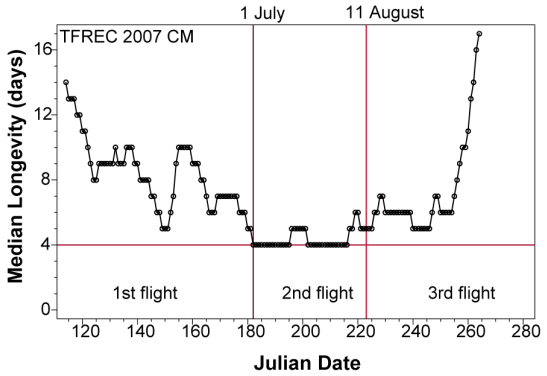
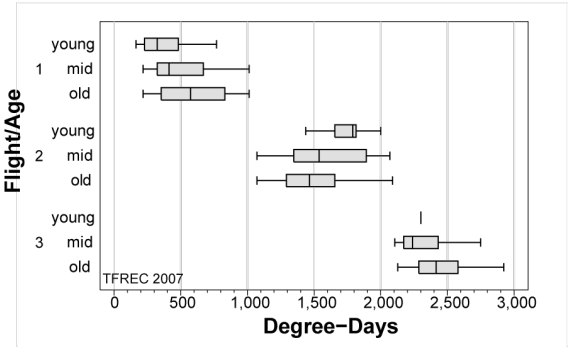


Fig. 3. Boxplots showning time of occurrence of the different CM age classes in each flight at TFREC 2007. The left edge of the box is when 25% emergence occurs, the right edge 75%. The line in the center is 50%. The whiskers show extreme values.

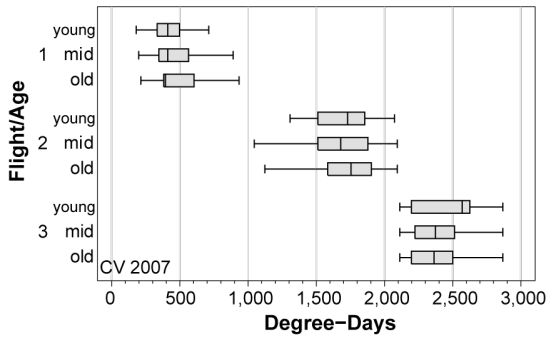


We used boxplots to summarize the flights of the different aged moths on a DD scale. Boxplots show variability by constructing a box whose edges are when 25 and 75% emergence occurs and the line in the middle is 50% emergence. The whiskers extend to extreme values. At TFREC, we see in the first flight that the young individuals emerge and are caught first, then middle aged, then old individuals (fig. 3). However, in the second generation, the quick aging and low capture of young individuals skew the timing. In the third flight, the trend is similar to the first generation, although trap catch of young individuals was more rare.

At Columbia View, we caught 2,154 moths over the season and the percentage of trap captures by moth flight and age looks virtually identical to what we saw at TFREC (fig. 1). The boxplots of flight by age (fig. 4) are also similar to the TFREC boxplots (fig. 3), but because of the larger sample size, we have more young moths caught in the second and third flights.

The OBLR studies were not as successful as the codling moth studies in terms of trap catch and the ability to age grade the moths. We caught 72 OBLR over the season at TFREC and 231 at a commercial site near Orondo. Overall, there were very few differences in male characteristics with which to determine age; however, mating status was easily detected. The difficulty in determining age may be related to the shorter lifespan of OBLR compared to CM (50% mortality at 73 vs. 102 DD), which results in 50% mortality by 3 days in the middle of the summer.

Fig. 4. Boxplots showning time of occurrence of the different CM age classes in each flight at CV 2007. The left edge of the box is when 25% emergence occurs, the right edge 75%. The line in the center is 50%. The whiskers show extreme values.



Plan for next year. We need to perform lab studies to see if we can quantify how long male codling moth retain the color change in the seminal duct which signifies mating has occurred. We also need to examine the effects of lure type on male age in CM and the effects of MD on age captures and mating status. We need to find several orchards that have large OBLR populations so that we can further examine how to age them. It is possible that we will have to be satisfied with just looking at mating status and two age classes with OBLR rather than the three we can detect with CM.

Objective 2. We are acquiring the flight mills and should have them ready for use in the late spring.

Plan for next year. As indicated in the original proposal, we will be flying moths various distances and then examining the reproductive rate of flown versus control moths (not flown). This will occur throughout the summer and fall of next season.

Objective 3. Methods: This year, we concentrated on evaluating the effects of Assail and Guthion on CM movement using the protein markers. Two plots were set up in a large Brewster, WA orchard. In the first plot, trees were planted on a 12' x 18' spacing, and were 24' x 18' spacing in the second. The trapped area of both plots was roughly 12.6 acres, but the first plot was longer and narrower (1476' x 360') than the second (1115' x 492'). We placed 60 traps in the first plot and 44 in the second; in both plots, we used the Combo DA lures in standard LPD traps. Both plots were under mating disruption using a full rate of Isomate C+ dispensers.

Traps were placed uniformly throughout both blocks and checked three times per week during a three-week period of the first and second flights. All moths were returned to the lab and dissected to determine mating status and age of the moth. Moths were aged by evaluating the internal coloration, size of the fat body, size of female ovaries and number of eggs present or condition of male testes. We also determined the number of spermatophores in females and the mating status of males (by examining the color of a specific segment of the male reproductive tract).

Treatments: We applied 150 gallons of a 10% egg whites solution to the center 1.5 acres in both plots on the same dates. Egg whites were applied once per generation at the time that roughly 25% of the adults would have emerged. In the first generation, Assail was applied to plot 1 and Guthion to plot 2. During the second generation, the treatments were reversed, so that Assail was applied to plot 2 and Guthion to plot 1.

During the first generation, we had a small amount of rain one week after treatment, and then an additional 0.3" fell (according to the Brewster Flat PAWS station) during the third week, that would

have affected the data. In the second generation, 0.2" fell two days after our treatments were applied, again potentially affecting marking throughout the experiment. The actual amounts of rainfall could have been much higher, because the rain gauges are notoriously unreliable; the Agri-Met station in Omak and Chief Joe dam recorded considerably higher rainfall totals during the same periods.

Results: We caught 333 moths in the two plots over both generations; 144 in plot 1 and 189 in plot 2. In plot one, 137 (95%) were caught in the first generation. In plot 2, 61% were caught in the first generation. The overall sex ratio was 89.2% males, which is similar to other studies we've performed with the Combo DA lures.

We caught 29 marked moths, 13 in plot 1 and 16 in plot 2. The low percentage marking is likely a result of the rain and restricts the complexity of the analysis that we can perform. First, there were no significant differences in dispersal distances related to the pesticide used. This is contrary to data we obtained two years ago and is likely related, in part, to the low power of the test caused by the low marking rate. The previous data had a much larger marked sample size, so we still think there is likely a significant difference related to treatments.

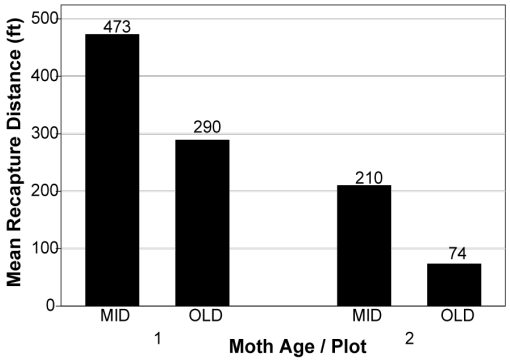
The differences between ages showed that moths classed as old dispersed an average of 160' and middle-aged individuals averaged 364'. In each plot, the average dispersal distance was significantly lower for older individuals compared to middle-aged ones (fig. 5). Only 3 marked females dispersed out of the area treated with egg whites, and they showed similar results to the males, with the two middle-aged females dispersing an average of 567' versus 154' for the single old female.

In terms of plot differences, the average dispersal distance in the first plot (395') was significantly higher than the second plot (142') (fig. 5). This was consistent between generations and age classes, regardless of which treatment was applied. The reasons for this difference is unclear, but may be related to tree density. The first plot was planted at roughly 2x the density of the second plot and it was not on flat ground (it sloped downward from east to west roughly 82'). Our previous studies have shown that wind velocity in higher density orchards tends to be lower than in low density orchards, suggesting that moths may be able to fly a greater percentage of the time (our studies in another project showed flight at wind speeds above 3.3 mph is rare and that moths are unable to locate lures at wind speeds above 3.3 mph). The potential difference in the amount of time moths are flying would tend to allow them to move further in the high density orchard where wind velocity would be lower than the 3.3 mph threshold a greater percentage of the time.

The evaluation of mating status also suggested that mated individuals fly further than unmated individuals. The results were especially pronounced in the first plot (higher tree density plot), with mated individuals flying twice as far.

Plan for next year. We need to repeat this experiment again next year. This will require different orchards with high populations because we found the population density dropped dramatically between the two generations, suggesting the population density next year would be low at this site. We also need to find orchards to test the effects of kaolin border treatments on migration rates and initiate those studies as well. Our age grading of CM worked well and should greatly enhance our understanding of movement patterns when a larger data set is available.

Fig. 5. Average recapture distance for middle-aged and old CM in Brewster 2007.



CONTINUING REPORT **YEAR 1 OF 3**
WTFRC Project Number: AE-06-603/ARS No.5853 52-7-368

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

PI:	Peter Landolt	**Co-PI(2):**	Greg Glenn
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Cooperators: Lerry Lacey, Gary Judd

Other funding Sources: None
Agency Name:
Amount awarded:
Notes:

Total Project Funding: $62,300

Budget History:

Item	**Year 1:** 2006	**Year 2: 2007**	**Year 3: 2008**
Salaries	$21,000		$21,700
Benefits	6,400		6,600
Wages			
Benefits			
Equipment			
Supplies	3,000		3,000
Travel	600		600
Miscellaneous	0		
Total	$31,000	No-cost extension	**$31,300**

Budget Notes: We requested that the WTFRC 2006 funding be used by us in 2007, and that additional funding be postponed one year. This requested change is reflected in the budget table.

Salary requests are for partial support of a biological technician at Wapato (½ time), and partial support for a chemistry technician at Albany, CA (½ time). Travel request is to cover Dr. Glenn's travel from California to participate in WTFRC meetings.

Supplies include materials for preparation of foams, a foamer applicator, olfactometer and arena assay supplies, banding, pesticides and other chemicals, codling moth rearing materials, and nematodes.

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

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

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

Significant Findings:
1. Comparisons of polyurethane foam and cardboard banding showed a superiority of the foam in recruiting greater numbers of larvae that are seeking spin up sites.

2. Laboratory evaluations of several additional materials showed a clear connection between foam cell (bubble) size and efficacy, and superiority of a starch based material over others.

3. An industrial foamer (texture sprayer) has been modified and shown to be useful for both mixing of experimental materials and application to tree trunks in an orchard.

4. Candidate materials have been selected and tested that meet the criteria of low cost, biodegradable,

Methods:

Polyurethane foam (commercial Great Stuff ®) was applied to tree trunks in a two inch wide band. Other trees were banded with 2 inch wide cardboard banding. The materials were removed in late September and numbers of codling moth cocoons counted.

A series of materials were manufactured in the Albany laboratory to provide candidates for testing. These materials were both natural and synthetic, but were all designed to be light weight, porous, and inexpensive. Base materials were cardboard, wood fibers, starches, straw, polystyrene, polyurethane, and concretes. A range of densities of materials were also tested. .

Candidate materials were tested in the laboratory to rank types of materials and material consistencies for their acceptance by mature codling moth larvae. Larvae from the USDA laboratory colony were removed from cocoons and placed individually in 16 oz plastic cups with pieces of test materials. Materials were scored at 1 and 24 hours for larval contact with and entry into the material. Great Stuff polyurethane foam and cardboard bands were used for comparison, as positive controls. A series of materials were tested using this arena assay. These comparisons provided candidate types of

materials for use in more advance testing and formulations.

Preliminary field testing was conducted using foaming formulations of milled wheat straw and a combination of milled wheat straw and softwood fiber. At material costs of $0.22 to $0.25 per tree, bands of these materials were applied to sets of 5 trees, in swaths of 5 to 8 inches, about 6 inches above the soil. The foams were applied using a commercial "texture" air gun sprayer. Applied materials were evaluated for weathering over a 6 week period.

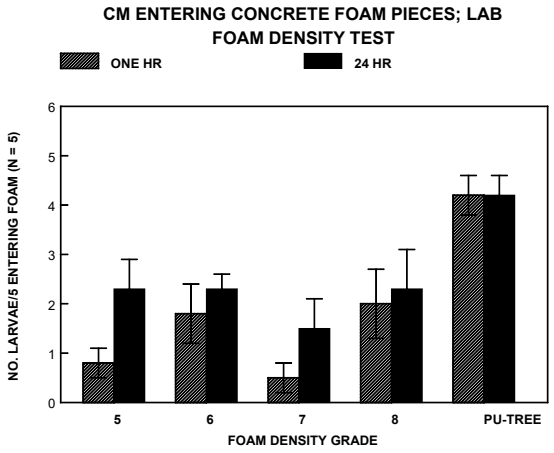
Results and Discussion:

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

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

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

Comparisons of densities of ultra-light concrete did not show an improvement in efficacy with decreasing density and all densities were inferior to cardboard banding. The lack of acceptance by some larvae may have been due to the toughness of the concrete, despite the presence of numerous small air pockets. It was surprising nonetheless to see codling moth larvae bore into soft forms of concrete and spin up cocoons within the concrete.



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

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

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

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

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

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

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

CONTINUING PROJECT REPORT **YEAR**: 1 of 2
WTFRC Project Number: CP07-709

Project Title: Fate of codling moth in apples after harvest

PI: Lisa G. Neven
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City: Wapato
State/Province/Zip WA, 98951

Total project funding request: **Year 1:** $58,815 **Year 2:** $55,000 **Year 3:**

Other funding Sources

Agency Name: **None**

Budget 1:
Organization Name: USDA-ARS **Contract Administrator:** Bobbie Bobango
Telephone: 509-454-6575 **Email address:** bobbie.bobango@ars.usda.gov

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

OBJECTIVES

1) Determine the critical duration of chilling needed for diapause-destined larvae to break diapause.

2) Determine the fate of diapause-destined larvae under tropical environments (short photoperiod, elevated temperatures, high chilling temperatures, short chilling period).

3) Determine the proportion of field codling moth population entering diapause at each harvest date.

4) Determine the proportion of both field and laboratory codling moth diapause-destined larvae surviving cold storage.

SIGNIFICANT FINDINGS

To date, at least one moth has emerged from every harvest date. The first moth to emerge was from the second harvest date (8/15/07) and had been cold stored at 1°C (34°) for 28 days, then transferred to 20°C (68°F) under a 12:12 (L:D) photoperiod. Based on preliminary studies (Neven, unpublished) we would not have expected any moths emerging from diapausing and diapause destined larvae (harvest dates 3-7).

METHODS

Two separate series of experiments are proposed; one involving laboratory reared-diapausing codling moth and the other field collected diapausing codling moths. Fresh batches of laboratory reared codling moth eggs will be exposed to organic thinning apples every week and subjected to 3 different rearing regimes. The rearing regimes will consist of a 12:12 L:D photoperiod at 20°C (68°F), 8:16 (L:D) at 20°C, and 15:9 (L:D) at 20°C (68°F). Infested apples containing immature codling moths will be taken at 0 to 28d after planting (on a weekly basis) and stored at 1.1°C (34°F) for 0 to 126d (on a bi-weekly basis). Following cold storage, infested, apples will be placed at 10°C (50°F) for 0 to 63d (on a weekly basis) at a 12:12 (L:D) photoperiod. Larvae will be assessed for survivorship after the first cold storage period. Any survivors will be re-introduced to thinning apples prior to placement at the 10°C (50°F) chilling temperature. Following the 10°C (50°F) storage period, infested apples will be returned to a 12:12 (L:D) photoperiod at 20°C (68°F) and monitored weekly for cocooning. We will also take a sample and hold at 20°C (68°F) under a 16:8 photoperiod as an additional control.

Cocooned larvae will be held at 12:12 (L:D) photoperiod at 20°C (68°F) for up to 6 months and monitored weekly for pupation and adult emergence. Following the 6 months holding period, strips will be dissected and cocoons assessed for stage of development and survivorship.

Field collected codling moths were obtained from infested, unsprayed abandoned orchards on a bi-weekly basis beginning on August 1 and ending October 24 for a total of 7 harvest dates. Trees will be banded 2 weeks prior to the first harvest date and replaced at each harvest date. Harvested, infested fruit will be held at 1.1°C (34°F) for 0 to 119d (on a bi-weekly basis). The 0d storage period will be used to assess stages of insects in fruit at time of harvest. We will include an additional control at 20°C (68°F) at 16:8 (L:D) to assess the number of larvae not diapause destined in each sample. Recovered bands will also be examined for number of cocoons and physiological state of larvae. Following cold storage period, samples will be handled in the same manner as in previous experiment. Infested fruit was (and will be) stored at 10°C (50°F) for 0 to 63d (on a weekly basis) at a 12:12 L:D photoperiod. Following storage, infested fruits were (and will be) placed at a 12:12 L:D photoperiod at 20°C (68°F) and monitored on a weekly basis for cocooning. Cocooned larvae will be held at 12:12 L:D photoperiod at 20°C (68°F) for up to 6 months and monitored weekly for pupation and adult emergence. Following the 6 months holding period, strips will be dissected and cocoons

assessed for stage of development and survivorship. Temperature, humidity, and photoperiod were recorded in the rearing rooms using Hobo data loggers with the data being downloaded every month.

We will need to replicate these experiments at least 3 times (1 per year) to account for natural variation in response to environment and other endogenous factors.

RESULTS & DISCUSSION

Field Studies:

We harvested codling moth infested apples from 6 orchards over 7 harvest dates. One harvest date was prior to the critical photoperiod (8/1/07), another on the critical photoperiod (8/15/07) and the last 5 after the critical photoperiod. The critical photoperiod is the date at which 50% induction of diapause in codling moth is predicted. We averaged 22-25 cocooned larvae from each block in controls from harvest dates 1& 2. We observed 12 cocooned larvae from each block in controls from harvest dates 3 and 4. Harvest dates 5, 6 and 7 averaged 6, 1, and 1 cocooned larva per block, respectively. At the 7th harvest date we took a sample of fruit and determined that there was only 1 larva per 30 harvested fruit. The decision was made to terminate any further harvests.

To date, at least one moth has been obtained from each harvest date from the field collected samples. The first moth to emerge was from the second harvest date (8/15/07) and had been cold stored at 1°C (34°) for 28 days, then transferred to 20°C (68°F) under a 12:12 (L:D) photoperiod. It took 16 days at 20° for this moth to emerge (Table 1), which was the shortest emergence duration for all moths to date. It is possible that this moth was from a non-diapausing larvae since only 50% of the population should have been induced to diapause at that date. To date, the maximum number of days at 20°C for adult emergence is 85 days with an average of 52 days. A total of 6 moths emerged from apples that were not stored for any duration at 1.1°C. Only 1 moth emerged from apples not stored at 10°C, but did receive 28 d of storage at 1.1°C. The shortest duration of 10°C storage for moths emerging from apples not stored at 1.1°C was 14 d. We did not observe any moths from fruit during the 10°C storage period. Moth emergence was only observed in apples held at 20°C.

Moth emergence appears to be protracted and asynchronous, as one would expect from a review of the literature. The number of emerged moths is low compared to the estimated sample size. We would expect at least 22-25 moths from each sample from harvest dates 1 and 2, 12 from harvest dates 3 and 4, and 6 from harvest date 5. This is probably due to the 12:12 L:D photoperiod used at the 20°C rooms. We will add an additional control of 20°C at 8:16 L:D to obtain optimal emergence data from each harvest date for next season.

Table 1. Emergence data from field collected samples.

Harvest Date	Harvest #	Days @ 1.1°C	Days @ 10°C	[1]Days @ 20°C	# Moths
8/1/2007	1	42	28	85	1
8/1/2007	1	42	56	43	1
8/15/2007	2	0	63	50	1
8/15/2007	2	14	21	78	1
8/15/2007	2	14	49	57	1
8/15/2007	2	28	0	16	1
8/15/2007	2	42	42	50	1
8/29/2007	3	0	49	50	1
8/29/2007	3	0	63	50	1
8/29/2007	3	28	49	50	1
8/29/2007	3	84	21	22	1
9/12/2007	4	14	14	85	1
9/12/2007	4	14	42	57	1
9/12/2007	4	14	56	43	1
9/12/2007	4	28	28	57	1
9/26/2007	5	0	42	57	2
9/26/2007	5	14	14	71	1
10/11/2007	6	0	35	36	1
10/25/2007	7	0	14	43	1
10/25/2007	7	0	35	36	1

[1]Also indicates number of days at 20°C, 12:12 L:D needed for adult emergence.

Laboratory Studies:
We obtained our thinning apples for the laboratory studies in late July 2007. At that time we determined that it was better to hold off on the laboratory portion of the research until the field harvests were completed. We did experiment with moths obtained from an outside source that were reported to be disease free (*nosema* and CpGV). Unfortunately, they did not perform well under our laboratory conditions. We attempted to use egg sheets for apple infestation to help reduce the spread of disease and scale. However, this resulted in an uneven distribution of larvae in the apples. We have returned to applying moth to trays of thinning apples to infest the fruit for the laboratory studies. We will have enough room in the rearing rooms in January to begin the full scale study of the laboratory colony.

It is important to have the laboratory colony studies included in this project since it will address many of the potential questions importers may have concerning the field collected studies. There will be questions as to the number of larvae in the test as well as not knowing whether the insects were really non-diapause or diapause destined. The laboratory studies will use codling moths reared under 3 photoperiods, 12:12, 8:16, and 15:9 (L:D). We expect to see moth emergence at the 12:12 and the 15:9 (L:D) photoperiods. We expect to obtain diapausing larvae from the 8:16 (L:D) photoperiod. If we do not get moth emergence at the 12:12 photoperiod, this might well make the case for discontinuing the 'three-strikes' policy with Taiwan. We will need to show efficacy of this procedure with over 5,000 to 30,000 individuals. This will be determined at a later date.

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

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

PI:	Stephen F. Garczynski	**Co-PI(2):**	Laura S. Lavine
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Address:	5230 Konnowac Pass Rd	**Address:**	P.O. Box 646382
City:	Wapato	**City:**	Pullman
State/ Zip	WA 98951	**State/ Zip:**	WA 99164-6382

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

Total funding request: **Year 1:** $40,000 **Year 2:** $40,000 **Year 3:** $40,000

Other funding Sources

Agency Name:	USDA- National Research Initiative
Amount requested:	$ 189,622 (2008-2009)
Title:	Identification and Characterization of Codling Moth Chemosensory Receptors
Notes:	The grant was submitted in June, updated August, and awarded October of 2007. Preliminary data generated for this proposal was generated in great part by completion of goals related to our proposal. Tree fruit industry support therefore was critical in winning this grant.

Budget 1:

Organization: USDA-ARS		**Contract Administrator: Bobbie Bobango**	
Telephone: (509) 454-6575		**Email:** Bobbie.Bobango@ars.usda.gov	
Item	**Year 1: 2007**	**Year 2: 2008**	**Year 3: 2009**
Supplies	$20,000	$20,000	$20,000
Travel			
Miscellaneous	$5,000	$5,000	$5,000
Total	$25,000	$25,000	$25,000

Budget 2: (*Complete only if funding is split between organizations***)**

Organization: Washington State University		**Contract Administrator:** ML. Bricker / Barb Smith	
Telephone: (509) 335-5504		**Email:** mdesros@wsu.edu / niehoff@wsu.edu	
Item	**Year 1: 2007**	**Year 2: 2008**	**Year 3: 2009**
Wages	6,240	6,490	6750
Benefits	150	156	162
Equipment			
Supplies	8,110	7,854	7,588
Travel	500	500	500
Total	$15,000	$15,000	$15,000

Comment on funding: The recently awarded NRI funding is for fundamental work on characterizing chemosensory receptors of the **adult** codling moth. The objective of the WTFRC proposal will now be largely devoted to characterizing expression and importance of the receptors of **neonate larvae**.

OBJECTIVES:

The following are our original objectives to provide critical new information that will build our fundamental knowledge toward the accomplishment of our *long term goal* of developing specific and potent odorant lures or disruptants for use in codling moth control programs.

1) Construct cDNA libraries from codling moth sensory organs and neuroendocrine tissues.

2) Sequence cDNA libraries and perform searches to identify target receptors.

3) Clone target receptors into expression systems suitable for analysis in insect cell lines.

4) Initiate assays to identify receptors for pheromones and kairomones used for codling moth control.

5) Convert cell based assays for practical use in high-throughput screening programs.

SIGNIFICANT ACCOMPLISHMENTS:

- Procedures were developed to rapidly and cheaply identify genes expressed in insects without a sequenced genome (including codling moth and other tree fruit pests).
- These developed procedures were used to identify pheromone receptors in codling moth males. This represent the FIRST time that chemosensory receptors have been identified in an insect species without a sequenced genome!
- In addition, the procedures developed for codling moth worked for ANY lepidopteran pest tested demonstrating the universality of the methods.
- Use of specific primers designed internally from sequenced regions allowed elaboration of full length sequences including five “pheromone family” receptors, one general odor receptor, and the ubiquitous or “helper” odor receptor
- Full length DNA sequences were also acquired for the olfactory g-proteins required for successful cell culture expression and assay of olfactory receptor protein activities.

METHODS

See original proposal.

RESULTS AND DISCUSSION:

Objective 1. Silk moth and tobacco budworm odor receptor sequences were aligned and regions of modest similarity was discovered in the 3-prime end of the receptors. These areas were used to design degenerate primers. Figure 1 shows an example of one area of similarity at the amino acid level.

```
BmOR3       --MIFVDDAVIGIKDPREYRHLRVLRTSLRLLGAWPGHYLGEETGSKYECAPMFLLMFIK
Hvir_OR13   MKILSDGSDLEGVEKVEDIFYINLARKSMWILDSWPKAFN-------ASSKYRYFVLALN
                       *             * *   *  **

BmOR3       IACLYLTIVYLRNNADVLGFFELGHVYLTIFMTFVTLSRGFSLTWNPNYHKVVKKFITEM
Hvir_OR13   VATLIGGAIYLRNNTGVLSSFELGHTYITVFMNCITCSR-CLMILSKDYNHVMTLFVQKI
             * *     *****  **  ***** * * **   * **         *  *   *

BmOR3       HLLYFKDNSEYAMKTHRRVHKISHFYTVFLKVQMIAGLTLFNVIPMYNNYRQGNYASDRP
Hvir_OR13   HLFHHKHKSDYAYLTHIFIHKISHFYTVYLLGLALNGLFLFNMIPFYNCYSRGMFRDVIP
            **   *  * **  **   ********* *      ** *** ** ** *  *      *

BmOR3       ANITYDLSIYYET-FDILNTPNGYIFICVFNWFASYICCSFFCSFDLILSLMISTVSGHF
Hvir_OR13   ANATYDHAVFYSVPFDYTTKFKGYLAMTSFNVFISYTCTSYFCVVDLTISLVIFHLWGHM
            ** ***    *   **      **     ** * ** * * **  **  ** *    **

BmOR3       RILIHNLLTFPLPEAITASKKFVDKHRCNGNRSEFVLEEAKLYSPAEMWQVTDRLRQCID
Hvir_OR13   RLLTYHLANFKKPASVLESNDNNKDEIKDHS----------YTEEELKEVFSKLREYIQ
            * *   *  *  *     *                      *   *   *   **  *

BmOR3       YHRKLVEFTGDISEAFGPMLFVYYLFHQVSGCLLLLECSQLNTAALVRYGVLTVVLYQQL
Hvir_OR13   HHNLILEFSSEMSNAFGPALLAYMVFHQVSGCILLLECSQLDTKTLVRYGPLTIVIFQQL
             *    **    * **** *  *  ******* ******** *  ***** ** *  ***

BmOR3       IQLSVIVESVGTVTGRLKDAVYEVPWEYMDTSNRKTVAIFLMNVQEPLHVNALGLAKVGV
Hvir_OR13   IQISVIFELLGSSNDKLIDGVYLVPWEYMDTKNRKLVFTMLRQSHRSINLTMMSMVTVGV
            ** *** *  *     * * ** ******** *** *   *               ***

BmOR3       QSMAAILKTSFSYFTFLRTVSE--
Hvir_OR13   QTMTAILKTSFSYFVMLKTVAEEE
            * * **********  * ** *
```

Figure 1. Amino acid sequence alignment of two odor receptor proteins from silk moth (BmOR3) and tobacco budworm (HvirOR13). Note absence of similar sequence identities throughout much of the remaining sequence. Degenerate primers were designed from the area in red.

Codling moth cDNA libraries were created using the SMART cDNA synthesis system (Clonetech). Using a degenerate primer and 3' RACE strategy (see proposal methods) we isolated and cloned multiple bands. These bands were cut from gels, cloned, and DNA sequences obtained. Figure 2. shows an example of bands as they appeared following separation in gels.

Subsequently we used the DNA sequences obtained from the 3'RACE (partial sequences of the receptors). The deduced amino acid sequences of five of these odorant receptors are provided in Figure 3 in alignment format that shows the very low similarity they have to the odor receptors of silkworm and budworm.

Subsequently we designed specific primers within the sequenced region and performed 5'RACE to obtain the remaining (5-prime) sequences for receptors. To date we have the full-length cDNA sequences that encode five pheromone family receptors and two additional odor receptors that are expressed in male CM antennae. Figure 4 displays a genetic tree which summarized the genetic relatedness of these genes with those of other moths. The five in the lepidopteran pheromone receptor family are highlighted. The amino acid sequences of the putative codling moth pheromone receptors are highly similar; CpOR3 vs. CpOR4 share 84.9% identity and 90.8% similarity, and CpOR3 or CpOR4 vs. CpOR1 share >59% identity and >70% similarity.

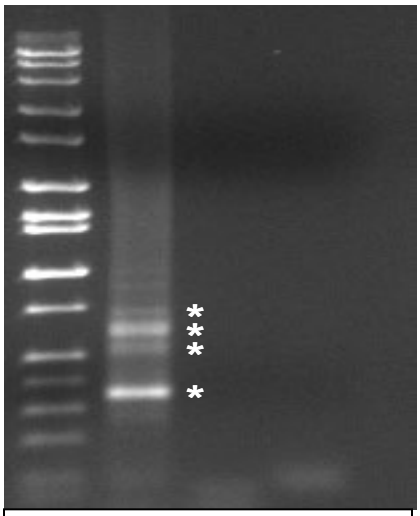

Figure 2. Example of PCR product using degenerate primers and normalized male antennal cDNA. Bands with asterisks were cloned and sequenced

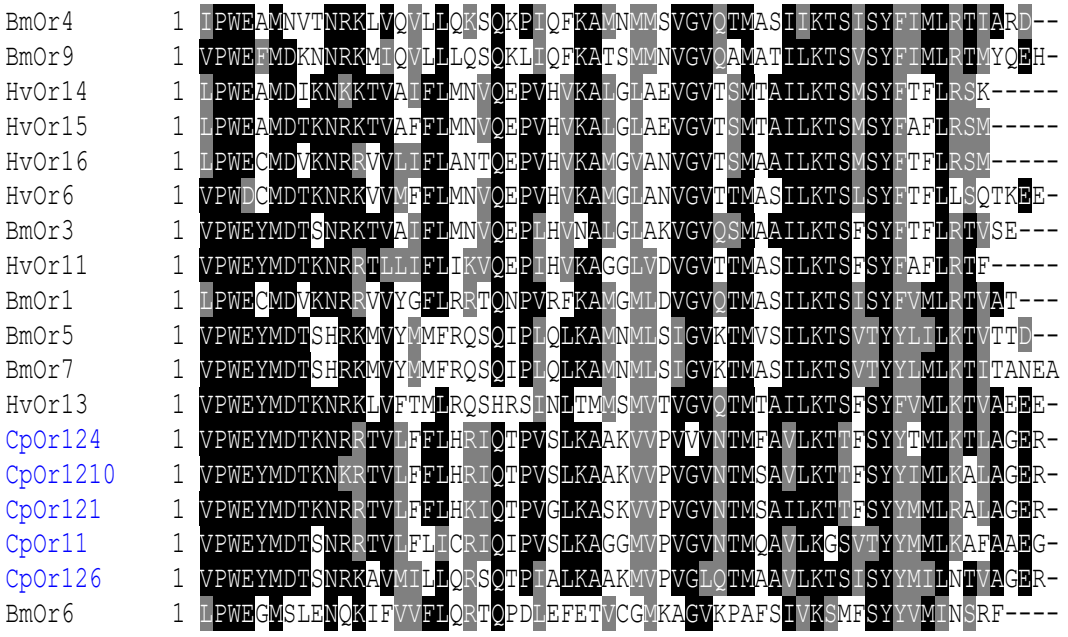

Figure 3. Alignment of 3-prime regions of silkmoth (Bm), budworm (Hv) and codling moth (Cp) odor receptors. The leftmost 8 amino acids identify the region from which degenerate primers were designed.

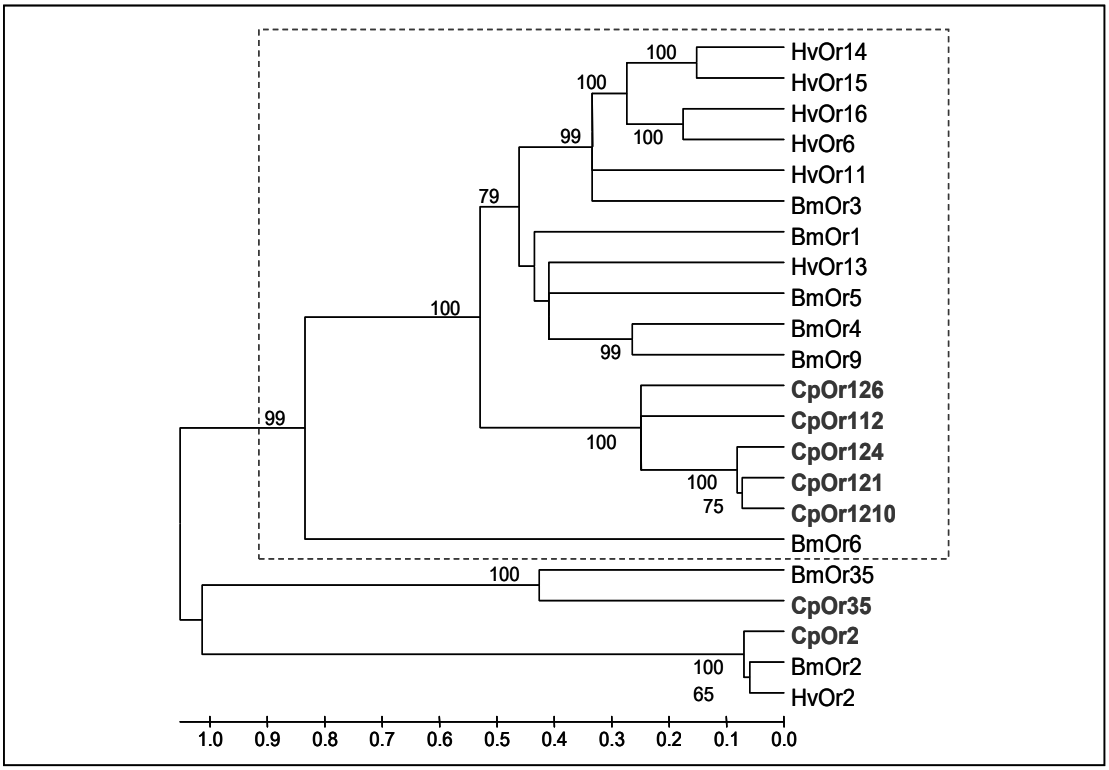

Figure 4. Neighbor joining tree of 22 **full-length** odorant receptors derived from an antennal cDNA library. Dotted line encloses pheromone family. **CpORxx**= *Cydia pomonella receptors*; Bm= *Bombyx mori*; Hv= *Heliothis virescens*.

The cDNAs encoding the pheromone receptors appear to be products of five separate genes based on unique nucleotide sequences of the 3' and 5' untranslated regions of their transcripts (data not shown). The cDNA sequences of two other ORs, CpOR2 and CpOR35, have also been determined. The deduced amino acid sequence of CpOR35 is most closely related to an OR identified in Bombyx mori (BmOR35) sharing 41% identity and 59% similarity. CpOR2, the ubiquitous receptor is nearly identical to that found in other insects including other moths and flies.

***Revision to proposal in light of NRI-funding*:**

The degenerate primer and 5'/3' RACE strategy to identify codling moth odorant receptors has been very successful. However, the lack of known lepidopteran odorant receptors will prevent us from designing primers to isolate and sequence all 58-60 receptor sequences we expect from codling moth. These will be resolved using 454-pyrosequencing technology and this aspect of the work as it pertains to adult CM receptors will be largely supported by the NRI award. Our emphasis for 2008 will be on objectives 1-3 applied to chemosensory (olfactory and gustatory) receptors of neonate larvae. In addition to chemosensory receptors we will also identify, sequence and clone in preparation for cell-based studies the suite of receptor proteins that regulate development, feeding, and reproduction. To this end we also will continue to discover, clone, and sequence the secondary messengers associated with chemoreception (g-proteins, tyrosine kinases) and other members of the secondary message cascade that apply to reproductive biology and development. We will also clone complete sequences in expression vectors in preparation for cell based assays. We have complete sequence for the general g-protein and the olfactory g-protein expressed in adult male antennae, and many partial sequences of other receptors that were collateral benefits of the sequencing conducted during discovery of the chemosensory receptors. Application of 454-pyrosequencing to the cDNA pool from the chemosensory regions of neonates (heads) will dramatically increase the pace of discovery of neuron-endocrine receptors as well as chemosensory receptors. This will be supported with WTFRC funds.

CONTINUING PROJECT REPORT (EXTENSION REQUEST) **YEAR:** 1 of 3

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

PI:	Stephen F. Garczynski	**Co-PI(2):**	Laura S. Lavine
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Address:	5230 Konnowac Pass Rd	**Address:**	P.O. Box 646382
City:	Wapato	**City:**	Pullman
State/ Zip	WA 98951	**State/ Zip:**	WA 99164-6382

Total funding request: **Year 1:** $30,000 **Year 2:** $0 **Year 3:** $30,000

Budget 1:

Organization: USDA-ARS		**Contract Administrator:** Bobbie Bobango	
Telephone: (509) 454-6575		**Email:** Bobbie.Bobango@ars.usda.gov	
Item	**Year 1: 2007**	**Year 2:** 2008	**Year 3:** 2009
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$10,000		$10,000
Travel			
Miscellaneous	$5,000		$5,000
Total	$15,000	0	$15,000

Budget 2:

Organization: Washington State University		**Contract Administrator:** ML. Bricker / Barb Smith	
Telephone: (509) 335-5504		**Email:** mdesros@wsu.edu / niehoff@wsu.edu	
Item	**Year 1: 2007**	**Year 2** 2008	**Year 3** 2009
Salaries			
Benefits			
Wages	6,240		6,750
Benefits	150		162
Equipment			
Supplies	8,110		7,588
Travel	500		500
Miscellaneous			
Total	$15,000	**0**	$15,000

The objective and methods of this proposal have not changed but work has only just begun because of delays in receiving funds and further delays in purchasing needed equipment. Specifically the fast-liquid chromatography system was just delivered and installed in mid-November. **Therefore we respectfully request a "no-cost" extension of this work for 2008.**

OBJECTIVES

The long-term goal of this project is to identify novel targets in the codling moth for use in pesticide discovery programs. In this proposal, we will determine the mode of action of toxins produced by the bacterium *Bacillus thuringiensis* (Bt) using codling moth larvae and a codling moth derived cell line as our experimental systems. Understanding the mechanism by which Bt toxins kill insect pests will provide us with the new targets that we can use for the bio-rational development of novel insecticides.

The specific objectives of this proposal include:

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

METHODS

Several studies in the scientific literature document susceptibility of codling moth larvae to Bt toxins. Due to codling moth feeding behavior in the orchard, formulations of Bt toxins have not met the desired control levels. We would like to take advantage of Bt toxin mode of action in codling moth larvae and use this information to identify novel targets for pesticide development. We will be using the following approach:

1) Determine the potencies of 10 different Bt toxins against codling moth larvae and codling moth derived cell line. The information from this objective will allow us to identify toxins of interest.

2) Once potencies of Bt toxins are determined in Objective 1, we will use the most active toxins to detect cell surface receptors. After we detect the receptors, we will characterize and identify the molecules to which Bt toxins bind at the midgut cell surface.

3) Using the codling moth derived cell line, we will determine the effects of the most active toxins on various signal transduction pathways.

4) Once the affected signal transduction pathways are identified, we will characterize the protein components of the system. After characterizations of the affected pathways are completed, we will clone the transcripts encoding the protein components of the various pathways.

5) The cloned transcripts will be transfected into insect cell lines and assays will be developed to monitor the protein activity. This assay can then be used to screen for chemicals that alter protein activity.

FINAL PROJECT REPORT
WTFRC Project Number: AH-05-508

Project Title: Employing Biological Elements of Orchard Ecosystems

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City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Ray Fuller, Dr. Gennaro Fazio

Other funding Sources: USDA Integrated Organic Research Program
Agency Name: USDA CSREES
Amount awarded: $303,267
Notes:

Total Project Funding: $166,640

Budget History:

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	22,800	23,940	25,137
Benefits	6,840	7,182	7,541
Wages	12,000	12,000	12,000
Benefits	3,600	3,600	3,600
Equipment	0	0	0
Supplies	8,000	8,000	8,000
Travel	800	800	800
Miscellaneous			
Total	**54,040**	**55,522**	**57,078**

Justification

For sites lacking significant lesion nematode populations, pre-plant *Brassica napus* seed meal amendment used in conjunction with a post-plant mefenoxam (RidomilGold EC) soil drench has provided levels of replant disease control, growth and yield equivalent to pre-plant soil fumigation (Mazzola & Mullinix, 2005). Preliminary studies with alternative seed meals suggest that disease control can be improved upon and may circumvent need for the post-plant mefenoxam application. Realization of this outcome would allow for the implementation of this disease control strategy in organic production systems.

The overall objective of this program is to develop an integrated management method compatible to conventional and organic apple production systems that provides the shortest time frame to initial commercial harvest when re-establishing orchards on sites previously planted to apple. As similar biological entities appear to have a role in replant problems encountered in pear, peach and cherry (Mazzola, unpublished data), it is plausible that such a system would have utility across tree fruit production systems.

Specific objectives:
1.) Examine the capacity of Brassicaceae seed meals to suppress the biological complex inciting replant disease and enhance tree growth in replant orchard soils.

2.) Determine the mechanism(s) by which these soil amendments provide control of the various plant parasites and pathogens that incite replant disease development, with emphasis on *Rhizoctonia solani*.

3.) Assess the influence of rootstock genotype on composition of resident *Streptomyces* populations and the efficacy of RSM-induced disease suppression

SIGNIFICANT FINDINGS:

- Pre-plant brassicaceae seed meal (BSM) soil amendment in conjunction with a post-plant mefenoxam (RidomilGold) soil drench provides initial increase in tree growth & yield equivalent to soil fumigation.

- The biological need for the post-plant mefenoxam soil drench differs with BSM.

- When used as a singular treatment (without mefenoxam), only *Sinapis alba* (yellow mustard) seed meal significantly improved tree performance.

- In field trials, initial tree growth in fumigated soils at times was inferior to or equivalent to other treatments, even those that did not provide disease control.

- The mechanism of pathogen suppression provided by BSM differs by target pathogen species, time and space.

- Growth performance in seed meal amended soils differed by rootstock.

- A composite seed meal compatible with organic production systems was formulated and in the field generated first-year tree growth comparable to soil fumigation.

RESULTS AND DISCUSSION

Tree Growth Performance in Orchard Trials

2005-2007 CV orchard trial

A field trial was established at the Columbia View Research and Demonstration Orchard, and was initiated with tree removal (Red Delicious/Seedling) from the site in October, 2004. In addition to *B. napus* cv. Dwarf Essex (canola), seed meal of *Sinapis. alba* cv IdaGold (yellow mustard) and *B. juncea* cv Pacific Gold (oriental mustard) were employed with or without a post-plant mefenoxam (Ridomil) soil drench. These seed meals were chosen based upon our data obtained in controlled environment studies, and data from the literature, suggesting the relative activity of these materials toward the pathogens and parasites causing replant disease. Seed meal was applied in April 2005 at a rate of 3.08 ton per acre and incorporated into the soil profile by rotovation. The site was planted to Gala/M26 on 19 May with 10 trees per plot with five replicates. A split-plot design was used with five trees per plot receiving a post-plant mefenoxam soil drench on 24 May.

All seed meal amendments significantly improved tree growth over three growing seasons when used in conjunction with the post-plant mefenoxam soil drench (Figure 1). Among seed meal amendments, only *S. alba* significantly improved growth relative to the control in the absence of the mefenoxam soil drench. Mefenoxam (Ridomil) alone did not improve tree growth over the three year study.

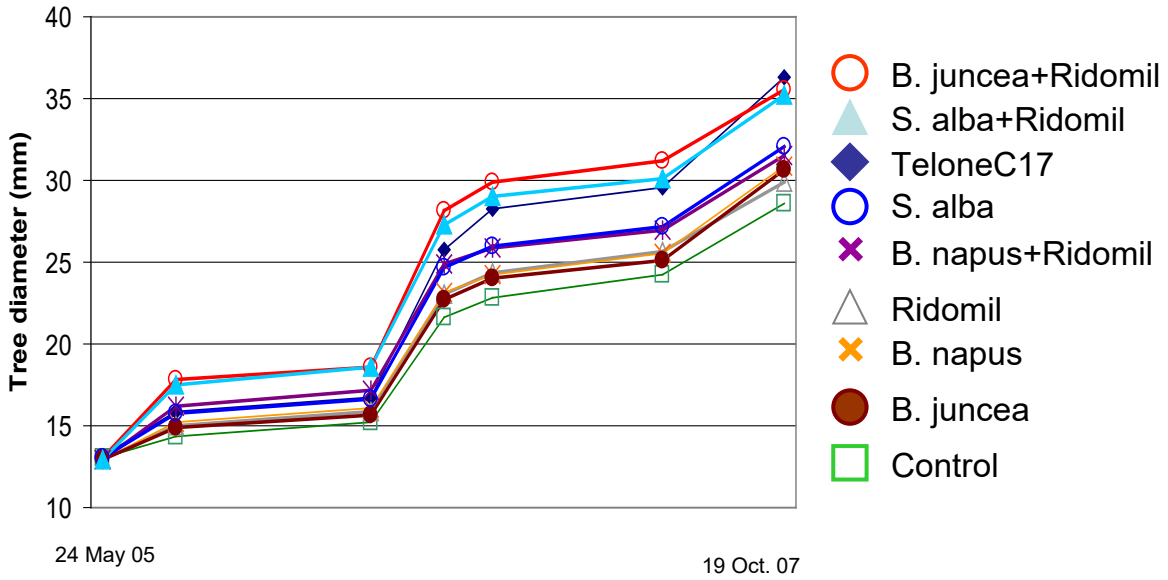

Figure 1. Impact of soil treatments on growth of Gala/M26 at the Columbia View Research and demonstration orchard.

Soil treatments had similar impacts on tree yield as was observed for tree growth. All seed meal treatments that included a post-plant mefenoxam soil drench significantly improved initial fruit yield relative to the control, and yields from *B. juncea* or *S. alba* + mefenoxam treated blocks were significantly greater than the Telone-C17 fumigated blocks. *B. napus* seed meal, *B. juncea* seed meal or mefenoxam alone did not significantly improve yields. As with tree growth, only *S.*

alba was the only seed meal when used singularly significantly improved yields relative to the non-treated control.

Table 1. Impact of soil treatments on fruit yields (kg per tree) from Gala/M26 established at the Columbia View Research and Demonstration orchard in May 2005.

Treatment	2006	2007
Control	0.0	4.71a
Control + mefenoxam	0.17	5.76ab
Telone-C17	0.20	6.94bc
B. napus	0.78	4.70a
B. napus + mefenoxam	0.94	7.83cd
B. juncea	0.95	4.96a
B. juncea + mefenoxam	1.49	9.29d
S. alba	0.45	7.01bc
S. alba + mefenoxam	1.55	8.67d
P=	0.234	0.002

A similar trial was initiated in 2002 at the Columbia View orchard, but included only *B. napus* seed meal. Results were similar to those attained for the trial initiated in 2005. Using the seed meal as a singular treatment did not improve tree growth or yield. Only when used in combination with mefenoxam did this seed meal improve performance of Gala/M26 in replant orchard soil (Table 2). As previously reported, this treatment did not improve tree growth or yield on a site that possessed significant lesion nematode populations (Mazzola and Mullinix, 2005).

Table 2. Mean cumulative fruit yields (2003-2006) of Gala/M26 established at the Columbia View orchard in May 2002.

Treatment	Yield (kg/tree)
Control	4.33a
Telone-C17 fumigation	7.53b
B. napus seed meal + mefenoxam	7.86b
B. napus seed meal	4.97a

Interestingly, initial year growth of trees established in fumigated soils tended to under-perform or was not significantly different from that of other treatments, even those treatments that tended to increase the inoculum potential of pathogens known to reside in these soils (e.g. *Pythium* spp.). This has been observed on multiple occasions during our trials conducted in conventional orchard systems and suggests that some resident biological community essential to optimal tree growth was negatively impacted in fumigated soils.

Growth performance attained was directly related to control of the pathogen complex resident to this site. All seed meal amendments significantly reduced root infection by *Rhizoctonia* spp. and both *B. juncea* and *S. alba* significantly reduced infection by *Cylindrocarpon* spp. (Table 3). As expected, *B. napus* and *S. alba* amendments dramatically increased populations of *Pythium* and the effectiveness of the post-plant

mefenoxam treatment in these seed meal treated soils is due to the control of this oomycete group. Unexpectedly, our data demonstrated the need for the post-plant mefenoxam drench for effective use of *B. juncea* seed meal, even though *Pythium* spp. were effectively controlled (Table 3). Analysis of root systems demonstrated that the enhanced tree growth resulting from mefenoxam application to *B. juncea* amended soils resulted from control of *Phytophthora cambivora* and *Phytophthora megasperma*.

Table 3. Impact of soil treatments on soil populations and Gala/M26 root infection by fungal pathogens.

Treatment	*Pythium spp.* per g soil	*Cylindrocarpon* root infection (%)	*Rhizoctonia* root infection (%)
Control	265b	19.5a	14.0a
Telone C17	65a	9.0b	11.7ab
Brassica juncea PG	75a	1.9b	5.0b
Brassica napus DE	5320c	18.0a	6.6b
Sinapis alba IG	4515c	7.1b	2.8b

Significance to industry: These findings are very promising due to the fact that the rootstock employed (M26) is highly susceptible to the pathogen complex resident to this site. Thus, it is plausible that further progress could be made towards a viable alternative to fumigation through the addition of host tolerance to the production system.

Based upon these trials, a composite seed meal amendment was formulated with the goal of developing a product that would not require a post-plant mefenoxam soil drench and has capacity to provide lesion nematode control. Such a material may be compatible with organic production systems. These trials are discussed in the following section:

2006 Orchard Rootstock Trial:

A field trial was established at a commercial organic orchard to evaluate the efficacy of a *Brassica napus/Brassica juncea* composite seed meal amendment for control of replant disease, and to assess whether the response was rootstock dependent. Trials were conducted in a commercial organic orchard. Composite seed meal or *B. napus* seed meal alone was applied on May 3 at 3.08 lbs per linear foot of tree row and rotovated into the soil profile. The site was planted with G11, G16, G30, M7, M9, M26 and seedling rootstocks. All G11 rootstocks died irrespective of soil treatment.

B. juncea seed meal suppressed the repeatedly observed *B. napus*-induced proliferation of *Pythium* spp. (Table 4). In assessments completed to date, the *B. napus/B. juncea* composite amendment significantly reduced lesion nematode populations recovered from roots of M26 and M7 rootstocks to a level comparable to or better than that attained through pre-plant soil fumigation. However, the level of nematode control attained was not comparable to what has been achieved with *B. juncea* alone in greenhouse trials (Table 5). Optimization of nematode control with the combination seed meal may require modification of application method to enhance soil retention of the *B. juncea*-derived allyl-isothiocyanate (AITC).

Table 4. Effect of treatments on *Pythium* soil populations.

	Pythium soil populations (cfu/g soil)	
Treatment	At planting (5.29.06)	At harvest (10.26.06)
Control	550b	604b
Telone-C17 fumigation	135a	350a
B. napus Athena	3890c	3917c
B. napus Athena+*B. juncea* PG	120a	300a

Table 5. Impact of soil treatments on recovery of lesion nematode from roots of apple rootstocks planted at Stormy Mountain Ranch, Chelan, WA

	Pratylenchus penetrans populations (#/g root)	
Treatment	M26	M7
Control	114b	189b
Telone-C17 fumigation	59a	104ab
B. napus Athena	109b	85a
B. napus Athena+*B. juncea* PG	42a	92a

2007 Orchard Field Trial

A further field trial was established at Stormy Mountain Ranch to evaluate the composite seed meal amendment for replant disease control. The site was treated in April 2007 with a *B. juncea/B. napus* (1:1; 3 ton/acre) seed meal amendment and rotovated, fumigated with Telone-C17 or not treated (control). The site was planted with Gala/M26 on 23 May. Root samples were collected 23 October.

Initial tree growth in seed meal treated soil was comparable to that attained in Telone-C17 fumigated soil, and significantly better than the non-treated control (Fig. 2).

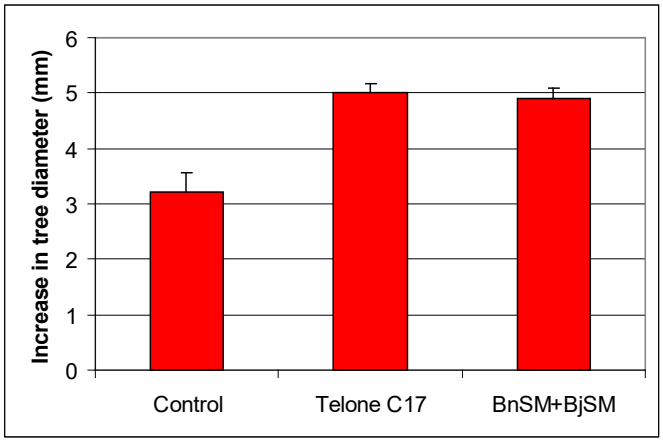

Figure 2. Initial year increase in trunk diameter for Gala/M26 at Stormy Mountain Ranch organic orchard.

As observed in previous trials, increased tree growth was associated with pathogen suppression. The seed meal treatment was as effective as soil fumigation in the initial suppression of lesion nematode root populations (Fig. 3). *Pythium* spp. were not stimulated by the composite seed meal amendment and *Pythium* root infection was not different from the fumigated check, and numerically, but not statistically, lower than the non-treated control (Fig. 4).

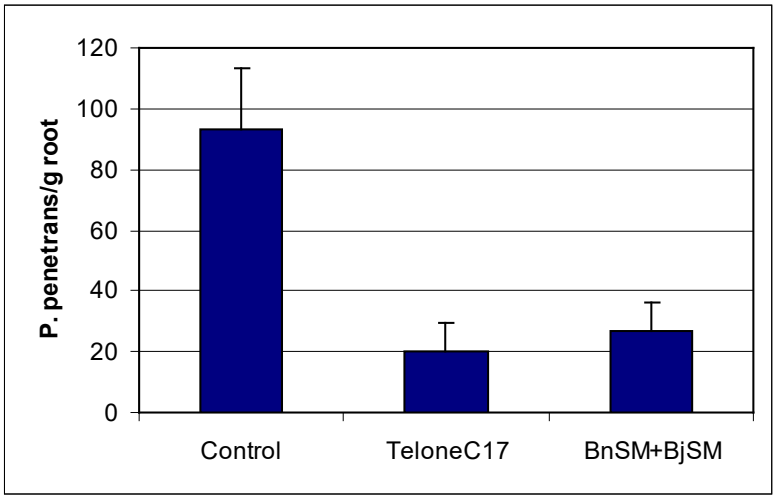

Figure 3. Impact of soil treatments on recovery of lesion nematodes (*P. penetrans*) from the roots of Gala/M26 at the Stormy Mountain Ranch.

Significance to industry: These preliminary findings indicate that this alternative treatment may be of value for control of replant disease in organic orchard systems. As this material also provides a significant source of N, P, K and S, the economic value of this strategy may surpass that resulting from disease suppression.

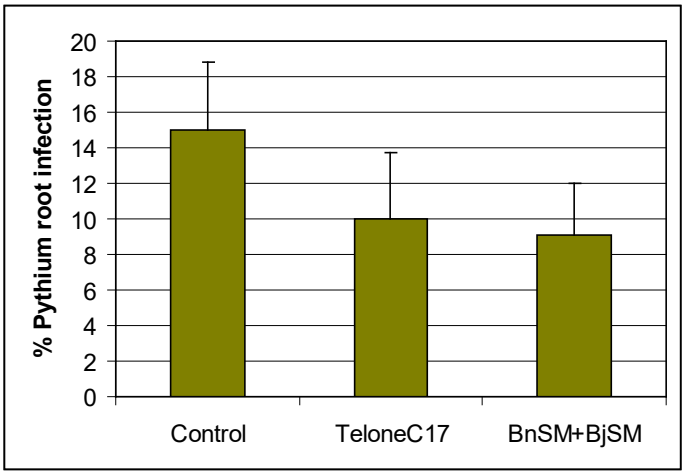

Figure 3. Impact of soil treatments on recovery of *Pythium* spp. from the roots of Gala/M26 at the Stormy Mountain Ranch.

Greenhouse rootstock trials

Multiple rootstock trials were performed in the greenhouse in addition to the field trial cited above. In general, rootstocks from the USDA-Geneva/Cornell breeding program

demonstrated lower levels of susceptibility to the pathogens (e.g. *Pythium* spp.) and parasites (e.g lesion nematodes) than those from the Malling series. G11 and G30 rootstock, in general, supported lower lesion nematode reproduction relative to other rootstocks evaluated (Table 6). Likewise, these rootstocks exhibited higher tolerance to infection by *Pythium* spp. relative to M26, MM106 or MM111.

Significance to industry: These findings support previous and ongoing trials (conducted by G. Fazio, T. Auvil) which indicate that apple rootstocks differ in relative performance on orchard replant sites, and by association tolerance to the causal pathogen complex. Thus, use of the Geneva series rootstocks is likely to provide growers with improved productivity on orchard replant sites.

Table 6. Impact of rootstock on recovery of *Pratylenchus penetrans* (lesion nematode) and *Pythium* spp. from apple roots when grown in GC orchard replant soil

Rootstock	# *P. penetrans*/g root Experiment			% *Pythium* root infection
	A	B	C	
Bud9	374b[z]	142a	578a	16.6b
G11	113a	-	434a	13.4b
G16	292ab	196a	1269cd	9.5b
G30	136a	-	472a	10.7b
M7	535b	710c	930	23.0ab
M9 Pajam	416b	254a	672ab	15.0b
M9 Nic29	430b	284a	1556d	13.2b
M26	562b	480b	393a	35.0a
MM106	478b	1754d	499a	34.8a
MM111	597b	850c	1085c	39.1a
Seedling	380b	371ab	956bc	20.5b

[z]Values in the same column followed by the same letter are not significantly (P>0.05) different.

Brassica seed meal mechanism(s) of action

Knowledge concerning the means by which any treatment provides disease control is imperative for the effective use of a practice beyond the site in which initial experiments are conducted. The inability of producers to effectively, consistently and predictably utilize many 'alternative' treatments, such as composts or compost teas', for disease control results from an absence of information relative to why disease control was attained, or conversely, why a treatment failed. Numerous studies have reported that the efficacy of brassica plant residues, applied either as a green manure or in the seed meal form used in these studies, is due to the process termed biofumigation. This process relies upon the hydrolysis of chemicals (glucosinolates) in brassica tissues that when hydrolyzed yield various active compounds including isothiocyanates, related to the active compound in the fumigant metam sodium.

Our studies demonstrated with absolute certainty that the mechanism by which brassica residues induce disease control can be pathogen specific, involve a biological rather than a chemical mechanism, and can change over time. This can be demonstrated in the example concerning control of *Rhizoctonia solani* in response to *B. juncea* amendment. Allyl-

isothiocyanate (AITC) is generated in response to *B. juncea* seed meal amendment. Production and release of AITC from a test soil was complete within 24 of seed meal amendment (Fig. 4A). When *R. solani* was introduced into soil at the same time as seed meal application (0h), disease was controlled. When introduction of the pathogen was delayed for 24 h, disease in seed meal amended soils is as severe in the control (Fig. 4B). This demonstrated that **initial** disease control was dependent upon production of AITC.

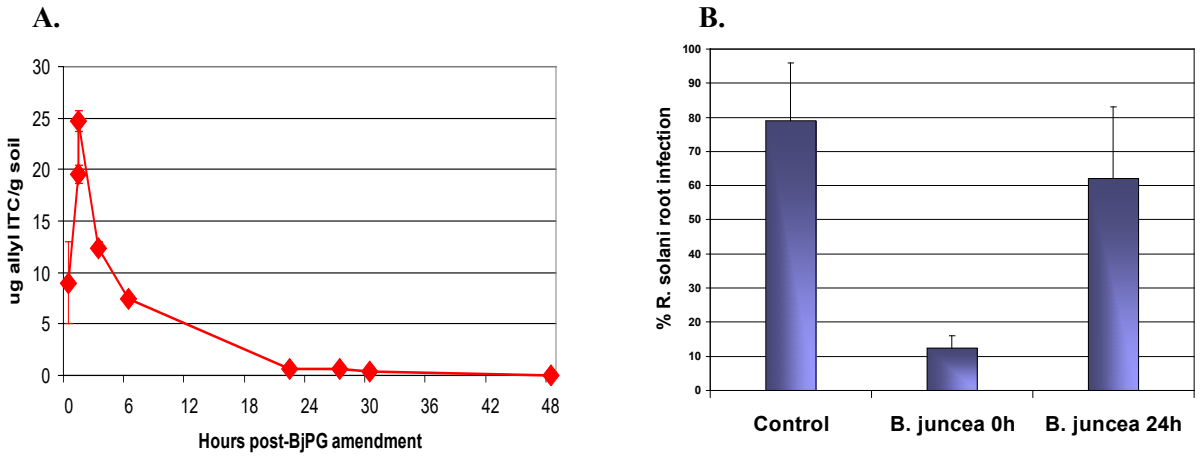

Fig. 4. Release of allyl isothiocyanate from *Brassica juncea* seed meal amended soil over a 48 h period (A). Relative control of *Rhizoctonia solani* when the pathogen was added to soil at the time of seed meal amendment (0h) or when delayed until 24 h after seed meal amendment.

However, the active mechanism changed with time, and disease suppression was restored simply by incubating soils for 4-6 weeks (Table 7). Restoration of disease suppression was dependent, in part, upon proliferation of bacteria belonging to the genus *Streptomyces*, and these bacteria are also responsible for control of *R. solani* attained with *B. napus* or *S. alba* seed meal (Mazzola et al., 2007). Sterilization of the soil prior to adding the pathogen abolished the disease control ability of these seed meals (Cohen and Mazzola, 2005; 2006), providing further support for the role of the resident soil biology in seed meal-induced suppression of *R. solani*. Numerous *Streptomyces* isolates were found to control *R. solani*, and did so through the induction of host defense responses (Cohen and Mazzola, 2006).

Table 7. Control of Rhizoctonia root infection and populations of *Streptomyces* in *B. juncea* seed meal amended soil incubated for four weeks prior to addition of the pathogen.

Treatment	% *R. solani* root infection	*Streptomyces* soil population
Control	79b	1.25×10^5
B. juncea seed meal	28a	3.75×10^7

Significance to industry: These findings are important as they have identified a biological indicator (*Streptomyces* spp.) which can now be monitored to determine when seed meal treated soil has developed suppressiveness to *Rhizoctonia solani*. Likewise, this "indicator species" can now be used in attempts to identify other materials that may be suitable for the control of *Rhizoctonia*. In contrast to *R. solani*, AITC production by *B. juncea* seed meal is the dominant mechanism responsible for control of *Pythium* spp. However, emerging data from this program indicate that an as yet unidentified biological mechanism is responsible for long-term suppression of this pathogen.

Impact of rootstock on Streptomyces populations and seed meal disease control

As cited above, rootstock performance in seed meal amended soils was variable, both in the greenhouse and in the field. In the field, root biomass production for G30 was superior to all other rootstocks examined. However, the short-term duration of these trials and variability among trials preclude the formulation of additional firm conclusions.

Rootstocks did vary in the capacity to support resident populations of *Streptomyces*. G30, Bud9, and G11 supported the largest populations on a per gram root basis, while populations recovered from the roots of M9 were significantly lower (Fig. 5). This is a clear association, but at this time there is not sufficient data to conclusively state a causal role for this association in the relative differences in disease tolerance observed amongst rootstocks.

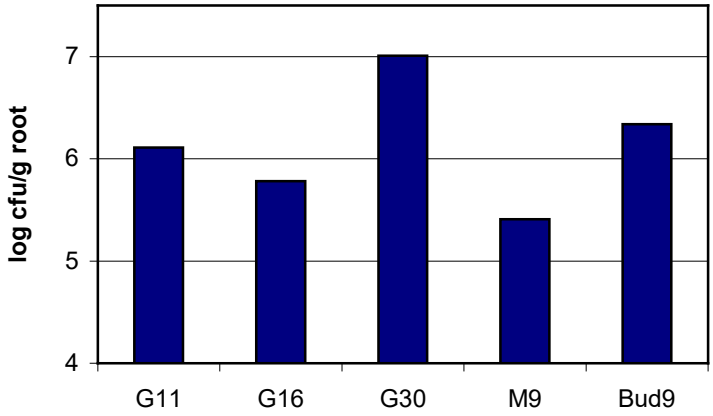

Figure 5. Relative recovery of *Streptomyces* spp. (log cfu/g root) from apple rootstocks grown in GC orchard soil.

Significance to industry: *Streptomyces* are a known to produce a broad-spectrum of antifungal and antibacterial compounds. In our work, we have demonstrated that these bacteria can also induce apple host defense responses. Should additional studies support these initial findings, as well as the importance of these bacteria in disease control, the capacity to support *Streptomyces* populations could serve as a relevant bio-marker for the selection of disease-tolerant rootstocks.

Citations:

Cohen M. F., and **Mazzola, M.** 2006. Impact of resident bacteria, nitric oxide emission and particle size on root infection by *Pythium* spp. and *R. solani* AG-5 in *Brassica napus* seed meal amended soils. Plant and Soil 286:75-86.

Cohen, M. F., Yamasaki, H., and **Mazzola, M.** 2005. *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of Rhizoctonia root rot. Soil Biology & Biochemistry 37:1215-1227.

Mazzola, M., Brown, J., Izzo, A, and Cohen, M. F. 2007. Mechanism of action and efficacy of seed meal-induced suppression of pathogens inciting apple replant disease differ in a Brassicaceae species and time-dependent manner. Phytopathology 97:454-460.

Mazzola, M., and Mullinix, K. 2005. Comparative field efficacy of management strategies containing *Brassica napus* seed meal or green manure for the management of apple replant disease. Plant Disease 89:1207-1213.

FINAL PROJECT REPORT
WTFRC Project Number: CP-07-703

Project Title: DNA arrays for monitoring orchard soil microbial communities

PI: Mark Mazzola
Organization: USDA-ARS
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Cooperators:

Other funding Sources:
Agency Name: USDA-ARS, Postdoctoral Associate Award
Amount awarded: $27,000
Notes:

Total Project Funding: $35,000

Budget History:

Item	Year 1: 2007	Year 2:	Year 3:
Salaries	20,000		
Benefits	6,000		
Wages			
Benefits			
Equipment			
Supplies	9,000		
Travel			
Miscellaneous			
Total	**35,000**		

The goal of these studies was to develop a DNA array-based method that could be used in the analysis of fungal community composition and function in orchard soils, for rapid identification of prominent taxa in mixed samples, and that is also more accessible and flexible than current options. As a more easily accessible and cost-effective technology relative to slide-based DNA arrays, we utilized nylon membranes as the printing medium with a colorimetric labeling and detection system. To avoid the time and costs needed to design and validate oligonucleotide probes, we employed the entire **Internal Transcribed Spacer** (ITS) region (ITS1/5.8S/ITS2) of the fungal ribosomal DNA as both the target on the array and the probe. The fungal ITS regions are well known to be particularly useful for species-level distinctions in fungi. While the 5.8s region is generally conserved, the ITS1 and ITS2 regions are quite variable and collectively should allow maximal potential for distinguishing fungal taxa.

OBJECTIVES

1) Develop DNA microarrays containing a comprehensive collection of DNA sequences from fungi found in orchard soil and apple roots in central Washington.

2) Evaluate the capacity of the DNA microarray to measure community structure and function for predictive purposes.

SIGNIFICANT FINDINGS

- The method developed allowed for distinguishing fungi that differed by approximately 5-10% in ITS DNA sequence
- Using an artificially constructed fungal community, the DNA array identified greater than 80% of the members in the community
- The array effectively described an orchard soil fungal community of known composition and identified previously known and unknown changes in composition induced by seed meal amendments
- The array identified the majority of fungal lineages in a previously undescribed and geographically distant (Quebec, Canada) orchard soil
- Based on these findings the method developed appears to be practical for the rapid detection of multiple genus-level lineages of fungi in complex orchard root and soil samples

RESULTS AND DISCUSSION

Fungal samples used in array construction came primarily from the rhizosphere of apple or soils from the Columbia View Research and Experimental (CV) Orchard (Orondo, WA). The fungal community was sampled in a number of ways to maximize the taxonomic diversity obtained. Fungi were sampled from soils directly or 5 days following amendment with various Brassicaceae seed meals in the greenhouse as described previously (Mazzola et al. 2007). Fungi were recovered from soils by manual isolation of visibly apparent hyphal growth, as well as by plating serial dilutions of

a soil suspension onto 1/5 strength potato dextrose agar amended with ampicillin (100 µg ml^{-1}). DNA was isolated from these individual isolates and the ITS region was amplified.

Alternatively, mass recovery of fungal ITS products were obtained by PCR amplification of DNA extracted from Gala/M26 roots collected at 24 plots across the CV orchard that had been planted either in native soils or soils previously amended with the same Brassicaceae seed meal amendments. The resulting DNA products were cloned and unique individuals were identified by sequence analysis. Sequence types were assigned a unique name based on their coarse affinities to taxa based on BLAST searches of Genbank.

Arrays were constructed by linking the unique ITS products onto Hybond+ membrane in triplicate. Various probe construction methods were used to test the utility of the array. These included:

1.) Probe consisting of PCR product from three different *Pythium* species.

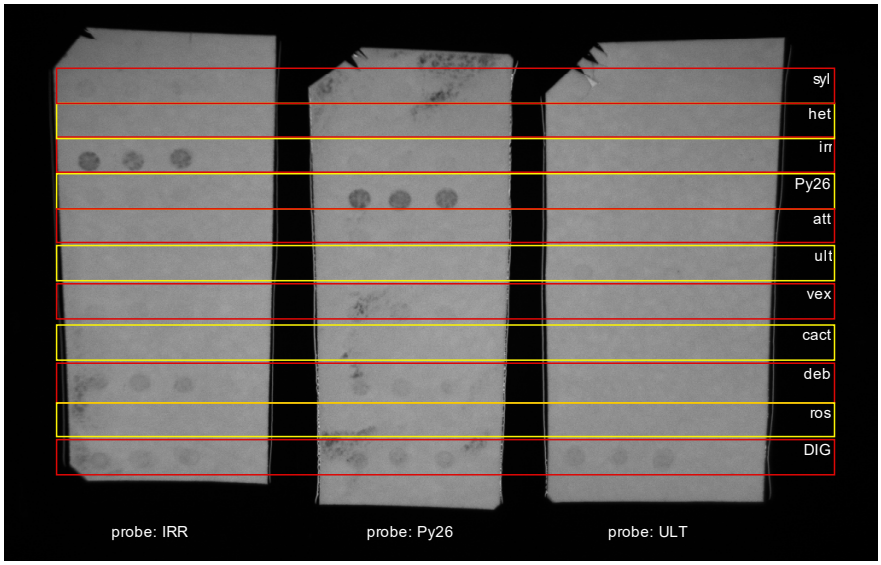
2.) Probe consisting of a mixed community of known composition constructed by amplifying 10 different environmental clones

3.) DNA was isolated from brassica seed meal amended soil at the Columbia View orchard and the ITS region was amplified to generate the probe.

4.) DNA was isolated from soil and roots obtained from an orchard in Quebec Canada and the ITS region was amplified to generate the probe

Experiment 1.

Under high stringency hybridization conditions, probes composed of the ITS region from *Pythium. irregulare* and an unidentified *Pythium sp*. ("Py26") each hybridized to targets on the array corresponding to the closest related sequences, with the strongest signal detected for the perfect match to itself (Figures 1 and 2). For example, when the *P. irregulare* probe was used, substantial hybridization signal was only detected for *P. irregulare*, *P. sylvaticum*, and *P. debaryanum* all of which possessed ITS sequence similarity greater than 90%, and the array targets corresponding to *P. irregulare* yielded the strongest signal. Similar results were obtained with the *Pythium sp.* "Py26" probe, which prominently detected *Pythium sp* "Py26" and showed lower signal for the two other most closely related species (*P. sylvaticum* and *P. debaryanum,* both possessing sequence similarities between 80-90%). The *P. ultimum* probe detected *P. ultimum,* however the signal strength was considerably lower. Under low stringency DNA hybridization conditions, the array signal remained limited to species of *Pythium* most closely related to the probe with the identical target sequence showing the strongest signal (data not shown). However, the lower stringency conditions also led to signal being detected from *Pythium* species that possessed sequence similarity of only 75-80% of the probe utilized. Even under low stringency conditions certain *Pythium* species targets were not detected with each probe, and these generally had the lowest sequence similarity to the probe species. *Phytophthora cactorum* – a representative of the genus highly related to *Pythium* - was not detected with any of the *Pythium* probes.



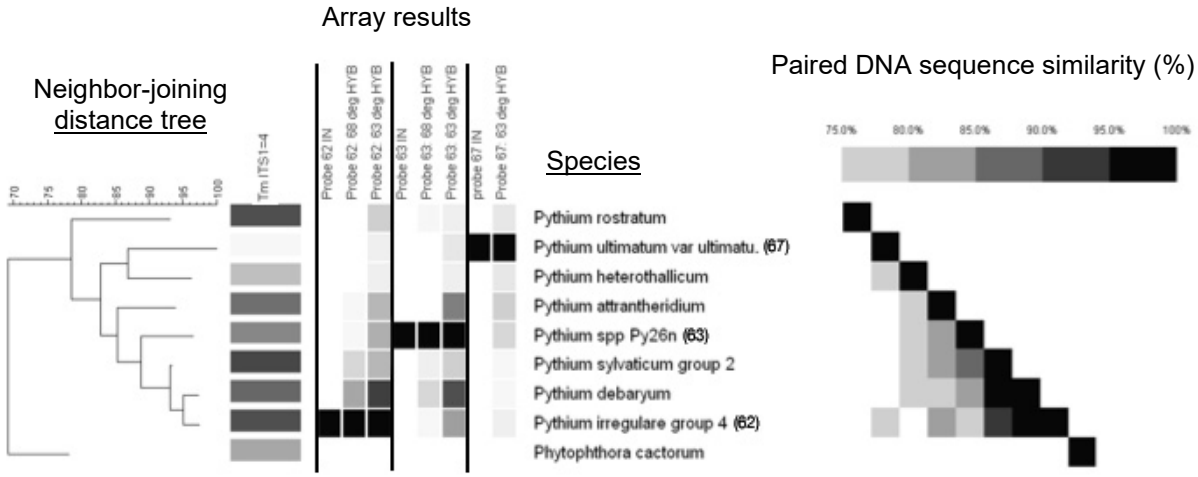


Figure 1. Identification of lineages within the genus *Pythium* using ITS PCR amplicon probes. Top: Developed miniarray blot of hybridizations performed at high temperature (68°C). Bottom: Relative hybridization signal obtained when using either "high" (68°C) or "low" (63°C) stringency temperatures with single species probes relative to DNA relatedness of species and target melting temperatures. Array signal is colored on a relative scale of 0-1.0 (white-black). Melting temperatures (Tm) of the PCR product resulting from amplification using the primers ITS1 and

ITS4 are colored on a scale from 84-95°C (white-black). The *Pythium* species used to generate the probe is noted by black box under the "probe" heading.

Experiment 2 and 3.

Analysis of artificially constructed fungal communities

A mixed community of known composition was constructed by amplifying 10 different environmental clones using the primer pair ITS1f and ITS4a. The majority (8/10) of the probe taxa that were used to construct the known community probe were successfully detected on the array under high stringency conditions (Figure 2). Additionally, targets that were less than 90% similar (based on DNA sequence distance) to samples contained in the probe typically produced weak or no signal. The two samples that were not detected were both from the *Mortierella*-related lineage which previously was found to have lower melting temperatures for the ITS target region. Thus, at the hybridization temperature (68°C) used in these studies, the probe would dissociate from the corresponding target on the array. Lowering the stringency condition (63°C) allowed detection of more of the known community (9/10 samples), however this resulted in false detection of many other array targets that were not in the probe (Figure 2B).

Analysis of seed meal amended soils

Array analysis detected a range of fungi from CV orchard soils amended with *B. napus* (Figure 2C). Following hybridization with the fungal probe generated from the *B. napus* treated soil, 18 of the 95 taxonomic array spots were visually positive. Based on DNA analysis, these 18 spots represented at least 8 unique lineages (>10% different from each other). Genera represented in array spots with signal included *Fusarium* (2 lineages), *Trichoderma*, *Acremonium*/*Nectria*, *Arthrobotrys*, *Cylindrocarpon* (2 lineages), *Mariannae*, and *Neonectria*. No signal was detected on array targets with affinities to *Mortierella.* Notable differences were observed in the fungal community detected in *B. juncea* amended soils via array analysis. Specifically, fewer taxa were identified and neither *Cylindrocarpon destructans* nor *Cylindrocarpon olidum*, both important pathogens of apple, were detected. This corresponds to results based upon the plating of Gala/M26 root samples on agar media, where infection by Cylindrocarpon spp. was not modified by *B. napus* soil amendment but was virtually eliminated in *B. juncea* amended soils (Table 1).

Table 1. Impact of soil treatments on Gala/M26 root infection by *Cylindrocarpon* spp.

Treatment	*Cylindrocarpon* root infection (%)
Control	19.5a
Brassica juncea PG	1.9b
Brassica napus DE	18.0a
Sinapis alba IG	7.1b

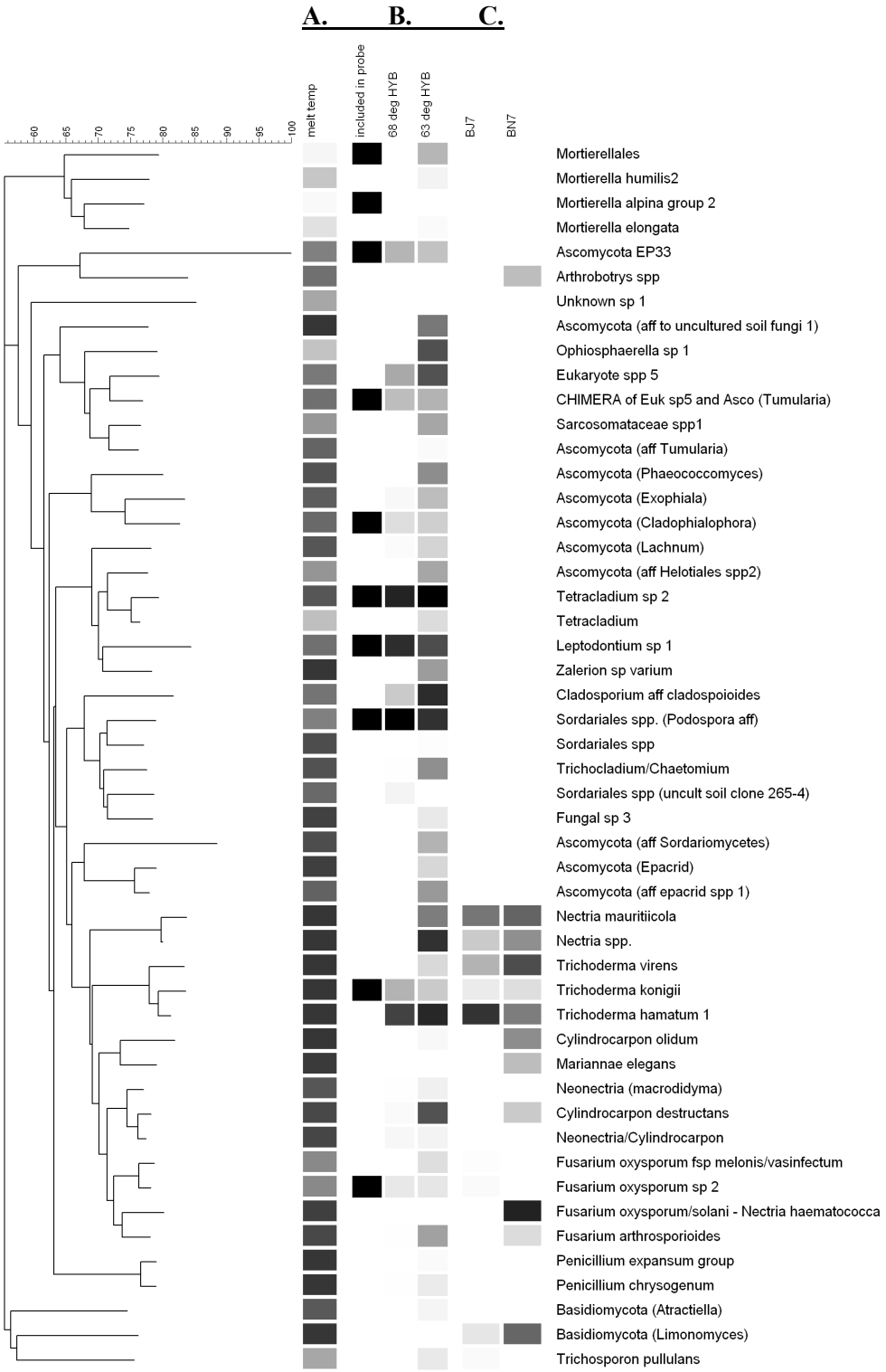

A.
B.
C.
melt temp
included in probe
68 deg HYB
63 deg HYB
BJ7
BN7
60
65
70
75
80
85
90
95
100
Mortierellales
Mortierella humilis2
Mortierella alpina group 2
Mortierella elongata
Ascomycota EP33
Arthrobotrys spp
Unknown sp 1
Ascomycota (aff to uncultured soil fungi 1)
Ophiosphaerella sp 1
Eukaryote spp 5
CHIMERA of Euk sp5 and Asco (Tumularia)
Sarcosomataceae spp1
Ascomycota (aff Tumularia)
Ascomycota (Phaeococcomyces)
Ascomycota (Exophiala)
Ascomycota (Cladophialophora)
Ascomycota (Lachnum)
Ascomycota (aff Helotiales spp2)
Tetracladium sp 2
Tetracladium
Leptodontium sp 1
Zalerion sp varium
Cladosporium aff cladospoioides
Sordariales spp. (Podospora aff)
Sordariales spp
Trichocladium/Chaetomium
Sordariales spp (uncult soil clone 265-4)
Fungal sp 3
Ascomycota (aff Sordariomycetes)
Ascomycota (Epacrid)
Ascomycota (aff epacrid spp 1)
Nectria mauritiicola
Nectria spp.
Trichoderma virens
Trichoderma konigii
Trichoderma hamatum 1
Cylindrocarpon olidum
Mariannae elegans
Neonectria (macrodidyma)
Cylindrocarpon destructans
Neonectria/Cylindrocarpon
Fusarium oxysporum fsp melonis/vasinfectum
Fusarium oxysporum sp 2
Fusarium oxysporum/solani - Nectria haematococca
Fusarium arthrosporioides
Penicillium expansum group
Penicillium chrysogenum
Basidiomycota (Atractiella)
Basidiomycota (Limonomyces)
Trichosporon pullulans

Figure 2. Results of three tests are presented in context of DNA sequence similarity. A) melting temperature of array targets; B) composition of an artificially constructed community probe and resulting array relative target signal in assays conducted under high or low stringency conditions; C) array results for probe composed of soil fungal community generated using DNA isolated from soil amended with *Brassica napus* or *Brassica juncea* seed meal. Scales are the same as Fig. 1.

Experiment 4.

Array analysis and cloning of uncharacterized environmental sample

Community composition based upon DNA sequence analysis

DNA extracted from an orchard soil in Quebec was used in amplification reactions using ITS primers to generate the probe used in experiment 4. Resulting product was also cloned and transformed into *E. coli* prior to screening by RFLP analysis to identify unique sequences. The clone screening revealed 50 unique RFLP types. DNA sequence data was obtained from 44 of the unique RFLP types and these accounted for 138 of the 144 clones screened. Approximately 40% (55/138) of the sequences contained in the probe were 90-100% similar to at least one sequence known to exist among the array targets. This portion of the probe community was composed of taxa with affinities to the genera *Leptodontium*, *Geomyces*, *Nectria*, *Tetracladium*, *Zalerion*, and *Mortierella*. A number of these probe constituents were 90% or higher similar to more than one target spot on the array. Two clone RFLP types with affinity to *Leptosphaerulina* dominated the probe community with (32% of total), but were only 80% similar to the closest DNA sequence match among array targets. Of the 17 RFLP types that were predicted to be detected on the array based on DNA sequence similarity to an array target, 14 were detected based on signal shown in the corresponding array target. Of the three that were not detected, two had affinities to the *Mortierella* group.

Community composition based upon DNA array analysis

Hybridization of the 190 taxon array with the probe generated from Quebec orchard soil fungal community DNA resulted in the detection of 26 array targets (Figure 3). Target spots with affinities to the genera *Leptodontium*, *Geomyces*, *Zalerion*, *Cadophora* were among those yielding the strongest array signals. Targets generating 0.50 or higher relative signal could be accounted for by the presence of cloned probe sequences that were 90-100% similar and that made up a large portion of the detectable clone sequences. Of the array targets yielding relative signals within the 25-50% range, three of the five had close sequence matches in the probe community. Although there were a few exceptions, most of the target spots below a relative signal of 0.20 possessed matches in the probe community however the similarities were much lower.

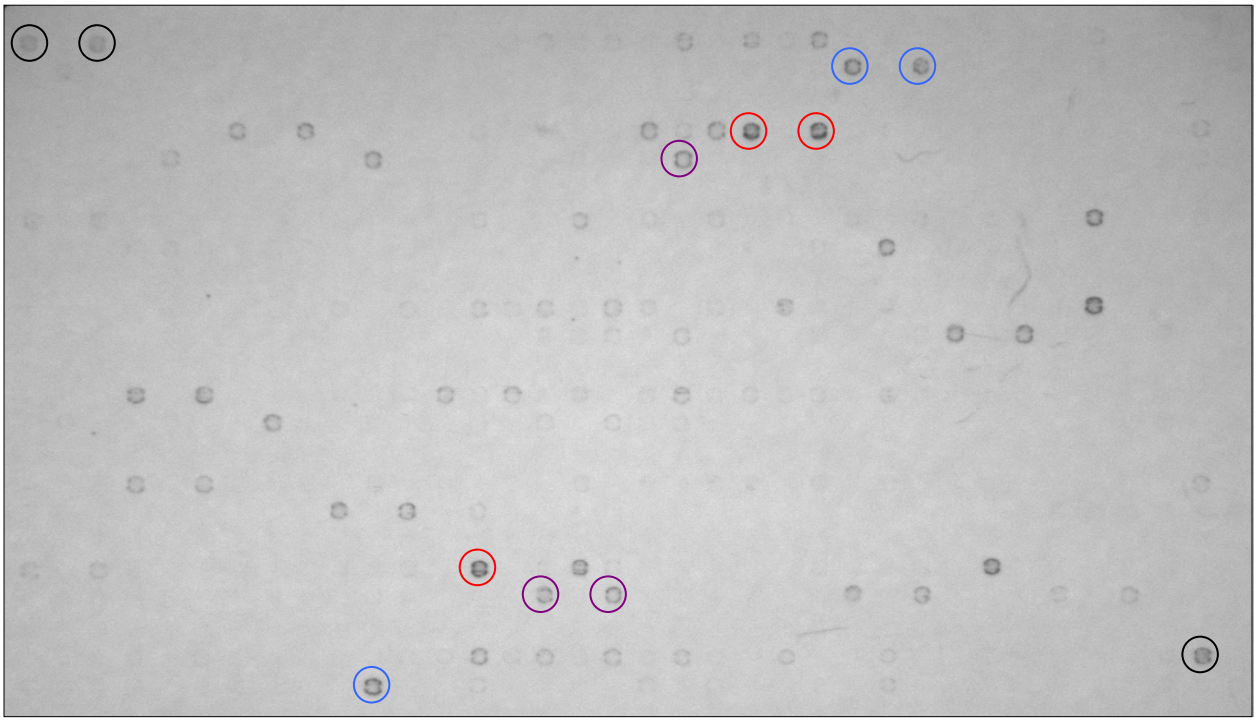


Figure 3. Fungal ITS array probed with environmental fungal community PCR product (primers ITS1 and ITS4) amplified from a Quebec orchard soil DNA sample. A DIG-labeled positive control and three prominent samples are noted by circles. Each array sample is replicated with three spots, one of which is in the mirror opposite position. ○ Positive internal DIG control; ○ *Leptodontium*; ○ Unknown cultured fungus; ○ *Geomyces*.

A DNA array approach utilizing fungal ITS PCR product as both the probe and target proved adequate for the identification of fungal genera present in orchard soil samples. Given the genus-level resolution this method is suitable to assess broad-scale patterns and to attain initial views into fungal community composition.

The general utility of the DNA array technique was demonstrated in studies which successfully described composition of the fungal community from a Quebec orchard soil that was geographically distant from that (CV orchard soil) which was utilized to generate the array targets. By cloning and sequencing the same amplicon community used to generate the probe for the Quebec orchard soil, we were able to confirm that a) probe constituents possessing sequences within 90% similarity to at least one array target were detected, and b) array targets exhibiting strong relative signal corresponded with an element of the probe population possessing sequence similarity at these same levels. As with all array-based approaches, the utility of a given array is limited to the degree to which the overall population is represented on the array. One fungus in

particular, *Leptosphaerulina*, was prominent in the Quebec orchard soil but it possessed an ITS sequence that was not within 90-95% similar to any array target. As a result, the fungus was detected in the cloning screen but not by array hybridization. This problem can be addressed in the construction of future arrays by improving the breadth of taxonomic sampling within genera such that all sequences known to exist, based on larger databases like Genbank, are 90% or more similar to at least one target spot. Despite the potential to overlook such samples, the ability to account for even 200 lineages should provide a considerable advantage when compared to culture-based analyses or molecular techniques that require continued screening, such as the cloning and sequencing of DNA.

Significance to industry:

Most of our understanding of root disease in orchard systems has been generated through culture-based studies. As is often seen when contrasting approaches are used, revisiting root disease with molecular-based approaches may potentially reveal other species not previously appreciated to be associated with root health. The approach we have developed offers that opportunity to obtain a unique view into these systems and can be utilized to revisit previously addressed questions that will further our understanding of fungal ecology in orchard soils and the rhizosphere of apple. The method has also revealed its capacity to identify prominent changes in fungal community composition in response to disease management strategies. For instance, the eradication of *Cylindrocarpon* spp. from *B. juncea* seed meal amended soil, which was predicted by DNA array analysis, was documented to be valied based upon the recovery of this root pathogen from trees grown in the treated soil. A previously unrecognized functional group of potential importance to disease management was also documented. *Arthrobotrys* was a significant component of the fungal community in *B. napus* as determined by DNA array analysis. Members of this fungal genus are known to be significant predators of plant parasitic nematodes and may contribute to the suppression of these parasites, including the lesion nematode. Finally, such a methodology would enhance the capability to conduct more powerful long-term research at sites such as the newly purchased WSU-Sunrise orchard at Quincy