APPLE HORTICULTURE AND POSTHARVEST COMMITTEE

30-Jan-14

Time	Page	PI		Yrs
			APPLE CROP PROTECTION COMMITTEE	
10:15		Hanrahan	Introduction & housekeeping	
10:20		McFerson	Research update	
			Final Reports	
10:30	1	Johnson	Fire blight management in conventional and organic apple	12-13
10:45	10	Knight	Integrating CM granulovirus into conventional orchards	12-13
11:00	17	Brunner	Identification of resistance to codling moth and leafroller in *Malus*	11-13
11:15	26	Jones	Enhancing BC in apples	11-13
Group			**Continuing Projects: 3:30-5:00**	
1	34	Beers	*Amblydromella caudiglans:* A new predatory mite for Washington	2
1	41	Landolt	Chemical mediation of aggregation by BMSB	3
1	45	Landolt	Codling moth attract-and-kill with kairomonal lures	3
1	53	Garczynski	Olfactory proteins as targets for enhanced codling moth control	3
1	59	Garczynski	Study of molecular mechanisms to preserve codling moth control	3
2	64	Jones	Models to assess pesticide impacts on CM, OLBR & C *nigricornis*	3
2	72	Norelli	Incorporating fire blight resistance into WA apple cultivars	3
2	80	Smith	Improving management of two critical pome fruit diseases	3

FINAL PROJECT REPORT **YEAR**: 2 of 2

Project Title: Fire blight management in organic and conventional apple

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Cooperators: Tim Smith, WSU Extension, Wenatchee; Rachel Elkins, UC Extension, Lakeport

Other funding sources

Agency Name: USDA NIFA OREI
Amt. awarded: $476K to Johnson, Elkins, and Smith 10/11 - 9/14
Notes: Objectives 1 and 2 of this proposal are matching objectives for the above NIFA OREI project

Total Project Funding: $44,660

Budget History:

Item	**2012**	**2013**	
Salaries Faculty Res. Assist.	11,000	11,330	
Benefits OPE 56%	6,160	6,345	
Wages undergrads	1,800	1,854	
Benefits OPE 8%	144	148	
Equipment			
Supplies	1,896	1,953	
Travel	500	515	
Miscellaneous			
Plot Fees	500	515	
Total	**$22,000**	**$22,660**	

Footnotes: Annually**,** FRA 3 mo plus fringe, 150 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES

1) Understand the relative toxicity of fruit crop load (bloom) thinning materials to the fire blight pathogen, and to bacterial and fungal biological control agents.

2) Achieve an improved understanding of floral colonization by the yeast biological control agent, *Aureobasidium pullulans.*

3) In the field, evaluate an inducer of systemic acquired resistance for protection of apple from fire blight and as an aid to cutting of blight in scions of young apple trees.

SIGNIFICANT FINDINGS

- Oversprays of the bloom thinning agent, lime sulfur, suppressed populations of the fire blight pathogen and of biological control agents after their establishment on apple flowers.
- Treatment with *A. pullulans* (Blossom Protect) after lime sulfur and fish oil reduced fire blight infections by 91% compared with water only; this level of control level was similar to streptomycin against a strep-sensitive pathogen strain.
- The yeast, *A. pullulans* (Blossom Protect), is an excellent colonist of both the floral stigma and floral cup, which differentiates it from bacterial biocontrol agents that colonize only the stigma.
- In parallel trials at Corvallis, OR, Wenatchee, WA, and Lakeport, CA, *A. pullulans* colonized nearly 100% of flowers on trees treated once with Blossom Protect at early to mid-bloom.
- Over three seasons, the addition the systemic acquired resistance material, acibenzolar-S-methyl (Actigard) to antibiotic treatments significantly enhanced fire blight control.
- Paints of Actigard onto EMLA 26 rootstocks reduced canker size and tree death after inoculation of the rootstocks with the fire blight pathogen.

RESULTS & DISCUSSION

Obj. 1a. Effect of fruit crop load thinners on microbial populations. Compared to the untreated control (UTC), lime sulfur oversprays onto pre-established epiphytic microbe populations on Golden Delicious flowers reduced significantly ($P < 0.05$) the population sizes of the fire blight pathogen (*Erwinia amylovora)* and the biocontrol agents, *Pantoea agglomerans* (Bloomtime Biological) and *A. pullulans* (Blossom Protect), regardless of sampling date or the rate of lime sulfur applied (Fig.1). Similarly, for the first sampling date from Rome Beauty apple, epiphytic populations of *E. amylovora* and *A. pullulans* were significantly reduced by all rates of lime sulfur, but the effects of lime sulfur on the population size of *P. agglomerans* were inconsistent (Fig. 1). On the second sampling date, only the *E. amylovora* populations on the Rome Beauty flowers were suppressed significantly by the lime sulfur treatments.

Discussion. We have shown that lime sulfur partially suppresses fire blight (see Fig. 2 below). The reason is likely twofold: 1) the treatment causes flower abscission, which reduces the number of flower clusters that become diseased, and 2) lime sulfur is directly toxic to epiphytic microbes on the flowers. Recent surveys that we made on the detectability of epiphytic *E. amylovora* in pear and apple flowers sampled from commercial orchards found that the likelihood of positive pathogen detection is relatively small (< 5%) during early to mid-bloom when thinning agents are typically applied, but increases five- to 20-fold by petal fall. Consequently, because of its

antibacterial properties, lime sulfur is likely sufficient in most orchards to delay/suppress the epiphytic increase of *E. amylovora* in early bloom, and that the biological materials specifically registered for fire blight control can be implemented after the bloom thinning protocol is completed. The deleterious effects of lime sulfur oversprays onto biological antagonists (*P. agglomerans* and *A. pullulans*) also indicates that antagonist treatments should be delayed until after the bloom thinning protocol is complete.

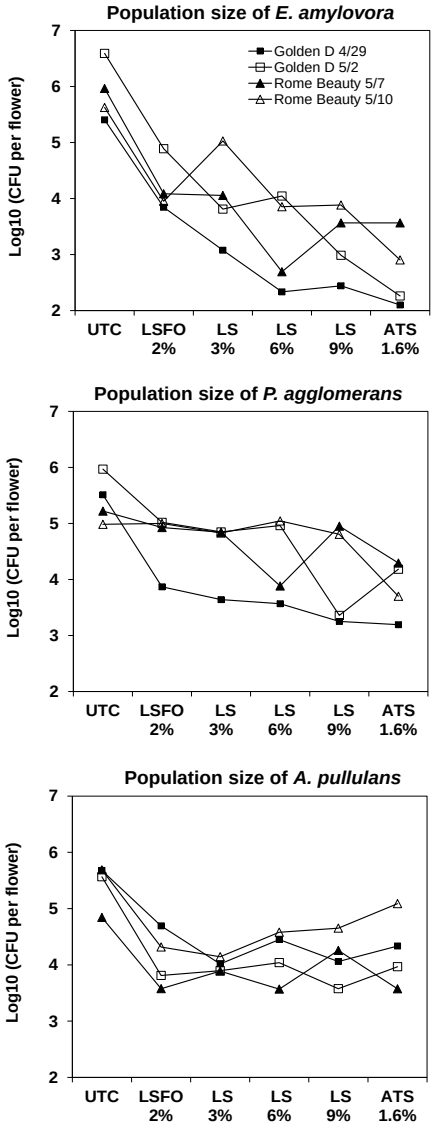

Fig. 1. Log_{10} (population size) of A) *Erwinia amylovora*, B) *Pantoea agglomerans* and C) *Aureobasidium pullulans* on apple flowers sprayed to runoff with the fire blight pathogen (1 x 10^6 CFU/ml) or with maximum labels rates Bloomtime Biological or Blossom Protect at 60 to 70% bloom (27 April and 2 May 2012 for cvs. 'Golden Delicious' and 'Rome Beauty', respectively) in experimental orchards located near Corvallis, OR. On the following day, inoculated trees were oversprayed to runoff with the fruit crop load thinning treatment lime sulfur (LS) (3, 6, or 9% v:v), with a mixture of lime sulfur and fish oil (LS+FO) (2%:2% v:v) or with ammonium thiosulfate (ATS) (1.6% v:v). Each point is the mean of three replications of five bulked flower clusters (~25 flowers per replicate) washed and dilution plated onto a semi-selective culture medium; standard errors for the points averaged 0.28 $\pm$ (s. d.) 0.21. Sample dates shown in the legend are 1 and 4 days after lime sulfur in Golden Delicious (squares) and 4 and 7 days after lime sulfur in Rome Beauty (triangles). UTC is the untreated control with respect to the fruit crop load thinning treatment.

Obj. 1b. Integrated fire blight control with lime sulfur thinning followed by Blossom Protect. We define 'integrated control' of fire blight as programs of different materials that when sprayed in sequence result in an improved (high) level of disease suppression. The improvement in control is achieved by suppression of the two distinct phases of the floral infection process: suppression the pathogen's prerequisite epiphytic phase on floral stigmas -- accomplished by a competing microorganism or by lime sulfur -- and suppression of infection by the pathogen via natural openings (nectarthodes) on the hypanthium -- accomplished with a chemical or by yeast colonization of this surface. For lime sulfur alone, treatment of apple trees at 30 and 70% bloom significantly ($P \leq 0.05$) reduced the incidence of blighted flower clusters (Fig. 2) compared to the water-treated control; the mean level of suppression achieved by LS+FO over all trials was 48 $\pm$ 10%. In the same orchard trials, an integrated program of two treatments of Blossom Protect (*A. pullulans*) following the bloom thinning protocol reduced the incidence of fire blight by an average of 91 $\pm$ 1% compared to trees treated with water only (Fig. 2). But within this four-spray program, the addition of Bloomtime Biological (*P. agglomerans*) to the full bloom Blossom Protect treatment did not improve control beyond that achieved by LS+FO followed by Blossom Protect alone (data not show). Overall, two applications of Blossom Protect after LS+FO provided a reduction of disease incidence that was

similar statistically to the level provided by the integrated program of *P. agglomerans* followed by oxytetracycline, and also to a single spray of streptomycin.

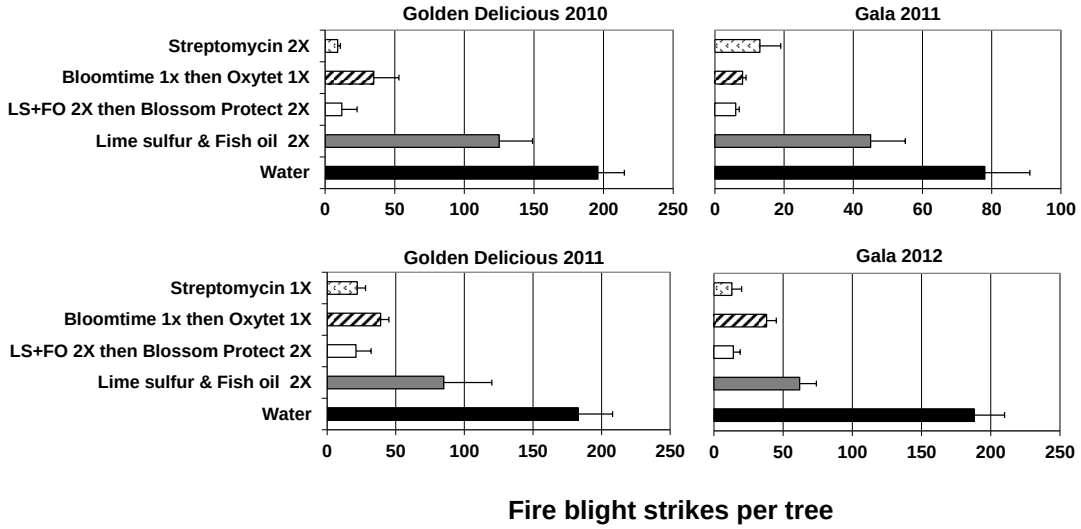

Fig. 2. Incidence of fire blight in Gala apple trees as affected by chemical and biological treatments sprayed onto the trees to suppress infection. The orchards were inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at full bloom. Lime sulfur and fish oil (2%:2% v:v) treatments were applied at 30 and 70% bloom. Bloomtime Biological and the first Blossom Protect treatment were applied at 80% bloom. Antibiotics and the second Blossom Protect treatment were applied 1 to 3 days after the pathogen inoculation. Error bars associated with each larger bar represent plus/minus one standard error of the mean.

Obj. 2a. Improved understanding of floral colonization by *Aureobasidium pullulans.*

Parts of the flower colonized by the yeast. In 2011 and 2012, overspray of a 80% bloom timing of Bloomtime Biological (*P. agglomerans*) with Blossom Protect (*A. pullulans*) at full bloom and petal fall revealed differences in the colonization of the flowers by these microbes. On stigmas, the incidence of both microbes was high (2011: 63 and 98% for *P. agglomerans* and *A. pullulans*, respectively; 2012: 71 and 94% for *P. agglomerans* and *A. pullulans*, respectively) (Fig. 3A, B). In contrast, for floral cups (hypanthial surface), the yeast again was recovered from 98% (2011) to 100% (2012) of sampled flowers but *P. agglomerans* was detectable on 9% and 18% of washed hypanthia in 2011 and 2012, respectively (Fig. 3C, D).

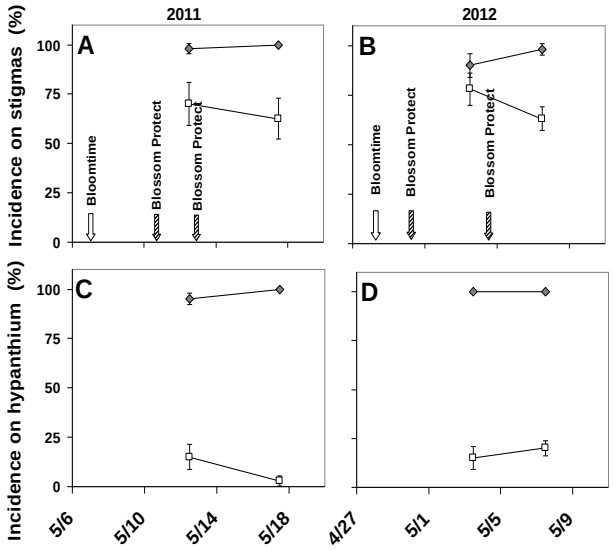

Fig. 3. Incidence of detection of *Pantoea agglomerans* (open squares) and of *Aureobasidium pullulans* (gray diamonds) on floral stigmas (A, B) and on hypanthia (C, D) by date of sampling from Gala apple trees treated with Bloomtime Biological at 80% bloom (open arrow) and with Blossom Protect at full bloom and prior to petal fall (hatched arrows) in an experimental orchard located near Corvallis, OR in 2011 and 2012. On each sampling date, incidence was determined by dilution plating dissected stigma and hypanthium subsamples from 10 flowers from each of four replicate trees. Error bars associated with each point represent plus/minus one standard error of the mean.

Environmental influences on yeast establishment in flowers. In 2012, at Corvallis and Wenatchee, treatment with Blossom Protect at 20, 80 or at both 20 and 80% bloom resulted in recovery of *A. pullulans* from nearly every flower sampled between full bloom and petal fall (Fig. 4A,C,D). Flowers sampled from the untreated control trees also had a measureable incidence of *A. pullulans* on flowers that ranged from 26 (apple Corvallis) to 80% (pear Corvallis). At Lakeport CA, pear trees treated with Blossom Protect in early bloom were sampled in mid-June when fruit were thumb-sized. Calyx ends of these fruit were washed and subjected to dilution plating. Nearly every calyx-end of sampled pear fruit had a recoverable population of *A. pullulans* (Fig. 4B). A repeat of the Lakeport experiment in 2013 had similar results (Fig. 5).

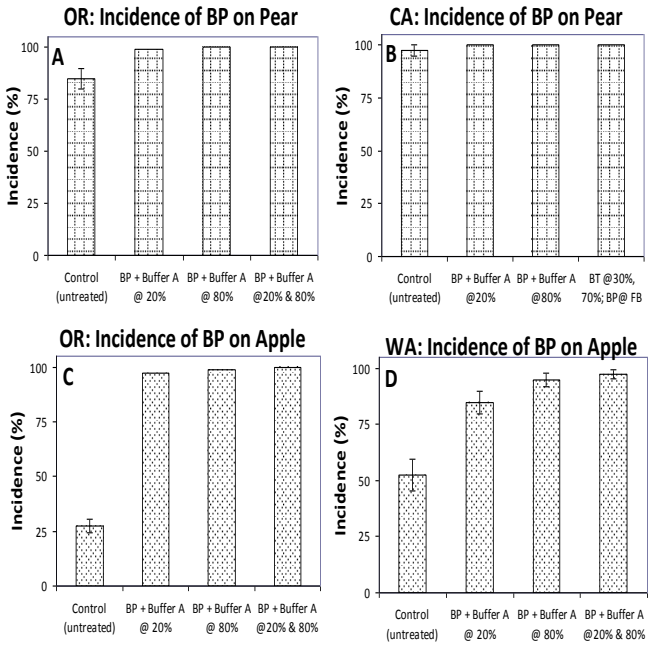

Fig. 4. 2012 experiments: Incidence of detection of *Aureobasidium pullulans* on pear (A, B) and apple (C, D) flowers after treatment with Blossom Protect at 20, 80 or both 20 and 80% bloom in orchards located near Corvallis, OR (A,C), Lakeport, CA (B), and Wenatchee, WA (D) in 2012. In Corvallis and Wenatchee, flowers were sampled twice between full bloom and petal fall. On each sampling date, incidence was determined by dilution plating 10 flowers from each of four replicate trees; the average incidence for the two sampling dates is presented in the figure. In Lakeport, samples were taken once when pear fruit were thumb-sized. The calyx ends of 10 pear fruit from each of four replicate trees fruit were washed and dilution plated. Error bars associated with each point represent plus/minus one standard error of the mean.

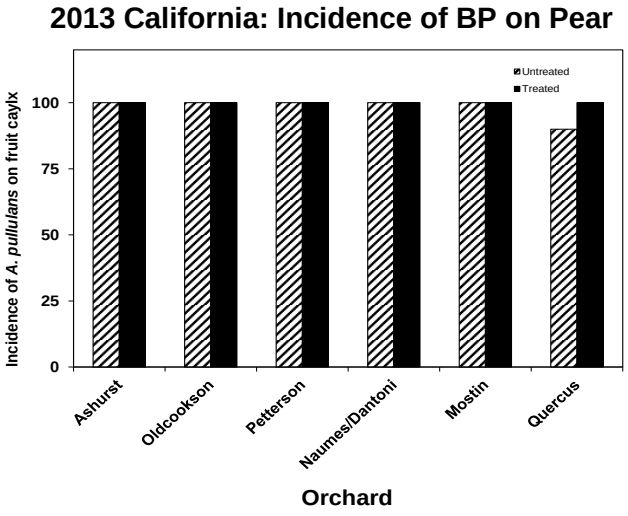

Fig. 5. 2013 experiment: Incidence of detection of *Aureobasidium pullulans* on Bartlett pear fruit sampled from six orchards in Lakeport, CA. Trees were treated with Blossom Protect once at 80-90% bloom. Samples were taken in mid-June when pear fruit were thumb-sized. The calyx ends of 10 pear fruit from each of four replicate trees per orchard were washed and dilution plated.

Discussion. The yeast, A. *pullulans*, is an excellent colonist of both the stigma and the hypanthium, whereas bacterial biocontrol agent, *P. agglomerans*, is only a good colonist of stigmas. The ability of *A. pullulans* to colonize the hypanthium may be a primary mechanism by which this

organism provides outstanding fire blight suppression (the hypanthial surface is the infection site for *E. amylovora*). Certainly, for yeasts used as biocontrol agents of postharvest fruit rots, the effectiveness of these antagonists is attributed to an ability to rapidly utilize nutrients available at the site of infection. As a biological product, Blossom Protect is produced to an excellent quality standard, which results in a high number of viable colony forming units (spores) in the spray tank. For three environments (Corvallis, Wenatchee and Lakeport), *A. pullulans* became established in nearly all pear and apple flowers to which Blossom Protect was applied. Moreover, this microorganism apparently spread flower-to-flower after initial establishment as evidenced by the high recovery of *A. pullulans* from flowers treated at 20% bloom, and from flowers and fruit sampled from the untreated control trees. After several years of orchard trials, non-antibiotic programs that utilize Blossom Protect continue to be the most effective and consistent for fire blight control.

Obj. 2b. Integrated fire blight control with Blossom Protect followed by Previsto copper. One potential drawback (expressed to us by growers) of a spray program where Blossom Protect follows lime sulfur is a *complete* reliance on a biological product (living microorganism) for fire blight control during the higher risk, late bloom period. A second potential drawback of Blossom Protect is a known ability of *A. pullulans* to russet fruit surfaces if extended wet periods occur during the period from late bloom to a few weeks after petal fall (Note: fruit russet caused by *A. pullulans* is generally unlikely on apples produced in semi-arid areas of central Washington). Consequently, we evaluated an alternative integrated program that began with one treatment of Blossom Protect (which could follow lime sulfur thinning in a commercial setting) followed by a treatment of Gowan's Previsto copper product, which is the (as yet unregistered) copper in ammonium/alginate complex that has shown less potential to russet fruit than other copper-based bactericides.

The integrated program of one treatment of Blossom Protect (*A. pullulans*) followed by Previsto copper reduced the incidence of fire blight by an average of 91 ± 2% compared to trees treated with water only (Fig. 6). Overall, this treatment program was similar to a single spray of streptomycin and superior statistically to the level of suppression provided by the integrated program of Bloomtime Biological (*P. agglomerans*) followed by oxytetracycline.

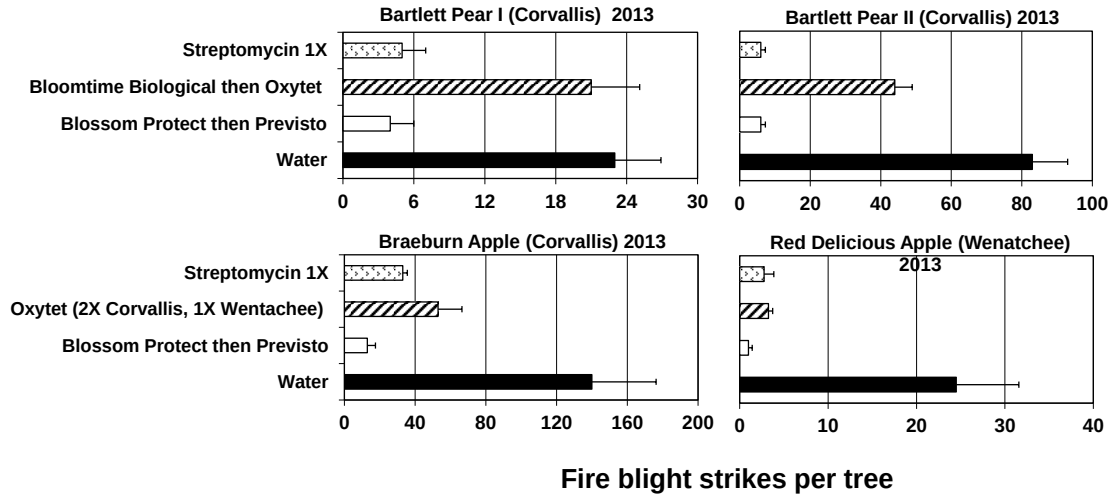

Fig. 6. Incidence of fire blight in experimental pear and apple orchards as affected by chemical and biological treatments sprayed onto the trees to suppress infection. The orchards were inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at full bloom. Biological treatments were applied at 70% bloom. Antibiotics and Previsto copper were applied 1 to 3 days after the pathogen inoculation. Error bars associated with each larger bar represent plus/minus one standard error of the mean.

An additional rationale to follow Blossom Protect with Previsto is that the copper product could suppress the yeast populations and potentially prevent yeast-induced fruit russet if excessively wet conditions were to occur in late bloom. In 2013, in a preliminary effort, we measured yeast populations on flowers oversprayed with Previsto. We observed a decline in the proportion of flowers with detectable yeast populations after treatment with Previsto copper (Fig. 7), which we have not observed when Blossom Protect was the last treatment sprayed onto the trees (see Figs. 4 and 5). Interestingly, however, we observed a larger decline in flowers with detectable yeast populations after treatment with Serenade Optimum. Serenade also has provided excellent fire blight control when sprayed after Blossom Protect (data not shown), is commonly used as a fungicide in other crops, and is considered fruit safe (D. Sugar, *unpublished data*). Further investigation of the response shown in Fig. 7 is an objective of our 2014-15 research proposal.

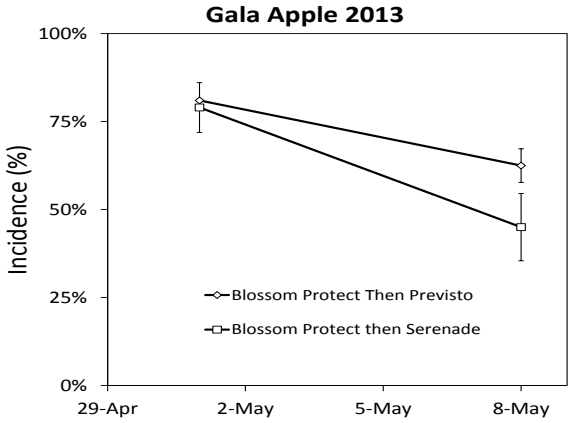

Fig. 7. Incidence of detection of *Aureobasidium pullulans* by date of sampling from Gala apple trees treated with Blossom Protect at 80% bloom and with Previsto or Serenade Optimum at full bloom in an experimental orchard located near Corvallis, OR in 2013. Flowers were sampled just prior to and six days after the Previsto or Serenade treatment.

Obj. 3. Systemic acquired resistance for protection of apple.

Actigard combined with antibiotics. From 2011 to 2013, Actigard (acetyl-S-methyl) was evaluated in combination with antibiotics for enhanced suppression of floral infection by the fire blight pathogen. Relative to the water-treated control, antibiotics alone significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree. In general, the addition of Actigard treatments in combination with streptomycin or oxytetracycline improved the control of fire blight compared to the antibiotics alone. (An additional 2013 pear trial (not shown) had results similar to Gala apple in 2011 and 2012.)

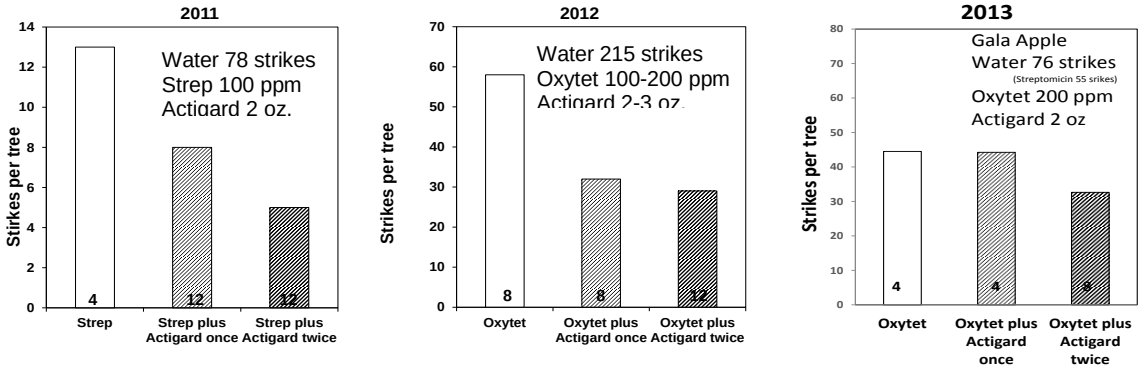

Fig. 7. Fire blight strikes per tree as affected by treatment with streptomycin (2011) or oxytetracycline (2012 and 2013) once or in a program with one or two additional Actigard treatments. Trials were conducted in a Gala apple orchard near Corvallis, OR. The antibiotic treatment was made at full bloom; timings of Actigard treatments varied from 30% bloom to petal fall. Numbers within bars are the number of replicate trees averaged for each mean.

Actigard as aid to cutting of blight in scions of young apple trees. Experiments were conducted in 2012 and 2013 to evaluate Actigard as an aid to cutting of blight in scions of young trees. In both years, the cutting experiment failed in apple because fire blight infections did not run in our young trees of cultivar 'Cameo' ('Red Delicious' parentage). We have removed the Cameo block and are re-planting with Gala and Pink Lady to continue this research. Several Actigard aid-to-blight-cutting experiments in pear have been successful (see pear report).

Actigard for protection of apple rootstocks from fire blight. We demonstrated previously that Actigard applied to potted trees of cv. 'Gala' on EMLA 26 provided a high level of protection from rootstock blight after the pathogen was inoculated directly below the graft union. In 2012 and 2013, we ran similar experiments in the field on 3- to 4-year-old 'Gala' on ELMA 26. In both seasons, the rootstocks were treated twice with an Actigard paint (30-45 g/L plus 1-2% Pentrabark, early and late June) prior to an early July inoculation of a high pathogen dose directly into the rootstock tissue. In these trials, cankers have formed on both the Actigard and untreated rootstocks, with a slight reduction in canker size on trees treated with Actigard. A larger difference among the treatments, however, has been observed in the appearance and health of the scion. For the 2012 experiment, 70% the untreated trees died in spring 2013 compared to 36% of the trees treated with Actigard; rootstock cankers in the Actigard trees had stopped expanding and had begun to heal. Similarly, for the trees inoculated in 2013, 45% of untreated trees showed decline of the scion in September (yellow foliage, early fall senescence and defoliation) compared to 10% of scions on Actigard treated trees. These trees will be evaluated again in the spring of 2014.

Discussion. We have made significant progress in understanding effective rates of Actigard for the various methods of application. Induction of systemic acquired resistance appears to have its greatest protective effect when blight symptoms are minimal (prior to or near time of infection, or after cutting). Actigard shows value as program partner with antibiotics during bloom, and perhaps more significant, it may be effective as long residual protectant for rattail and shoot infection phases of fire blight (e.g., in long blooming cultivars like 'Pink Lady'). Use of Actigard paints continues to show promise, although we are not convinced that protection of apple rootstocks with Actigard paints will be practical in commercial orchards given that the rates of Actigard required to obtain a response are high in relation to the probability of a tree developing a rootstock infection. Nonetheless, in pear, we continue to achieve promising results with Actigard as an aid to cutting of blight in scions of young trees (see pear report). It is likely that apple scions with running fire blight cankers would benefit from similar Actigard paint treatments to healthy tissue below the cut.

EXECUTIVE SUMMARY

Project Title: **Fire blight management in organic and conventional apple**
Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:

Integration of fruit crop load thinning with fire blight control:

- Oversprays of the bloom thinning agent, lime sulfur, suppressed populations of the fire blight pathogen and of biological control agents after their establishment on apple flowers.
- Treatment with *A. pullulans* (Blossom Protect) after lime sulfur and fish oil reduced fire blight infections by 91% compared with water only; this level of control level was similar to streptomycin against a strep-sensitive pathogen strain.

Industry implications: Chemical fruit crop load thinning with lime sulfur has become common practice in both organic and conventional apple production. Timing of these thinning treatments can coincide with treatments to prevent fire blight. Because of its antibacterial properties, lime sulfur is likely sufficient in most orchards to delay/suppress the epiphytic increase of *E. amylovora* in early bloom, and that the biological and chemical materials specifically registered for fire blight control can be implemented immediately after the second thinning treatment at 70% bloom.

Understanding floral colonization by the yeast biocontrol agent, *Aureobasidium pullulans*:

- *A. pullulans* (Blossom Protect) is an excellent colonist of both the stigma and floral cup, which differentiates it from other biocontrol agents that colonize only the floral stigma.
- In parallel trials at Corvallis, OR, Wenatchee, WA, and Lakeport, CA, *A. pullulans* colonized nearly 100% of flowers on trees treated once with Blossom Protect at early to mid-bloom.

Industry implications: *A. pullulans* ability to colonize the floral cup (hypanthium) may be a primary mechanism by which this yeast provides outstanding fire blight suppression. As a product, Blossom Protect is produced to an excellent quality standard, which results in a high number of viable yeast spores in the spray tank. This yeast became established and spread to nearly all apple and pear flowers on trees treated with Blossom Protect regardless of the trial environment or the timing of the treatment. Spray programs that utilize Blossom Protect continue to be among the most effective for fire blight control.

Systemic acquired resistance:

- Over three seasons, the addition the systemic acquired resistance material, acibenzolar-S-methyl (Actigard), to antibiotic treatments significantly enhanced fire blight control.
- Paints of Actigard onto EMLA 26 rootstocks reduced canker size and tree death after inoculation of the rootstocks with the fire blight pathogen.

Industry implications: Actigard, with its unique (host defense gene-inducing) mode-of-action, shows value as program partner with antibiotics for fire blight prevention during bloom. With regard to Actigard paints, even with very good products for fire blight prevention, the disease still occurs and its clean-up can be difficult, especially in young orchards. We continue to achieve promising results with Actigard as an aid to clean-up of blight in scions and rootstocks of young trees.

FINAL PROJECT REPORT

Project Title: Integrating codling moth granulovirus into conventional orchards

PI: Alan Knight
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Address: 5230 Konnowac Pass Rd
City/State/Zip: Wapato, WA 98951

Cooperator: Dr. Mike Dimmock, Certis USA

Other funding sources
Agency Name: California Pear Advisory Board
Amt. funded: $10,500

Total Project Funding: $42,000

Budget History:

Item	Year 1: 2013	Year 2: 2013
Salaries		
Benefits		
Wages	17,454	17,454
Benefits	1,546	1,546
Equipment		
Supplies		
Travel	2,000	2,000
Plot Fees		
Miscellaneous		
Total	21,000	21,000

OBJECTIVES

The overall objective of this project was to develop effective feeding stimulants for codling moth larvae that can significantly improve the performance of selective insecticides. Selective insecticides are materials that are toxic for codling moth and whose use will not disrupt the biological control of mites, aphids, mealybugs, or other secondary pests. These include the microbial insecticides, CpGV and Bt's, and synthetic compounds such as Intrepid and Altacor. Specific objectives of the project included conducting laboratory bioassays with possible feeding stimulants such as, naturally occurring yeasts isolated from codling moth larvae, commercial bread yeast, monosodium glutamate, L-aspartate, and Monterey Insect Bait. The second specific objective of this project was to evaluate a promising subset of the most effective feeding stimulants characterized in the laboratory bioassays with CpGV in field trials at the USDA Research Farm.

SIGNIFICANT FINDNGS

2012

- The addition of a*Metschnikowia sp.* Yeas, collected from codling moth larvae, with cane sugar significantly improved the efficacy of a CpGV insecticide (Cyd-X) in laboratory bioassays.
- Three additional species of yeast were isolated from codling moth field-collected larvae and were found to also improve the performance of CpGV in laboratory bioassays..
- Other materials, such as active bread yeast with sugar, the amino acid, L-aspartate with sugar, and the Monterey Insect Bait were found to be very effective in improving the activity of the CpGV in laboratory bioassays. MSG with or without sugar was not very effective in similar bioassays.
- Yeast adjuvants are not compatible with Bt insecticides.
- Field trials with *Metschnikowia* sp. yeast and bread yeast both combined with sugar and added with a low rate of Cyd-X both significantly increased larval mortality and reduced fruit injury in a season-long virus spray program. The addition of MSG or L-aspartate without sugar enhanced the kill of larvae, but did not add any protection of the fruit from codling moth injury.
- The addition of the microencapsulated pear ester formulation did not improve the efficacy of CpGV with or without bread yeast and sugar added.

2013

- Laboratory assays found that the addition of bread yeast and sugar improved the effectiveness of Altacor but not Entrust, Delegate, or Intrepid.
- The use of Cyd-X HP at a low rate alone with the addition of several adjuvants did not prevent codling moth injury on apples despite the use of 11 sprays timed every 5 - 12 d during the season.
- The addition of bread yeast plus sugar or Monterey Insect Bait significantly increased the proportion of dead larvae compared with the virus alone.
- The addition of the yeast *Cryptococcus tephrensis* (isolated from codling moth larvae) with sugar to the virus reduced the numbers of codling moth larvae overwintering in bands on tree trunks by 80% compared with the virus alone.

RESULTS & DISCUSSION

2012: Yeasts isolated from field-collected codling moth larvae were found to significantly increase the effectiveness of CpGV (Table 1). The addition of sugar alone had minimal effect but adding sugar to the yeasts significantly increased the toxicity of CpGV. Larval mortality was significantly increased with the addition of bread yeast with or without sugar to CpGV, but levels of mortality were not quite as high as with the field-collected yeasts. Rates of yeast higher than 3 lbs per 100 gallons have not been tested due to consideration of the economics of adding an adjuvant. The optimal level of brown cane sugar is also unknown but the effect of adding 1 or 3 lb seems to vary among tests with different materials. The higher rate was selected for field trials due to the possible effect of weathering. MSG was found not to be an effective additive. The amino acid, L-aspartate with sugar was effective. The Monterey Insect Bait provided the highest level of larval mortality with CpGV in 2012 bioassays.

The addition of the yeasts with sugar significantly reduced codling moth fruit injury compared with the virus alone. The virus applied at the low rate of 1 oz per 100 gal was not very effective in preventing fruit injury under these high pressure conditions. The use of PE MEC with CpGV provided no additional control. Both MSG and L-aspartate significantly increased larval morality but levels of fruit injury were not reduced compare with the virus alone. The addition of all adjuvants except PE MEC significantly increased the proportion of dead larvae and decreased the proportion of live larvae in or exiting the fruits in fruits (Table 2).

2013: Results clearly showed that the use of Cyd-X HP at such a low rate does not prevent codling moth injury on apples despite the use of 11 sprays timed every 5 -12 d during the season (Table 2). However, the use of the virus did significantly increase the proportion of dead larvae. The majority of dead larvae caused small 'stings' on the fruits but other larvae were found dead deep inside the fruit. Both the addition of bread yeast and sugar or MIB significantly increased the proportion of dead larvae compared with the water control or with the unsprayed trees. The proportion of dead larvae with both the bread yeast and sugar and MIB was also significantly higher than with the virus alone. The density of larvae found in bands placed on the trunk of trees varied among treatments (Table 2). The addition of the either of the two yeasts plus sugar or MIB resulted in a significant decrease in the number of larvae found in bands compared with the untreated trees. However, only the use of *C. tephrensis* and sugar significantly reduced the number of larvae in bands compared with the water control. No difference was found in the number of larvae in bands among all of the treatments that included the virus though the mean densities varied by 4-fold.

The addition of bread yeast and cane sugar only significantly improved the performance of Altacor at a 5% of the field rate (Table 3). These bioassays are continuing in order to get more replicates and studies with Assail will be completed. Also, we will run similar assays with 0.05% Delegate. Following the completion of the bread yeast and sugar bioassays these tests will be repeated with the addition of Monterey Insect Bait at 2.0 qts per 100 gallons.

Table 1. Summary of laboratory bioassays with various materials added to CpGV (Cyd-X).

Active material / rate per 100 gallons					Brown cane sugar (lb)	Mean proportion dead larvae
Untreated					0	0.08
CpGV / 1 oz					0	0.30
CpGV / 1 oz				+	3	0.34
CpGV / 1 oz	+	Inactive torula yeast / 3lb			0	0.38
CpGV / 1 oz	+	Active bread yeast / 3 lb			0	0.49
CpGV / 1 oz	+				1	0.50
CpGV / 1 oz	+	MSG / 1lb		+	3	0.51
CpGV / 1 oz	+	MSG / 1lb			0	0.53
CpGV / 1 oz	+	Active bread yeast / 1lb			0	0.57
CpGV / 1 oz	+	Inactive torula yeast/ 3lb		+	3	0.65
CpGV / 1 oz	+	L-aspartate / 1lb			0	0.65
CpGV / 1 oz	+	*Metschnikowia* spp. / 1 lb			0	0.66
CpGV / 1 oz	+	Active bread yeast / 3lb			1	0.68
CpGV / 1 oz	+	Active bread yeast / 3 lb		+	3	0.73
CpGV / 1 oz	+	Blossom Protect / 1.25 lb			0	0.73
CpGV / 1 oz	+	Active bread yeast / 1lb		+	1	0.74
CpGV / 1 oz	+	*Cryptococcus tephrensis* / 3lb			0	0.74
CpGV / 1 oz	+	*Metschnikowia* spp. / 3 lb			0	0.75
CpGV / 1 oz	+	*Metschnikowia* spp. / 3 lb		+	1	0.75
CpGV / 1 oz	+	Blossom Protect / 1.25 lb		+	3	0.78
CpGV / 1 oz	+	L-aspartate / 1 lb		+	3	0.80
CpGV / 1 oz	+	*Aureobasidium pullulans* 3 lb			0	0.81
CpGV / 1 oz	+	*Metschnikowia* spp. / 1 lb		+	1	0.84
CpGV / 1 oz	+	*Cryptococcus* sp. n / 3lb			0	0.86
CpGV / 1 oz	+	*Aureobasidium pullulans* 3 lb		+	1	0.89
CpGV / 1 oz	+	*Metschnikowia* spp. / 3 lb		+	3	0.90
CpGV / 1 oz	+	Monterey Insect Bait / 2 qts			0	0.92
CpGV / 1 oz	+	*Cryptococcus tephrensis* / 3lb		+	1	1.00

Materials in shaded rows were tested in field trials.

Table 2. Field evaluations with the granulosis codling moth virus (CpGV) during 2012-13 adding wild yeasts (WY) isolated from codling moth larvae, bread yeast (BY), monosodium glutamate (MSG), L-aspartate (L-Asp), and Monterey Insect Bait (MIB) alone or plus sugar (S) or pear ester (PE), N = 10.

Year / #	Treatment [a]	Mean (SE) %		
		% larval mortality	% uninjured fruit	No. larvae per band
2012 / 1	UTC	13.8 (3.2)b	60.3 (7.1)c	-
	CpGV	43.0 (4.1)b	66.7 (3.4)c	-
	CpGV + WY1 + S	80.8 (2.4)a	78.9 (2.7)b	-
	Insecticides [b]	48.0 (17.6)ab	98.5 (0.4)a	-
	ANOVA	$F_{3, 26} = 10.98$ $P < 0.001$	$F_{3, 26} = 90.39$ $P < 0.0001$	-
2012 / 2	UTC	17.8 (3.1)b	51.1 (5.1)c	32.8 (4.0)a
	CpGV	41.2 (3.9)b	61.7 (3.1)bc	17.6 (5.4)ab
	CpGV + PE	42.3 (2.1)b	66.9 (3.8)ab	14.1 (5.1)b
	CpGV + BY + S	81.4 (2.4)a	78.3 (2.4)a	9.5 (2.6)b
	CpGV + PE + BY + S	73.8 (2.2)a	74.3 (2.4)ab	13.6 (5.1)b
	ANOVA	$F_{4, 45} = 18.83$ $P < 0.0001$	$F_{4, 45} = 38.54$ $P < 0.0001$	$F_{4, 45} = 4.21$ $P < 0.01$
2012 / 3	UTC	9.8 (2.4)c	71.9 (2.0)b	-
	CpGV	47.0 (3.6)b	80.8 (2.7)a	-
	CpGV + MSG	81.8 (2.0)a	77.0 (2.8)ab	-
	CpGV + L-Asp	80.6 (1.8)a	83.4 (1.3)a	-
	ANOVA	$F_{3, 36} = 176.95$ $P < 0.0001$	$F_{3, 36} = 45.24$ $P < 0.0001$	-
2013 / 4	UTC	21.2 (2.1)c	54.3 (2.5)	10.6 (1.7)a
	Water only	16.0 (3.0)c	57.5 (2.6)	9.1 (2.3)ab
	CpGV	68.0 (5.0)b	60.9 (2.7)	6.8 (3.2)ab
	CpGV + BY + S	86.1 (2.4)a	58.4 (3.5)	2.9 (0.9)abc
	CpGV + WY2 + S	76.0 (5.9)ab	66.0 (5.2)	1.4 (0.5)c
	CpGV + L-Asp + S	81.7 (2.7)ab	61.2 (3.4)	5.1 (1.7)abc
	CpGV + MIB	89.1 (1.5)a	64.9 (2.1)	2.1 (0.7)bc
	ANOVA	$F_{6, 58} = 68.09$ $P < 0.0001$	$F_{6, 58} = 1.61$ $P = 0.16$	$F_{6, 58} = 5.77$ $P < 0.0001$

Column means for each orchard followed by a different letter were significantly different, $P < 0.05$.

[a] Wild yeasts include *Metschnikowia* sp. (WY1) and *Cryptococcus tephrensis* (WY2) .

Table 3. Summary of laboratory fruit assays comparing the effectiveness of several standard insecticides at 1 and 5% of the labeled field rate applied alone versus with the addition of bread yeast and sugar.

Insecticide	Rate	Mean (SE) proportion of injured fruits		Fisher's Exact Test
		Alone	W' Bread yeast and sugar	
Water	-	1.00	1.00	*P* = 1.00
Intrepid	1%	0.63	0.57	*P* = 0.79
	5%	0.40	0.50	*P* = 0.60
Entrust	1%	0.60	0.60	*P* = 1.00
	5%	0.33	0.27	*P* = 0.78
Altacor	1%	0.40	0.27	*P* = 0.41
	5%	**0.33**	**0.07**	***P* = 0.02***
Delegate	1%	0.19	0.09	*P* = 0.66
	5%	0.00	0.00	*P* = 1.00

N = 30, five neonate larvae were placed on the upper portion of each fruit. Fruit injury was scored after 14 days.

EXECUTIVE SUMMARY

Tremendous progress was made in the two years of this project in developing feeding adjuvants to enhance the toxicity of insecticides for codling moth. This is the first study to use live yeasts to enhance insecticides. The activity of several wild yeasts isolated from codling moth was characterized as high in combination with CpGV. However, an inexpensive and readily available yeast, *Saccharomyces cerevisiae* (bread yeast) was also found to exhibit significant activity. Previous studies reporting minimal activity from adding sugar alone to CpGV were confirmed. The addition of PE MEC with the virus provided no additional activity. The level of activity from adding MSG was shown to be low, but the amino acid L-aspartate looked promising when used with sugar. Trials also suggest that Monterey Insect Bait is a promising adjuvant for codling moth and should be tested with Bt insecticides.

Studies investigating the use of adjuvants with conventional insecticides have started and to date the use of bread yeast and sugar improves the activity of Altacor but not the spinosyns or the IGR, Intrepid. Studies will be conducted with Assail and each insecticide with Monterey Insect Bait during January. Differences in the chemical properties of insecticide formulations and their relative systemic penetration of plant surfaces are likely key factors influencing the benefit of adding a feeding stimulant for enhanced toxicity to codling moth. Altacor is known to be long-lived and has excellent systemic absorption into plant parts. Dupont personnel have shared with us that they believe that the diamide insecticides have an important oral route of entry for fruit feeding insects. They also expressed that the spinosyns are more active through dermal penetration than diamides. Our bioassays would support these general comments concerning the relative importance of different routes of entry into the target pest. Further laboratory bioassays with bread yeast and sugar as well as Monterey Insect Bait with the diamide insecticides are continuing.

A recently-registered microencapsulated formulation of pear ester has been effective in improving conventional insecticides used in apple, including organophosphates, neonicotinoids, diacylhydrazines, spinosyns, and diamides. Pear ester is not a feeding stimulant but increases larval wandering (host searching behavior). The enhanced wandering stimulated by pear ester increases larval exposure to insecticides. Thus, a cocktail made with a feeding stimulant and a wandering stimulant could provide significantly improved levels of control of codling moth with a range of insecticide classes. No previous studies of codling moth have combined the use of additives that can stimulate both host searching and larval feeding to improve conventional insecticide-based control.

The goal and potential benefit of this research to apple growers is the development and validation of pest management programs that avoid disruption of biological control and prevent problems with some secondary pests. Minimizing this impact through substitution of less disruptive materials, the use of fewer sprays, or the application of reduced rates are options which would also help to reduce the typically higher costs of the new chemistries. The use of behaviorally-active, inexpensive additives is a rational approach which may allow this integrated program to be implemented.

FINAL PROJECT REPORT

Project Title: Identification of resistance to codling moth and leafroller in *Malus*

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Other funding sources: NONE

Total Project Funding: **Year 1:** $37,904 **Year 2:** 53,399 **Year 3:** $53,348

Budget History

Item	2011	2012	2013
Salaries (grad student)	22,901	23,817	26,055
Benefits	1,895	1,970	2,207
Wages	10,094	15,840	15,120
Benefits	514	2,772	1,466
Equipment	0	0	0
Supplies	1,500	6,500	6,000
Plot fees	0	2,000	2,000
Travel	1,000	500	500
Miscellaneous	0	0	0
Total	37,904	53,399	53,348

Objectives:

1. Identify and characterize resistance in *Malus* accessions growing at the Sunrise Research Orchard to codling moth and leafroller.
2. Localize the genes that confer resistance to codling moth (CM) and leafrollers (OBLR).
3. Develop predictive genetic markers to identify codling moth and leafroller resistance in potential parents and seeding populations of the breeding program.

Significant Findings:

1. Leaf bioassays for OBLR were developed that revealed differences in larval survivorship, development time, and pupal and adult weight. A 21-day leaf bioassay will provide sufficient data on larval survivorship and development rate allowing a screen of more varieties.
2. Bioassays for CM using whole fruit appear to provide good data on larval survival and development time.
3. Twenty percent, 5 of 20 apple varieties evaluated, showed some degree of negative impact on OBLR suggesting resistance is present to some degree.
4. Some apple varieties showed signs of abnormal OBLR larval development expected from exposure to juvenile hormones.
5. A whole-leaf bioassay for OBLR, developed in year one (2011) was utilized to assess differences in larval survivorship, development time, pupal weight, and adult fecundity for different genotypes of *Malus*.
6. For some genotypes (e.g., Cox's Orange Pippen), resistance was expressed as a function of the plants' phenology (spring, summer, fall).
7. Some apple genotypes (e.g., Antonovka 1.5) appeared to disrupt normal hormone development in OBLR larvae, which inhibited the completion of pupal development, suggesting plant-produced juvenile hormone analogs might be involved.
8. Other mid-eastern genotypes (i.e., KAZ 96-07-06) expressed high mortality to OBLR larvae. No larvae survived to pupate. More importantly, prior to death, most larvae showed signs of hemorrhaging, suggesting the action of plant proteases on the digestive system of OBLR.
9. Oil as a residue on foliage was shown to be highly toxic to young OBLR larvae while the codling moth virus had no effects.
10. One genotypes, KAZ-181, showed almost no impact on OBLR in all bioassays. This genotype could therefore function as a susceptible model for comparison with other genotypes instead of using artificial diet.
11. We were not able to replicate the production of larval-pupal intermediates when only later instar larvae (5^{th} and 6^{th}) were exposed to leaves of genotypes that had produced larval-pupal intermediates in previous bioassays.
12. The use of a matrix population model provided a good method of synthesizing all parameters derived from the leaf bioassay for OBLR. This model provided an estimate of the intrinsic rate of increase and the net reproductive rate and replaces the index method used previously.
13. Using new versus old leaves in OBLR bioassays was demonstrated as critical to avoid overestimating resistance.
14. The variation of resistance of *Malus* genotypes to OBLR across different periods of the growing season has made it difficult to interpret results.

Results and Discussion:

Characterization of Malus resistance to OBLR.

Data on the development time and mortality of OBLR larvae reared on an artificial diet was collected as an independent internal standard to affirm the viability of OBLR in the colony and to compare with OBLR larvae reared on leaves of various *Malus* genotypes. (Fig. 1) Previously reported work showed that under controlled temperature conditions (22% RF; 23°C; 16:8 LD), OBLR larvae were primarily in the second instar after seven days, in the fourth instar after 14 days, in the fifth and sixth instars after 21 days and in the pupal stage after 28 days. These development data provided a timeline on which to evaluate OBLR development on leaves, and when to change leaves with minimal disturbance to larvae. Since transferring an insect larva during the sensitive molt period can increase mortality, checking leaves every seven days when most larvae were not in the process of molting was an attempt to introduce artificial mortality into the bioassay.

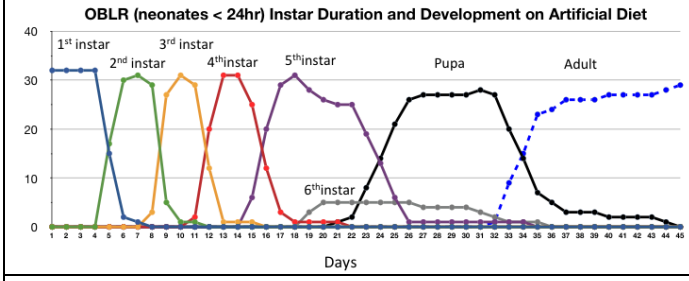

Figure 1. Number of OBLR larvae in a life stage when reared on artificial diet.

The bioassay method developed in 2011 provided leaf quality over time to measure key developmental parameters in 2012. The bioassay involved using a whole leaf placed in a large Petri dish (94 mm X 16 mm) with the leaf petiole placed inside an Eppendorf vial (2.0 ml) that contained water. The Eppendorf vial was inserted through a hole in the side of the plastic Petri dish and sealed with Teflon tape to prevent larval escapes (Fig. 2).

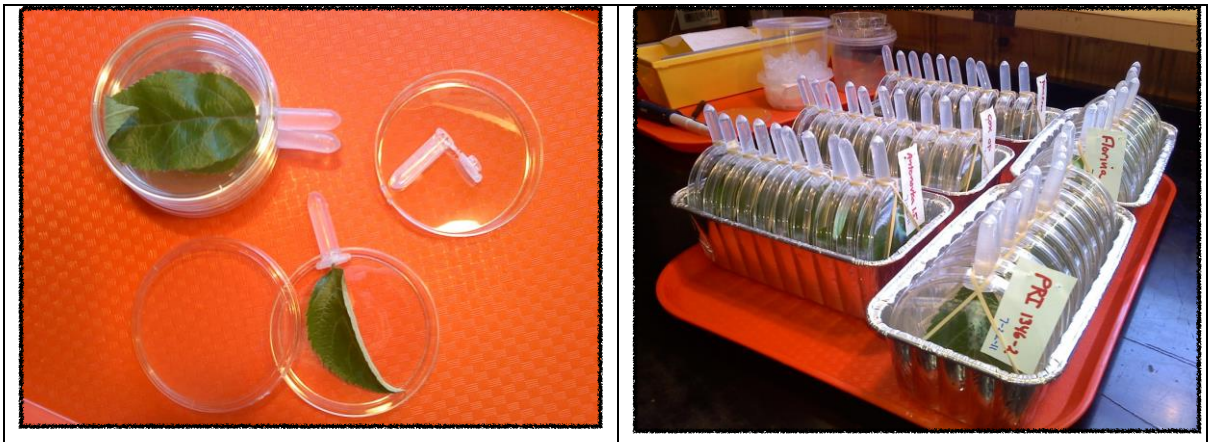

Figure 2. Whole leaf bioassay method developed for OBLR.

In 2011 twenty apple genotypes were evaluated for possible OBLR resistance. The genotypes were: Antonovka 1.5, PRI 1346-2, Redfree, Florina, Cox's Orange Pippin, Northern Spy, Liberty, Russian Seedling, Jonafree, Cortland, Yellow Transparent, Viking, Lady, Jonathan, Virginiagold, Trent, Delicious, Poeltsamaa Winter Apple, Haralson and Granny Smith. These varieties were only evaluated during the summer of 2011, thus did not represent possible resistance traits that could be expressed at other times of the growing season.

Figure 3 shows three types of survivorship curves for a select group of apple varieties, which represented patterns of survivorship curves for other apple varieties evaluated in 2011. There was high survival over 63 days for larvae reared on pinto bean diet (Type I). Florina (Type I) represents a variety where OBLR survival was high. Granny Smith (Type II) represents a group of other varieties, Group A (see Fig. 3). Lady and Trent (Type III) are varieties where there was high larval mortality in young instars. Northern Spy (Type II) also resulted in high larval mortality but most of the mortality occurred on later larval instars. Antonovka (Type II) is a variety in which mortality was expressed mostly in later instars. Larvae that fed on Antonovka showed developmental abnormalities that were similar to larvae that have been exposed to juvenile hormone at the wrong time in the life cycles.

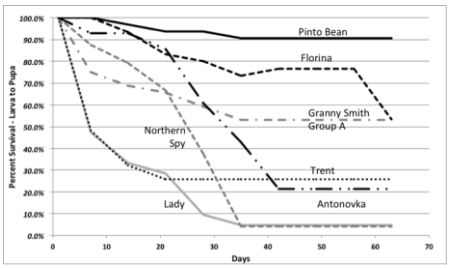

Figure 3. Average percent survival of OBLR larvae reared on different apple varieties. Type I – Florina, Delicious, Haralson, Poeltsamaa, PRI 1346-2, Russian Seedling, and Jonathan; Type II – Granny Smith, Northern Spy, Antonovka 1.5; Type III – Lady and Trent.

When mortality was examined for just the early instars (1-4 → Day 21), three genotypes had highest mortality, Viking (52%), Yellow Transparent (73%) and Lady (71%) (Fig 4). If induction of resistance is stimulated by feeding of OBLR larvae this early mortality might signal a particular mechanism of resistance that might be more important than others.

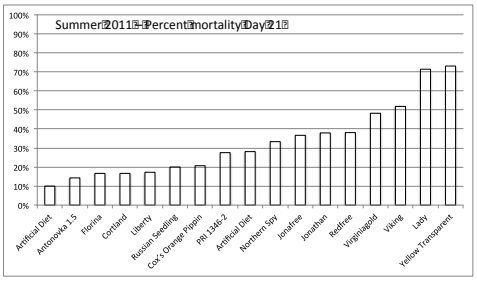
Fig. 4. Percent OBLR larval mortality after 21 days, 2011.

Other life history parameters that were measured in 2011 were development time (days to pupation) and pupal weight. A slower development time can be an indicator or plant resistance as can the weight, which is directly correlated with size, of pupae. The development time for OBLR reared on artificial diet was 25-26 days for males and 29-30 days for females. Development time on apple leaves was generally longer than on diet, but some varieties like Yellow Transparent, Viking and Liberty had exceptionally long development times of 40+ days. Pupal weight of OBLR reared on diet was 83-102 grams for males and 138-178 grams for females. Pupal weight for OBLR fed on leaves was almost always lower than when fed on diet, ranging from 80 to as low as 47 grams for males and 133 to as low as 58 grams for females. Liberty and Viking had very low pupal weights to go along with their slow development times.

In 2012, 25 *Malus* genotypes were evaluated for possible OBLR resistance, eleven from the "diversity map set"; Antonovka 1.5, Yellow Transparent, Northern Spy, Viking, Keepsake, Cox's Orange Pippin, Jonafree, Trent, Lady,

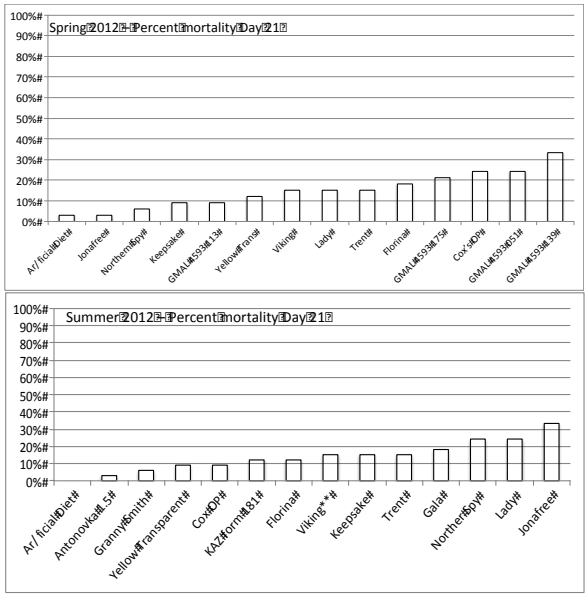
Fig. 5. Percent OBLR larval mortality after 21 days, 2012.

and Florina, and Granny Smith and fourteen from the "core diversity set" which originated from the middle east; KAZ form 181, Sieversii UZB GMAL 3265, KAZ 95-05-01P-22, KAZ 96-09-05, KAZ 96-07-06, KAZ 95-08-06, Sieversii TAJ GMAL 3244, Sieversii TUR GMAL 2251, Sieversii KYR GMAL 3158, KAZ 96-05-05, Sieversii KAZ GMAL 3310, Sieversii KYR GMAL 1750, KAZ 96-09-02, and KAZ 96-07-03. To determine if resistance was expressed differentially over the season for a specific *Malus* genotype, OBLR development was observed in three distinct periods (spring, summer and fall). Here we report on mortality of OBLR at Day 21 for thirteen *Malus* genotypes for the spring and summer periods, nine that showed variability across both time periods. The snapshot view at Day 21 showed relatively low larval mortality, however, there was differential mortality expressed in different genotypes in different periods. For instance Jonafree and Northern Spy showed low mortality in spring but high mortality in summer where as Cox's Orange Pippen (OP) showed high mortality in the spring but lower mortality in the summer.

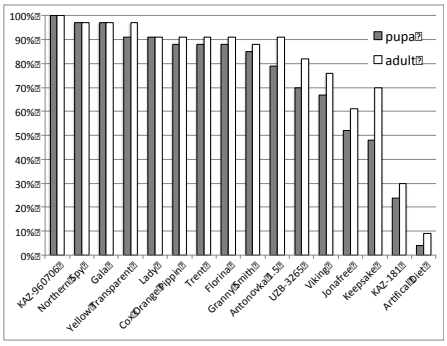

Fig. 6. Percent mortality of OBLR at the pupal and adult stages, summer 2012.

We know that this snapshot of mortality is not the total picture of developmental effects of *Malus* genotypes on OBLR development but it provides a common reference of comparison of larval mortality prior to pupal formation. Some of the larvae surviving at Day 21 did not complete normal development to the pupal or adult stage. Figure 6 shows the percent mortality of these *Malus* genotypes in the summer period to the pupal and adult stages. Clearly additional mortality occurs from larvae dying after Day 21, abnormally formed pupae or pupae that never emerge as adult.

We do not show data for the 14 middle eastern genotypes, however, one, KAZ form 181 had very low mortality, equal to the artificial diet while several KAZ and UZB genotypes had mortality at Day 49 (all adults or deformed/dead pupae) greater than 70%.

In 2013 we again followed *Malus* genotypes in the diversity map set for two periods, spring and summer. We intended to follow these genotypes through the fall but a hailstorm that severely damage foliage and fruit made it impossible to get good data for this period. Here we report on mortality of OBLR at Day 21 for twelve (spring) and eleven (summer) *Malus* genotypes, with nine that showed variability across both time periods. The snapshot view at Day 21 showed high relatively larval mortality in the spring and very low mortality in the summer (Fig. 7). Granny Smith, Golden Delicious and Viking had very high mortality in the spring but low mortality in the summer, along with other genotypes. These results suggest that induction of resistance in the spring could be different than in summer.

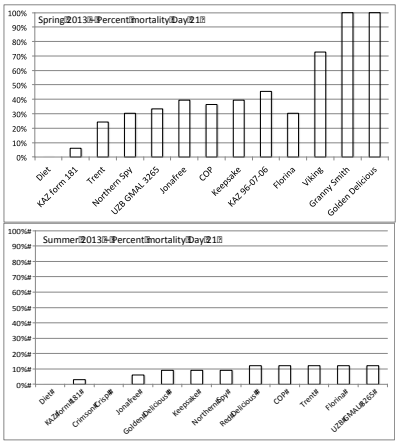

Fig. 7. Percent OBLR larval mortality on Day 21, 2013.

In 2013 we also evaluated for the first time without being compromised by insecticide treatments *Malus* genotypes in the Parent mapping set (Red Delicous, Arlet, Braeburn, Splendour, Cripps Pink, WSU-2, Coop-15, Honeycrisp, Sundowner, Crimson Crisp, Fuji, Cameo, Aurora, Enterprise and Jazz). We evaluated sixteen genotypes with the hope that these data allow us to move to Objective 2 - localization of genes that confer resistance to OBLR. However, results from this effort were highly unexpected and do not provide information we can use to identify a genetic basis for resistance. Larval mortality in the spring and summer exceeded 80% for each genotype. In fact, most of the

mortality occurred in late instar larvae with very little mortality during the first four instars (Day 21 or less). Virtually no adults were produced in the bioassays in either spring or summer periods.

Example of genotype variation across seasonal periods – Exposing OBLR larvae to *Malus* genotypes was limited due to the lack of availability of leaves on some genotypes and to human resources required to follow OBLR through development to the adult stage. However some genotypes in the *Malus* diversity set were followed through two years and two seasons, spring and summer. Figure 8 shows survival to the adult stage in the summer period for 2012 and 2013. Survival was high in 2013 for OBLR reared on the artificial diet, KAZ-181 and some of the other KAZ and UZB genotypes. There were much lower levels of survival to the adult stage in 2012 for Cox's Orange Pippen, Trent, Florina, Northern Spy and Gala than in 2013. Of all the Malus genotypes Gala, Northern Spy and Keepsake showed the highest level of resistance.

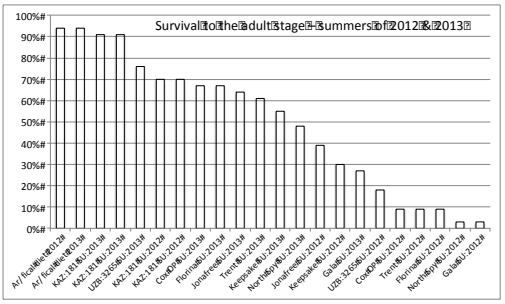

Fig 8. Percent survival to the adult stage of OBLR in the summers of 2012 and 2013.

Population Growth as an Assessment Tool – Synthesis of bioassay data for leaffrollers is a challenge as there are several factors measured, each of which could contribute to what might be called resistance in *Malus* genotypes. In 2012 we developed and index to incorporate multiple factors as way of synthesizing the development data collected. While this approach was useful it was not as robust as we desired. We therefore turned to a modeling approach using a PopTools matrix program that allowed us to make projections of population growth using the development data collected from our bioassays (development time for each stage, mortality, fecundity and sex ratio). Figure 9 shows results of running developmental data through the population matrix model. The intrinsic rate of increase is a measure of population growth. The artificial diet, KAZ-181, Trent and UZB along with Cox's Orange Pippen had high intrinsic rate of increase values for both periods of 2013. Many of the genotypes had intrinsic rate of increase equal to zero, indicating there would be no population growth due either to high mortality or no reproduction by adults that were produced. Of course these data are confounded to some degree by the low number of individual OBLR larvae we were able to rear (max of 33 per bioassay period). We have similar data for the 2012 season but there is not sufficient room to report it here.

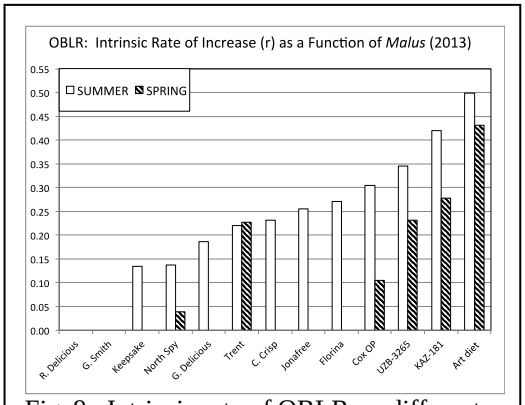

Fig. 9. Intrinsic rate of OBLR on different Malus genotypes based on 2013 bioassays.

Effect of leaf age on OBLR development – In 2013 we conducted a research project comparing the development of OBLR on three Malus genotypes (Red Delicious (RD), Golden Delicious (GD) and Crimson Crisp) where we collected old and new leaves from each to run our bioassay. Figure 10 shows that OBLR larvae exposed to old leaves of Crimson Crisp, RD and GD had much higher mortality at Day 21 compared to those exposed to

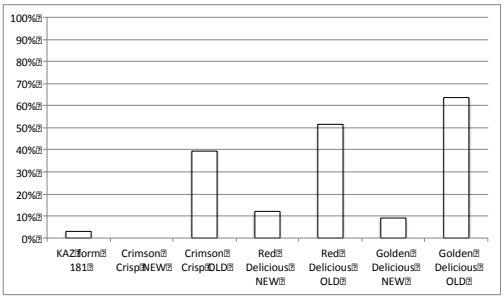

Fig. 10. Percent mortality of OBLR larvae on Day 21.

young leaves. Since OBLR larvae can readily move and choose a leaf that is a better food source, one that is not producing chemicals that inhibit development or increase mortality, it is important when conducting leaf bioassays to use as young of leaves as possible. While these results do not represent novel information regarding leaf age and impact on insect development they are instructive of how we might interpret some variations in our data from year to year when leaf availability in the diversity map set were limited in the summer relative to spring.

Other development parameters informing OBLR bioassay results – Several factors other than mortality inform the impact of Malus genotypes on OBLR. These include larval development rate (LDR), pupal weight (PW), adult mortality rates and adult longevity. We analyzed all our data looking at these parameters for Malus genotypes where we had data across two or more seasonal periods. Figure 11 shows data for LDR and PW. LDR was high in the artificial diet, due in part to a lack of disturbance of larvae and the high level of nutrition provided by the diet (Fig. 11 top). The LDR for KAZ-181, UZB3265, and Florina were close to that of the artificial diet. The LDR was lowest on Cox's Orange Pippen, Jonafree, Viking and Yellow Transparent. Pupal weight was highest for larvae reared on the artificial diet for reasons mentioned above (Fig. 11 bottom). The pupal weight for KAZ-181 was the closest to pupae from the artificial diet. Pupal weights for the other Malus genotypes were similar but about 50% lower compared to those from the artificial diet. There was a high degree of variability between AL and AMR with little difference in these parameters between the artificial diet and *Malus* genotypes (data not shown).

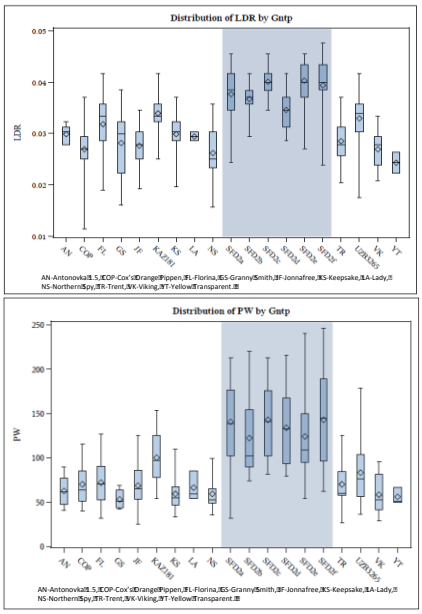

Fig. 11. Larval development rate (top) and pupal weight (bottom) for OBLR reared on different *Malus* genotypes (Gntp).

Hormonal effect on OBLR development – In 2011 and 2012 we observed that when OBLR larvae were reared on certain *Malus* genotypes, incomplete pupation or larval-pupal intermediates – see image at right - were produced that resembled exposure to a juvenile hormone (JH). Since high JH levels have an abnormal effect if they are present at the last larvae stage we conducted a study in an attempt to confirm whether certain *Malus* genotypes could consistently produce the larval-pupal intermediates. We reared OBLR larvae until the fifth instar on artificial diet and then transferred them to apple leaves using our bioassay method and follow them through to the adult stage. The genotypes evaluated were KAZ 95-05-01-P22, Antonovka 1.5, UZB GMAIL 3265, KAZ 96-09-05 and Red Delicious. While a few larval-pupal intermediates were produced the percent was far below what we had observed previously. It is possible that the cause of larval-pupal intermediates is driven by a lack of adequate nutrition when OBLR larvae are reared in foliage over their entire life and is not specifically related to the induction of a chemical that mimicked JH in the *Malus* genotypes.

Effect of oil and CM virus on OBLR – In 2012 we started using the parent block at Sunrise to assess the *Malus* genotypes impact on OBLR development. However, a mix up in the general pest control program resulted in Intrepid being applied in the spring to trees in the parent set causing high mortality in our bioassays. The intent was to shift the use of CM virus plus oil in the summer to conduct leafroller bioassays so we set out to test whether the CM virus and oil would have negative impacts on OBLR larvae. We found that there was no impact of CM virus

on OBLR larvae, however, we did discover that when oil was applied by an airblast sprayer at 1% concentration to apple trees, and leaves were then collected and ran through our bioassay, all neonate larvae died within the first 7 days.

Effect of fungicides on OBLR – The impact of mildew control on OBLR was evaluated as part of this project since fungicides were applied as part of a maintenance program to apple blocks where leaves were collected for bioassays. There is some literature that has shown negative effects of fungicides on insect or mites so to ensure our bioassay results were not compromised by fungicides. We conducted a bioassay using the diet incorporation of two fungicides, Procure and Rally, and evaluated the development of OBLR larvae. There was no difference in mortality of OBLR larvae reared on diet with concentrations of each fungicide ranging from 0 (untreated) to 300 ppm.

Codling moth resistance 2013 – At the end of the first codling moth generation, selected trees (16) in the GMAIL mapping set were evaluated for fruit injury. No insecticide sprays had been applied to the block and fruit injury was readily observed on most trees. The total number of fruit per tree was counted with the number injured by codling moth recorded. There was an average of 106 (42-175) fruit per tree and the average percent fruit injury was 7.8 (0.0-27.7). These trees were close together so it is doubtful spatial distribution of the codling moth population accounted for the observed differences. These results point to variation in the resistance of genotypes in the GMAIL mapping set to codling moth injury. Plans were to complete the codling moth survey at the end of the second generation; however, two hailstorms in the latter half of summer injured the fruit so that sampling was not possible.

Plans for 2014 – Leafrollers and Codling Moth. We are proposing with a new one year project to continue exploring genotypes with highest levels of resistance and those with high levels of susceptibility. We are proposing to work with Dr. Dhingra's laboratory to identify genes that are up-regulated when OBLR feeds on selected genotypes. We will not have access to the Parent map set in 2014 due to needs for these trees for other research projects, therefore, pursing additional data from these genotypes seems unlikely. We will complete assessment of the GMAIL mapping set and the HCxCP mapping set to codling moth injury in the field, through rearing of injury to fruit and through bioassay.

Localize genes for resistance. We intended to be at a point where we might be able to research the apple genome for expressions of resistance to leafroller by year three. Because we were not able to get data from the Parent set at Sunrise we likely do not have a robust enough data set to explore this objective. The confounding of multiple needs for the same mapping sets associated with the apple breeding program suggests that the genotypes in the parent and possibly the Pedigree set should be replicated by grafting to trees in the pest control plantings at Sunrise.

Develop predictive markers for resistance. This aspect of the project will not be completed within the time frame of the project unless some unexpected results are found in the search for localized genes in Objective 2.

Executive Summary:

This project was originally designed to explore possible resistance in *Malus* genotypes to a leaf feeding insect, OBLR, and a fruit feeding insect, codling moth. We focused our initial efforts on developing a bioassay that allowed us to rear OBLR through their entire life cycle with minimal disturbance and while preserving as best we could the integrity of the excised leaf. The bioassay seemed to work well, at least with regard to maintaining good leaf quality for seven days, which was determined as the best time interval for changing leaves. The first year of the study we evaluated 19 genotypes from the diversity map set at Sunrise. There were only two trees of each genotype and this restricted the population of leaves, which could be used in bioassays. In 2012 and 2013 we evaluated some of the same genotypes from the diversity map set with the objective of determining seasonal variation in resistance. There was much more seasonal variation in resistance expressed in the different genotypes than expected. We were able to evaluate genotypes from the Parent map set in 2013 and found that most of these caused high mortality in OBLR with few or no adults produced and with most of the mortality occurring in late instars. These results have made it difficult to interpret data and identify genotypes that show consistent levels of resistance to OBLR. We did identify one variety, KAZ-181 that caused almost no negative impact on OBLR. KAZ-181 could be a good susceptible genotype to use in future field studies to gage resistance of other genotypes. We showed that old leaves have a definite negative impact on OBLR compared to new leaves. Because OBLR can and does moves from one feeding site to another during its life history, always moving to newer leaves when the opportunity affords itself, we knew we needed to always use newer leaves in our bioassay. The number on trees in the diversity set most likely impacted our bioassay results in summer and fall periods by restricting the availability of new leaves.

We utilized a simple matrix population model to synthesize the life history parameters, stage specific mortality, development time, fecundity and sex ratio, derived from out OBLR bioassays. This method generated a couple of values, the intrinsic rate of increase and net reproductive rate, which were easy to understand was a better method than the indexing method we used in 2012.

We did demonstrate as part of this project that oil residues on foliage have a high negative impact on OBLR larval survival. We have not observed this before with larger larvae but when neonate larvae were exposed to oil residues we consistently observed high mortality. No negative effects on OBLR were observed when exposed to CM virus or the fungicides Rally and Procure.

Preliminary samples of codling moth injury to trees in the GMAIL mapping set showed a high degree of variability following the first generation in 2013. However, due to two hailstorms that injured foliage and fruit in late summer, additional surveys of possible codling moth resistance in the GMAIL and Honey Crips X Crips Pink mapping set could not be completed.

There is a great deal of research that needs to be done to explore insect and disease resistance in the *Malus* genome, however, the problem of using the same trees for horticultural and pest studies arose as a barrier in this project. A long-term plan should be developed to plant *Malus* genotypes of highest interest in areas of Sunrise where pests are not or marginally controlled. This would allow for more trees from which to harvest foliage or fruit without the risk of pesticide contamination and to challenge trees in the field with pests like OBLR and codling moth.

FINAL PROJECT REPORT

Project Title: Enhancing BC in apples: how do conventional and organic systems differ?

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Other funding sources

Agency Name: USDA-SCRI grant (Enhancing BC in Western Orchard Systems)
Amount awarded: $2.25M. Approx.
Notes: $60K from that grant was used for this project.

Total Project Funding: **Year 1:** $96,916 **Year 2:** $99,133 **Year 3:** **$**103,775

Budget History:

Item	2011	2012	2013
Salaries	55,000	55,000	55,000
Benefits	18,920	17,820	17,186
Wages	13,440	14,112	18,000
Benefits	2,016	2,117	3,042
Equipment	0	0	0
Supplies	4,600	6,997	7,306
Travel	2,940	3,087	3,241
Total	96,916	99,133	103,775

Objectives:

1. Compare the natural enemy (NE) complex in conventional and organic orchards to determine differences in diversity and abundance.
2. Evaluate low dose pesticide applications to minimize pesticide impacts on NE and reduce residues, while maintaining low pest damage.
3. Evaluate attractant traps' attractive radius and determine the feasibility of "herding" NE to improve BC and integrate BC better with chemical controls.

Significant Findings:

- The organic orchards evaluated tended to have larger NE populations and lower aphid populations.
- The pest control intensity is probably more important in predicting pest problems than the "conventional" versus "organic" labels. Many conventional orchards use very soft programs (virus, *Bt*) and can be actually softer than a harsh organic program.
- Comparison of the use of organic, full rate of Delegate and 10% field rate of Delegate at the WSU-Sunrise orchard showed that there was no sigificant change in damage from codling moth, leafroller, woolly apple aphid, green apple aphid, rosy apple aphid, or San Jose Scale between the treatments.
- Natural enemy populations increased from year to year in the three different treatment programs, likely because of decreased early season sprays. Second generation sprays tended to increase differences in NE numbers between the organic or reduced rate of Delegate treatments compared to the full rate of Delegate treatments.
- NE numbers increased around NE lures and resulted in lower aphid numbers two weeks after lures were placed in the field, suggesting that NE concentration via lures can contribute to localized suppression of aphid populations. The studies also showed greater NE egg-laying occurred near the lures, suggesting that the effect of short term baiting will have longer suppressive effects because after egg hatch, the relatively immobile immature stages would be present in higher numbers in the baited areas.
- Previous studies had shown the squalene lure-baited traps were highly attractive to the lacewing *C. nigricornis*. Those studies did show that >93% of the capture was male, which potentially meant that it would disrupt the mating process. However, studies in this grant showed that female *C. nigricornis* were also attracted near the lures (just not into the traps), so that squalene should be useful for herding *C. nigricornis* without concern that it would disrupt mating behavior of this key predator.

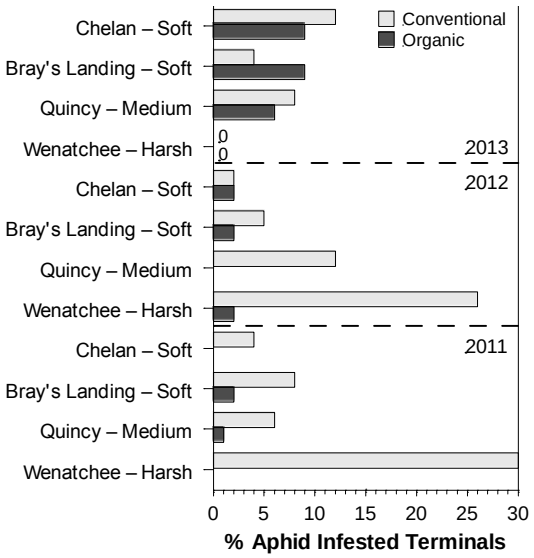
Results and Discussion:

Objective 1. Compare the natural enemy (NE) complex in conventional and organic orchards to determine differences in diversity and abundance.

Methods: We sampled four pairs of orchards (one conventional and one organic) where orchard pairs were separated by <0.5 mile. We sampled the orchards for differences in natural enemy (NE) populations using our HIPV and floral volatile traps and earwig (corrugated cardboard) bands. All orchards in this objective were under mating disruption. These orchards are classed in terms of program harshness to NEs: two pairs were very soft with few differences in the management programs between the conventional and organic treatments (e.g., virus + oil were used in both pairs of orchards – Bray's Landing and Chelan), in one pair both conventional and organic had harsh programs (large number of disruptive treatments - Wenatchee), and in the final pair (Quincy) both conventional and organic had medium programs (one to several harsh treatments and a few softer materials used at other times in the season). In 2013, we set up a new orchard pair in Quincy, because the orchards used in 2011-12 were pulled by the grower. We also changed the conventional orchard used in the Chelan location in 2013 to get a closer match of cultivars between the two orchards. The

discussion is focused on the aphid NEs for brevity. To simplify the analysis and presentation, we use a "natural enemy-day" analysis, which is calculated by taking the number of average density of the aphid feeding natural enemies between two sample dates and multiplying that by the number of days between samples. This gives us an estimate of the amount of potential predation/parasitism and can be accumulated over the season to give overall differences between orchards. One caution is that each of the NEs kill different amounts of prey and the generalists may eat more than just aphids, thus this is a simplification and the data is skewed by the most common aphid feeding NEs (the lacewings *Chrysopa nigricornis* and *Chrysoperla plorabunda*, the parasitoid of the woolly apple aphid, *Aphelinus mali*, *Deraeocoris brevis* and the European earwig). Aphids monitored included WAA, green apple aphid (GAA), and rosy apple aphid (RAA).

Fig. 1. Percentage terminals infested by aphids at the end of the season 2011-2013.



Results: Over the three-year period, percentage of aphid infested terminals tended to be highest in the conventional orchards (Fig. 1). The damage tended to be inversely related to the number of aphid natural enemies, which was greatest in the organic orchards in three of the four orchard pairs (Fig. 2). Abundance of aphid natural enemies, especially *C. nigricornis*, *A. mali*, and *D. brevis* was generally higher in the organic orchards at Bray's Landing, Chelan and Wenatchee. In contrast, the conventionally managed orchard in Quincy

Fig. 2. Cumulative aphid NE days versus day of the year for the orchard pairs, 2011-2013.

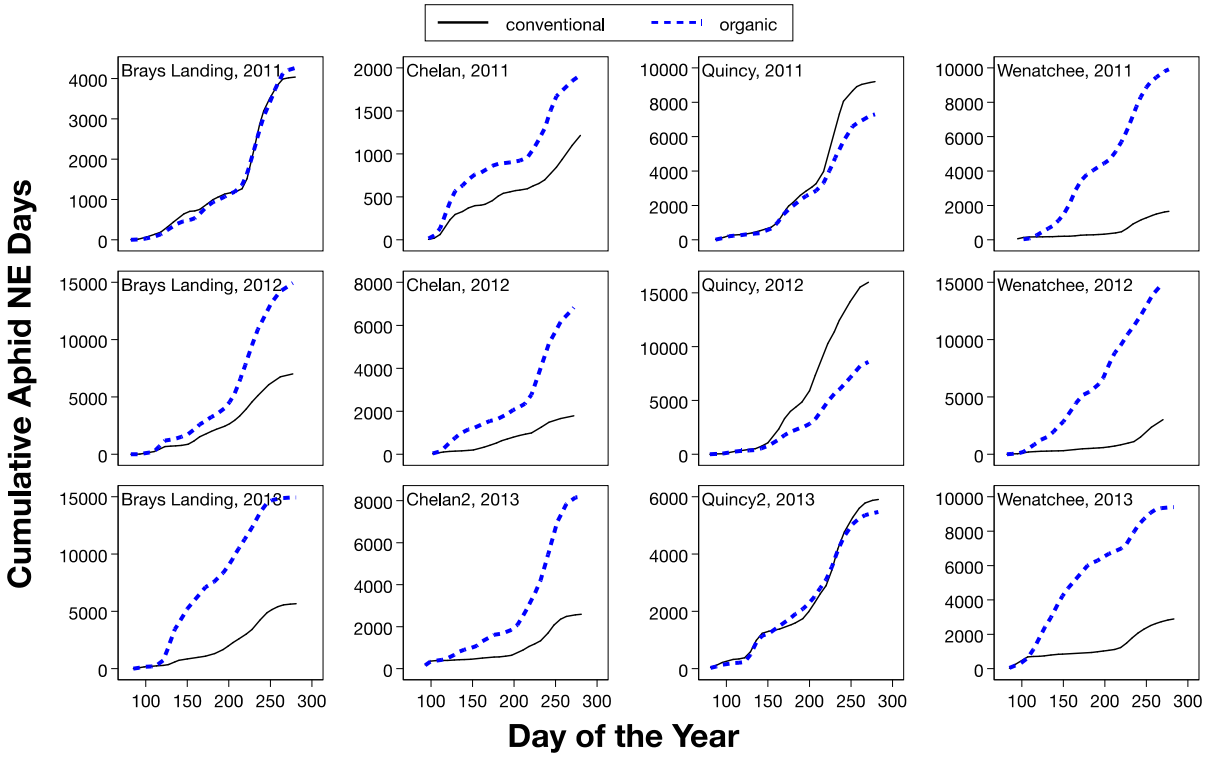
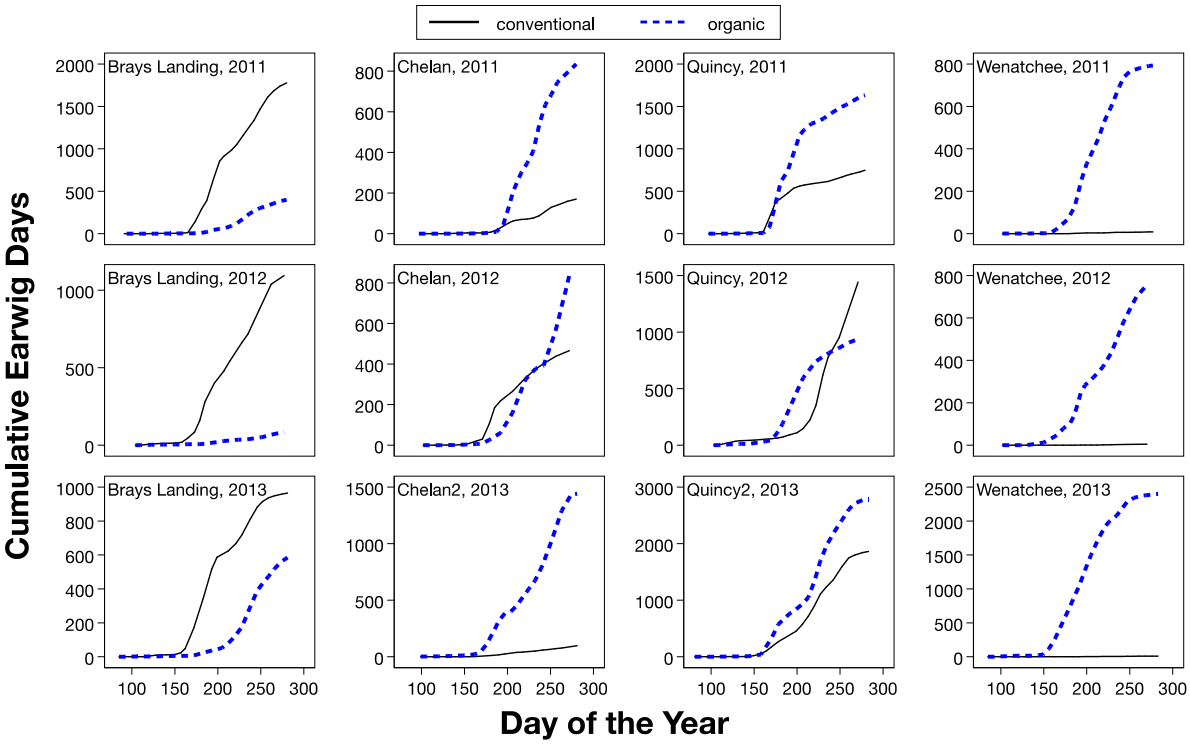


Fig. 3. Cumulative earwig-days from banding data versus day of the year for four pairs of orchards 2011-13.



orchard had consistently higher natural enemy abundance in the 2011-2012 seasons (there was no difference in aphid natural enemy days 2013 in the new orchard pair). The cumulative aphid natural enemy days started to diverge between the organic and conventional blocks about the time that five oil (+ virus) sprays targeted at codling moth were applied (2011) and four oil and either virus or Entrust were applied in the organic block (2012). In the conventional block, treatments at the time of divergence were minimal with two sprays in 2011 (Proclaim at the start of the divergence and Altacor near the end) and one spray in 2012 (imidacloprid). Even though there were more cumulative natural enemy days associated with the conventional block in 2011 and 2012, damage was still highest in the conventional block, which suggests that other factors may also be important in this orchard pair. Earwig densities were highest in the organic block in 2011 all year and 2012, until just before our aphid evaluation (Fig. 3). Thus, the earwigs appear to be a dominant factor in the suppression of aphids in the Quincy organic orchard.

Interestingly, the conventional orchard in Wenatchee accumulated aphid natural enemy days at only 16-31% of that in the organic orchard (both orchards were classified as harsh management programs, Fig. 2). We have not yet received spray records from the conventional Wenatchee orchard for 2012 or 2013, so we cannot yet explain the sharp drop in aphid populations in 2013 (we have been promised they will come soon).

Examination of the earwig banding data (Fig. 3) showed that the abundance of earwigs was typically greatest in the organic blocks, with the glaring exception of the Bray's Landing orchard pair. In that orchard pair, the organic block had under-tree irrigation that wet our bands resulting in them falling apart and extremely lowering earwig capture. However, we know that the earwigs occurred likely at higher densities in the organic block because our HIPV and floral volatile traps (which are ≈40 fold less efficient at trapping earwigs than the bands) captured ≈63% more earwigs in the organic block

than in the conventional block. The lack of earwigs in the Wenatchee conventional block was not caused by irrigation issues, but more likely by pesticide use as we caught only 1 earwig in our attractant traps over the three-year period we monitored that orchard.

Across all the orchards and years, we collected an average of 29 different natural enemy taxa that attack aphids. Despite this diversity, the dominant species was nearly always the lacewing *C. nigricornis* or the WAA parasitoid *A. mali*. The mirid bug *D. brevis* was the next most common predator (particularly in the Chelan orchard pairs), but also in 2012 in the Brays Landing and Quincy orchard pairs. Other natural enemies that were abundant at various locations and years included the lacewing *C. plorabunda*, the syrphid fly *Eupeodes fumipennis*, and the aphid parasitoid *Ephedrus* spp. Diversity indices done on the yearly data did not show significant differences trends between conventional and organic blocks, but further analysis examining the diversity at monthly intervals needs to be performed to see if there are trends masked by averaging over the entire season.

Objective 2. Evaluate low dose pesticide applications to minimize pesticide impacts on NE and reduce residues, while maintaining low pest damage.
Methods: For a three-year period, we have followed a 15-acre plot at WSU-Sunrise that was originally an organic block. In 2011, we divided the plot into twelve 1.25-acre plots, which were randomly assigned to either a conventional, organic or reduced rate treatment. All plots received mating disruption in all three years. The pesticide used in the conventional and reduced rate treatments was Delegate, which is considered to be relatively harsh to natural enemies, based on bioassays done in objective 1 of the SCRI "Enhancing biological control in Western apple, pear, and walnut orchards". The conventional treatment in 2011-2012 was the full rate of Delegate applied twice in the first CM generation with a delayed first cover. Thus, oil was applied at 375 DD and then at 525 DD the first Delegate spray was applied and ≈ 2 weeks later a second cover was applied. The organic treatment during this period also had a delayed first cover (i.e., oil applied at 375 DD) and the first cover of CM virus was applied at 525 DD, then three more times at ≈ 1-week intervals. The reduced rate treatments were applied at the same timing as the organic treatments, but used a 10% field rate of Delegate in place of the virus. These treatments would be considered very light conventional and organic treatments, with no other pesticide or oil sprays used other than as described above.

In 2013, we had an outbreak of apple mealybug (present throughout the entire northern part of the Sunrise orchard, both inside and outside our plots). Therefore, we used the same treatment timing as 2011-12 in the first CM generation, but added oil to all the treatments (e.g., virus + oil or Delegate + oil) and added an additional oil treatment at the end of the first and second CM generations on 17 June and 12 August to help suppress apple mealybug. We also added treatments for the second generation which were applied starting at 1375 DD (oil only to all treatments), the first cover (either Delegate + oil or virus + oil) at 1525 DD, and then applied the appropriate treatment 2 weeks later (full rate Delegate + oil) or at weekly intervals (virus + oil or the 10% field rate of Delegate + oil), in an attempt to see if second generation treatments affected natural enemy populations.

Results: In all treatments the aphid natural enemy populations built up over time, with increases occurring each year (Fig. 4). The first year showed very little differences between the treatments and even the full rate of Delegate not causing much disruption. Differences in the cumulative aphid natural enemy days started to appear in 2012 and expanded in 2013, with the full rate of Delegate being 20.7% and 28% lower in 2012 and 2013, respectively. The reduced rate of Delegate and the organic treatments were similar in all three years, with the largest discrepancy occurring in 2012 were the organic treatment showed a 15.9% reduction, but that disappeared in 2013. The heavier treatments in 2013 did not reduce natural enemy populations compared to the previous two years, but probably exacerbated the effects of Delegate by suppressing late season increases of earwigs, and the

lacewings *C. plorabunda* and *C. nigricornis*. During the three-year period, there was a relatively sharp drop off of *C. nigricornis* populations and equally sharp increase in *C. plorabunda*, probably because we eliminated the delayed dormant sprays that occur when *C. plorabunda* has already emerged. Earwigs were the dominant predator each year, and also increased steadily from year to year, without showing any fruit damage.

The aphid counts (WAA, GAA, RAA), leafroller, and codling moth damage estimates showed no significant differences occurred in any year for any pest between the different treatments. Peak damage of codling moth occurred in 2012, when 0.3, 0.1, and 0.1% of the fruit showed codling moth damage in the conventional, reduced rate, and conventional treatments, respectively, but no damage occurred in any treatment in 2011 or 2013. The percentage of terminals infested with WAA or RAA were < 0.5%, but GAA showed peak damage in 2011 at 2.25, 1.5, and 3.0% for the conventional, organic and reduced treatments, respectively. In 2012 and 2013, aphid infestations were only detected at 0.5% GAA (conventional) and 0.25% WAA (reduced treatments). We never observed SJS scale in any of the treatments over the three-year period. Leafroller damage did not appear to follow any particular trend in the conventional or reduced rate treatments between years, while the organic treatment had no detectable damage in 2011-2012 and only minor (0.4% terminals damaged) in 2013 (Fig. 5).

Fig. 4. Cumulative aphid NE days 2011-2013 at the WSU-Sunrise orchard in the conventional, organic and reduced rate treatments.

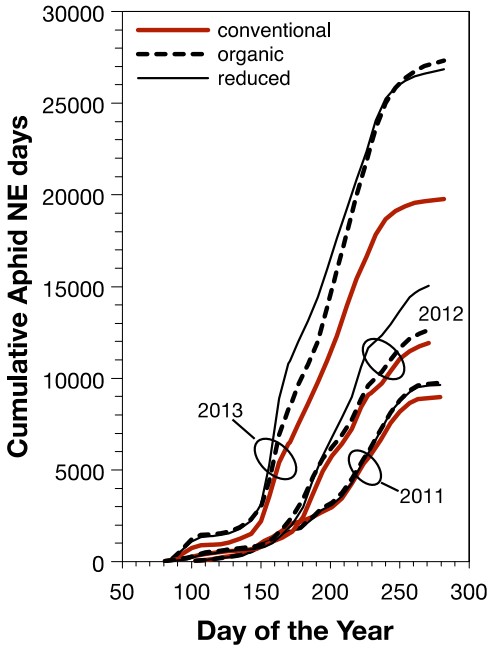
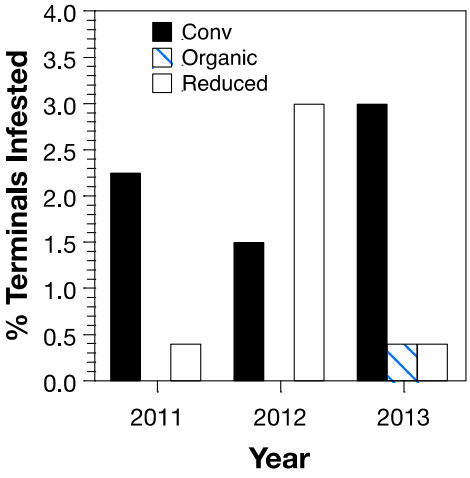


Fig. 5. Percentage of terminals infested by leafrollers at the WSU Sunrise orchard in the conventional, organic and reduced rate treatments 2011-2013.



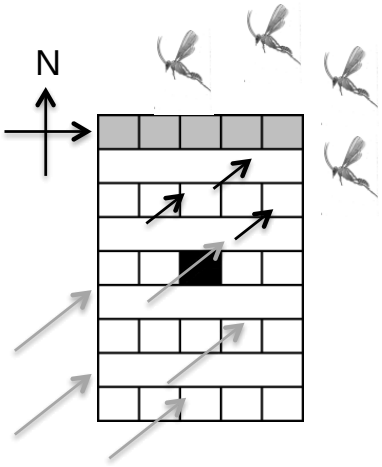
Objective 3. Evaluate attractant traps' attractive radius and determine the feasibility of "herding" NE to improve BC and integrate BC better with chemical controls.

Biological control contribution of NEs responding to HIPVs

Methods: A field study was conducted during June 2013 in an organic apple orchard (>6 acres) to evaluate the direct impacts of NEs, responding to HIPV lures baited with squalene, 2-phenylethanol + geraniol (PE+GER), and acetic acid + methyl salicylate + 2-phenylethanol (AMP), on aphid populations. Eight blocks consisting of 25 trees each were located where aphid populations were high, and during the first week of June, apple aphids (woolly, green, and rosy) were counted to determine a baseline infestation. Natural enemy eggs, larvae, and adults were also recorded. Lures were placed in the center tree of half of the blocks, while the other half were non-lured and served as controls, giving us four pairs of blocks to examine. To eliminate visual attraction, we used clear interception traps. Traps were placed on trees at various distances (0, 6, 12, 18, 15, 30, and 45 ft) and directions (north, south, east, and west) from the center tree (lured or non-lured) to trap NEs. Aphid and NE counts were taken again two weeks after lures were set in place. We anticipated lower aphid populations and higher NE populations in HIPV lured plots.

Results: Natural enemies caught in traps and observed on trees included green lacewings, brown lacewings, ladybird beetles, syrphid flies, the parasitoid wasp, *Aphelinus mali*, and braconid wasps. We found that traps in lured blocks caught significantly more NEs than non-lured blocks (9.3 ± 1.4, 4.75 ± 1.1, average ± SE, respectively) in the northeastern quadrants of blocks on trees 15 and 30 feet from the lure location. Natural enemies tend to fly upwind in response to odor plumes coming from the opposite direction, and because winds originated from the southwest (daily average 218°) during the time lures were in the field, NE most likely responded to volatile plumes coming from this direction (Fig. 6), resulting in higher NE response in the northeast portion of blocks. Surprisingly we did not see significant reduction in aphid colony counts in trees directly adjacent to the lure, but we did find a greater reduction in the most northern row of trees (30ft from lures) in lured blocks (lured, 11.3 ± 1.6; non-lured, 4.3 ± 2.6; average reduction per tree ± SE). These results suggest that NEs attracted to HIPV lures are contributing to biocontrol, but wind direction needs to be considered.

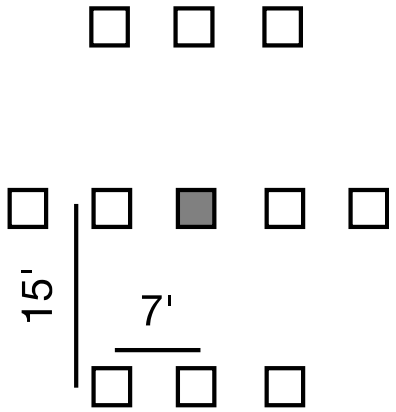
Fig. 6. NE are attracted to volatile plumes (black arrows) caused by SW wind (grey arrows), resulting in a reduction of aphid numbers in northern most trees (grey boxes). Boxes represent trees, and the black box represents the lured tree.



Are female C. nigricornis attracted to squalene lures?

Methods: During July and August 2013, field experiments were conducted to determine if female green lacewings, *C. nigricornis*, are attracted to (and not repelled by) squalene lures. Previous studies showed few females were caught (≈3.3%) in squalene-baited traps. In the first set of experiments, we placed squalene lures in specific trees, then captured adults using sweep nets and determined their sex. Our experimental unit was an 11-tree set of trees (Fig. 7), where the center tree had either a squalene lure or a control (blank) lure, where the two treatments were replicated four times each throughout the orchard. Our analysis tested if the numbers of adult *C. nigricornis* caught or *C. nigricornis* egg masses laid were significantly different between the treated and the non-treated replicates.

Fig. 7. The experimental unit consisted of 11 trees, the center (gray) tree had either a squalene or a blank lure.



Results: Similar numbers of males and females were captured on foliage near squalene lures on all four sampling dates (males 9.3 ± 0.5, females 7.5 ± 2.1). The number of egg masses laid was significantly higher in center row trees in the squalene-treated areas than the same location in the non-treated areas (10.7 versus 5.0 per block). However, the effect did not extend to non-lured areas adjacent to the row with the lure present (i.e., the top and bottom rows in Fig. 7), showing that the lures are acting on a relatively small spatial scale.

Our data shows that females are not repelled by squalene lures, similar numbers of males and females are present in foliage near lures, and females are ovipositing more eggs in the vicinity of lures. This sort of response had been previously found for the lacewing *Chrysopa oculata* where males are caught in high numbers in traps baited with the lure iridodial, but females were found on the foliage around the lure, but not in the trap. In addition, our 2011 and 2012 work with protein markers, suggests that green lacewings are quite mobile, easily moving in large numbers 200 feet or more in 2-3 days. These results suggest that effective recruitment of a generalist predator, like *C. nigricornis*, to pest hot spots with our NE lures is possible.

Executive Summary:

The organic versus conventional orchard evaluations showed that there are wide differences in the intensity of management even within each orchard type. Several of the conventional orchards used very soft programs, based on mating disruption plus virus and *Bt* for control of codling moth and leafroller. These orchards also tended to have lower pest populations, high natural enemy populations and little difference in damage from their organic comparison orchard. We noticed most of the differences occurred in our pairs of orchards where the pest control program was considered harsh or medium. In those situations, the organic orchards had a better overall balance of natural enemies and lower aphid damage. Comparisons of the diversity of natural enemies did not reveal consistent differences between the conventional and organic orchard pairs. This may have been at least partially caused by averaging the diversity over the entire season, and further analysis will be required to evaluate this factor. We collected an average of 29 taxa that attack aphids over the four orchard pairs, although an average of 2-3 taxa comprised 75% or more of the total numbers collected. The most common natural enemies were earwigs, the lacewings *Chrysopa nigricornis* and *Chrysoperla plorabunda, Deraeocoris brevis,* the syrphid *Eupeodes fumipennis*, and the woolly apple aphid parasitoid *Aphelinus mali*.

The test of using low rates (10%) of a normally disruptive material (Delegate) at the timing normally used for organic materials was highly successful. We observed no differences in damage or abundance for a broad range of pests (CM, OBLR, WAA, GAA, RAA, SJS) and natural enemy populations were similar in number to those found in the organic treatment. This sort of use does not lead to increased resistance because selection pressure for the development of resistance is lower and has the advantage of reducing costs, residues, and impacts on natural enemies. Another reason for the efficacy of this approach is that the more frequent applications used in organic programs allow less shoot/fruit growth between applications, so that there is less unprotected foliage/fruit, especially in the spring when shoot growth in a week can result in an average of three nodes (20% of the total) being unprotected (data from our other project). The change in rates and timing was done in the commercial section of WSU-Sunrise, so it is applicable to commercial settings and this appears to be a very promising technique that needs further investigation.

The work on use of NE attractants to herd natural enemies revealed three important points: (1) natural enemies do aggregate downwind of the lures and may contribute to lower pest population levels; (2) squalene, one of our more attractive lures, attracts both sexes near the lures, but only males enter the traps; and (3) lacewings laid more eggs adjacent to lures. These three points suggest that we should be able to deal with orchard pest hot spots using either HIPV or floral lures. Marking studies in 2011 and 2012 also showed movement of lacewings was common within 200 feet of a lure, so that we can attract and retain NE within a reasonable radius of a lure. We still need further work to decide which of 3-5 lures are best for suppressing aphids and how long they should remain in the field, but this also appears to be a promising tactic that we need to follow up on in the future.

CONTINUING PROJECT REPORT **YEAR**: Year 1 of 2
WTFRC Project Number: CP13-103

Project Title: *Amblydromella caudiglans*: A new predatory mite for Washington apples

PI: Elizabeth Beers
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Cooperators: David Crowder, Thomas Unruh, David Horton, James McMurtry

Total Project Request: **Year 1:** $23,419 **Year 2: $23,275**

Other funding sources

Agency Name: Washington State Commission on Pesticide Registration
Amt. requested/awarded: Awarded $13,690 (2013)/Requested $11,750 (2014)

Budget 1
Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181, ext. 221 **Email:**carriej@wsu.edu/joni.cartwright@wsu.edu

Item	2013	2014
Salaries[1]	14,198	14,766
Benefits	2,029	2,110
Wages[2]	4,733	4,922
Benefits	459	477
Equipment	0	0
Supplies	0	0
Travel	0	0
Miscellaneous[3]	2,000	1,000
Plot Fees	0	0
Total	23,419	**23,275**

Footnotes:
[1]Project Assistant (Graduate student, academic year).
[2]Summer wages (graduate student).
[3]Payment to Dr. James McMurtry for species identification consultations.

OBJECTIVES

1. *Conduct a survey of predatory mite (Phytoseiidae) species in Washington apple orchards to determine species prevalence and biodiversity.*

   To date, we have collected 110 leaf samples from apple blocks (plus 10 from other crops) throughout central Washington. This is an unusually large sample size for spatial analysis (Obj. 2). The primary focus of next year will therefore be the collection and analysis of site information needed to complete Obj. 2 and 3.

2. *Assess the effects of various factors, including climate, landscape, available prey species, and type of pesticide regime (conventional or organic) on the species composition of predatory mites found at each site using Geographic Information Systems (GIS) analysis. This will allow us to form a model which can be used to predict the species composition of individual orchards. The ability to inform growers which predator species they are likely to find in their orchard will allow them to adapt their management strategy to best suit the needs of that particular predator to maximize biological control of spider mites*.

   We have created a survey to collect information from pest consultants regarding management practices to include in the model. Questions asked included apple variety, block age, block density, weed levels, pest mite levels, and pesticide history. We have received 59 responses and will continue to collect this information throughout 2014. We have incorporated this data into a database that can be analyzed using GIS software and general linear modeling. This analysis is currently in progress.

3. *Compare the biology, pesticide tolerance, and predatory ability of A. caudiglans and G. occidentalis. This will provide growers with the information needed to adapt current IMM practices to their dominant predator*.

   We have completed basic comparative life history studies of the two predators, including determination of the average duration of each life stage (egg, larva, protonymph, deutonymph, and adult). For *G. occidentalis*, we have also determined the average longevity and fecundity for females taken from a laboratory colony. *Amblydromella caudiglans* was proven more difficult to rear in the laboratory than *G. occidentalis*, and attempts to use a similar system (whole bean plants infested with twospotted spider mite) were unsuccessful. Trials of several different leaf-disk and single-leaf arenas allowed us to determine the ideal rearing methods for this species for next year. As a result we will be able to evaluate the fecundity and longevity of this predator, as well as conduct trials to compare the pesticide tolerance of both species.

SIGNIFICANT FINDINGS

- 24% of identified mites were *Amblydromella caudiglans*
- *Amblydromella caudiglans* was the dominant species in 22% of samples
- Predatory mite species found in the survey include: *Amblydromella caudiglans, Amblyseius andersoni, Euseius finlandicus, Galendromus flumenis, Galendromus occidentalis, Neoseiulus fallacis, Typhldromina citri,* and *Typhlodromus pyri*
- *Galendromus occidentalis* spent significantly more time in the egg and larval stage than *A. caudiglans,* but *A. caudiglans* spent significantly more time in both nymphal stages, thus *G. occidentalis* has an overall shorter development time

- Survivorship for both species was similarly high at all life stages, except for the egg stage; fewer *G. occidentalis* eggs hatched
- The sex ratio for both species was similar (74% female *G. occidentalis*, 78% female *A. caudiglans*)

METHODS

1. Phytoseiid survey

A sample of 100 leaves per block was collected from apple orchards throughout eastern Washington. All phytoseiids found were removed from the leaves using a fine-tipped brush. Collected mites were slide-mounted using modified Berlese's solution and all adult females were identified to species. Identifications have been confirmed by Dr. James McMurtry (Professor Emeritus UC Riverside, Sunriver, OR).

Fig. 1. *Amblydromella caudiglans consuming a twospotted spider mite*

2. GIS analysis

ArcGIS software was used to map all sampling sites and determine land use in a 50 m radius around each sampling site. Growers and fieldmen are being interviewed to assess the history of pesticide use, and classify it as to intensity of organophosphate use. In addition to pesticide regime, the effects of climate, landscape, and available prey species on phytoseiid species composition, abundance, and diversity are being examined using regression. A survey was distributed to the pesticide consultants for each site sampled. The survey asks questions regarding grower practices at the sample site, including irrigation, dust control, pest levels, and pesticide application history.

3. Predator comparison

3a. Life history comparison. One female *G. occidentalis* or *A. caudiglans* were placed on a bean leaf disk provisioned with twospotted spider mites and apple pollen and held at 89 °F with a 16 h day length. These conditions were used to simulate a typical summer day in arid conditions. Observations of the females and their progeny were made at 12 h intervals until the individuals from the eggs reached maturity. The females were removed from the disks after they laid a single egg. The time to hatch, developmental period of each life stage, and mortality at each life stage were recorded for each egg individually. Fifty or more individuals were observed and recorded for each species.

To examine longevity and fecundity, leaf disk arenas identical to those previously described will be constructed. One female and two males (*A. caudiglans* or *G. occidentalis*) will be confined on each disk and provided with twospotted spider mite prey and apple pollen (25 replicates). Each leaf disk will be examined daily and data on longevity, egg production, and pre-oviposition, oviposition, and post-oviposition periods will be recorded.

3b. Predatory ability. To examine prey consumption, 50 leaf disk arenas will be created. The disks will be provisioned with 40 twospotted spider mite eggs. A single *A. caudiglans* or *G. occidentalis*

female will be placed on each leaf disk (25 replicates). The number of eggs consumed in a 72 h period will be recorded.

3c. Pesticide tolerance. A pesticide bioassay will be used to determine the tolerance of *A. caudiglans* relative to that of *G. occidentalis*. Bean leaf disks will be provisioned with twospotted spider mite eggs and apple pollen. A single female *G. occidentalis* or *A. caudiglans* will be added to each leaf disk. Altacor, Delegate, Imidacloprid, Guthion, Lorsban, or Sevin will be applied to the leaf disks using a Potter spray tower. Pesticides will be sprayed at the maximum label rate per acre. Each arena will be sprayed with 2 ml of the appropriate concentration; the checks will be sprayed with distilled water. Each species and rate will be replicated 25 times. The disks will be evaluated after 48 h, recording the number of live and dead phytoseiid females and the number of phytoseiid eggs. Any remaining phytoseiid females will be removed and the disks held to allow hatch of the phytoseiid eggs. After hatch is complete in the checks, the numbers of live phytoseiid larvae, and hatched and unhatched eggs will be recorded. The twospotted spider mite eggs and pollen will be left on the disk as a food source for the phytoseiid larvae.

RESULTS & DISCUSSION

Amblydromella caudiglans, *Amblyseius andersoni*, *Euseiulus finlandicus*, *Galendromus flumenis*, *Galendromus occidentalis*, *Neoseiulus fallacis*, *Typhlodromina citri*, and *Typhlodromus pyri* have been identified from the locations surveyed (Fig. 2). The majority of identified individuals were *G. occidentalis* (Fig. 2), but *A. caudiglans* was also present in significant numbers. Although *G. occidentalis* was the dominant predator in the majority of orchards, *A. caudiglans* was dominant at over 20% of the sites surveyed (Fig. 3). It was suggested by Downing and Moillet (1972) that a movement away from chemical control, especially organophosphates, could result in the replacement of *G. occidentalis* by *A. caudiglans*. *Galendromus occidentalis* has historically

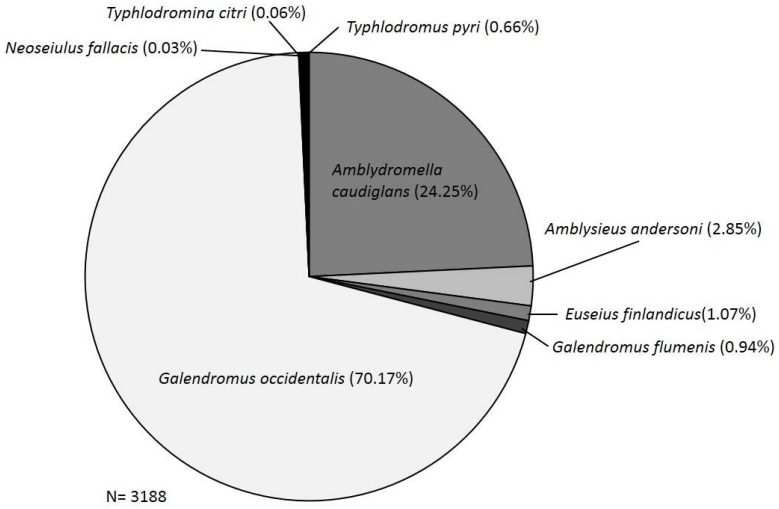

Fig. 2. Proportion of species found in all collected samples.

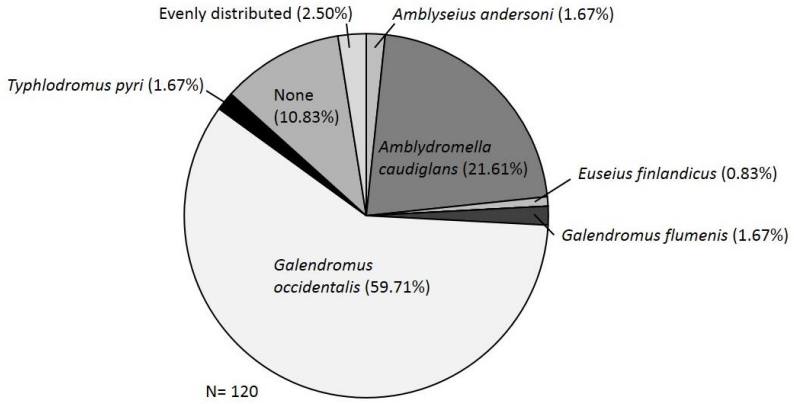

Fig. 3. Percent of samples where species were dominant.

been considered to be highly resistant to pesticides compared to other phytoseiid species (Downing & Moillet, 1972). Additionally, European red mite has replaced the McDaniel mite as the common outbreak pest mite species in Washington apple orchards (Beers & Hoyt, 1993). While spider mites that spin copious webbing, like the McDaniel mite, are the preferred prey of *G. occidentalis* (McMurtry et al., 1997). *A. caudiglans* has difficulty moving through webbing and prefers spider mites like *P. ulmi* that produce little webbing (McMurtry et al., 1997; Putman 1962). Therefore, the transition to a new predominant pest mite species may have promoted the increase in *A. caudiglans* populations.

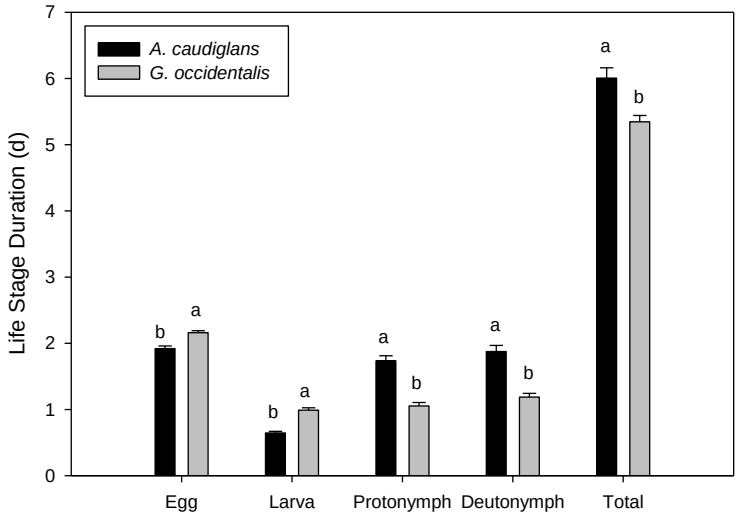

Fig 4. Life stage duration of predatory mite species.

To date, 59 surveys regarding grower practices have been completed. Preliminary analysis of the data collected from these surveys indicates that organic orchards tend to have fewer predatory mites than conventional orchards, likely due to their lower densities of pest mites. It is also of interest that most orchards where *A. caudiglans* was present in higher numbers were conventional orchards. Therefore, a more detailed statistical analysis of the types of pesticides used in these programs will be necessary before conclusions about what factors affect predatory mite species composition can be drawn. The determination of these factors will allow for improved performance of the predatory mite species in a given orchard; growers will know which practices to avoid in order to promote biological control.

Galendromus occidentalis has a shorter egg to adult development time than *A. caudiglans* at 89° F and 16 h day length (Fig. 4). Although *A. caudiglans* has shorter egg and larval stages, *G. occidentalis* has shorter nymphal stages. Unlike *G. occidentalis*, *A. caudiglans* larvae do not feed, thus rapid progression more quickly through its early life stages is advantageous. One implication of this life history trait is that *G. occidentalis* is capable of providing biological control earlier in its life cycle than *A. caudiglans.* This makes it less detrimental that it spends a greater amount of time as

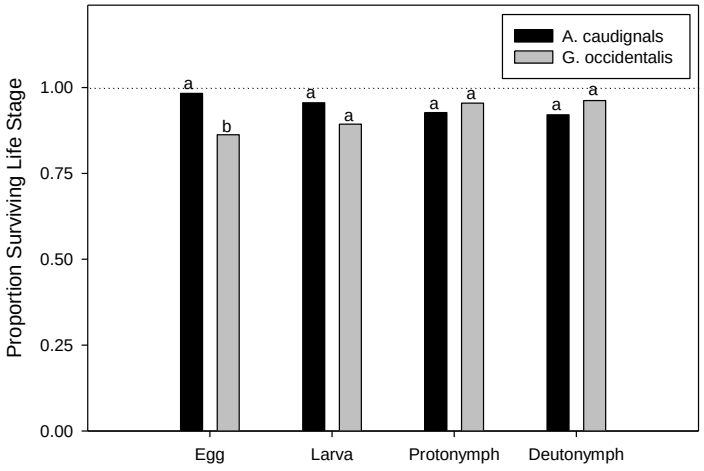

Fig. 5. Survivorship at each life stage for predatory mites.

a larva. Both species develop quickly, allowing for their success as predators of rapidly growing pest populations. Both predators also had similar proportions of individuals survive through each life stage, except as eggs (Fig. 5). At the specified conditions, *G. occidentalis* has lower percentage egg hatch. This could indicate that *A. caudiglans* eggs are more likely to remain viable in the warmer climates found in central Washington. Therefore, like *G. occidentalis*, this predator matures quickly, increasing its ability to control pest mite populations. Both species have similar sex ratios (74% female for *A. caudiglans*, 78% female for *G. occidentalis*).

Although data on *G. occidentalis* longevity, fecundity, pre-oviposition period, post-oviposition period, and prey consumption were collected (Table 1), the parallel data for *A. caudiglans* have not been collected due to difficulty establishing a colony of this species. However, we have found a successful rearing technique for *A. caudiglans* involving a whole bean leaf surrounded by a cotton barrier, and placed on water-saturated capillary matting. We will rear this predator from a wild population next spring. Collecting these data will allow for a better comparison of these two species as natural enemies of pest mites. Based on the prevalence of *A. caudiglans* in many orchards, including those with more intense pesticide programs, it may have the ability to provide biological control in orchards where *G. occidentalis* is lacking. Modifying integrated pest management to promote populations of this species has the potential to enhance mite control.

Table 1. Longevity, fecundity, oviposition periods, and prey consumption for *G. occidentalis* females of unknown age

Longevity (d)	Fecundity (total eggs/female)	Pre-oviposition period (d)	Post-oviposition period (d)	Prey consumption (Prey/female-day)
12.45	11.62	4.13	1.45	7.05

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-13-100A

Project Title: Chemical mediation of aggregation by brown marmorated stink bug

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Cooperators: Tracey Leskey, USDA, ARS, Kearneysville, WV
Helmuth Rogg and Todd Adams, Oregon State Dept. Agric., Salem, OR

Total Project Request: **Year 1:** $40,000 **Year 2: $40,000** **Year 3:** $40,000

Other funding sources: **None**

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

Item	2013	2014	2015
Wages	12,500	12,500	12,500
Benefits	1,500	1,500	1,500
Supplies	5,000	5,000	5,000
Travel	1,000	1,000	1,000
Total	$20,000	**$20,000**	$20,000

Footnotes: Personnel costs are for a ¼ time GS-5 technician to rear insects, conduct assays and field tests, and a summer student to assist with plant sampling and assays, and field tests. Supplies needed are GC gases, solvents, chemicals for EAD, assays and field tests, olfactometer glassware, and materials for traps for field testing of chemicals. Travel costs are for trips to multiple field sites in Oregon and Washington.

Budget 2
Organization Name:Univ. California, Riverside **Contract Administrator: Robert Chan**
Telephone: (951) 827-7986 **Email address: rchan@ucr.edu**

Item	2013	2014	2015
Wages	12,035	12,276	12,522
Benefits	4,828	4,915	5,013
Supplies	3,137	2,809	2,465
Total	$20,000	**$20,000**	$20,000

Footnotes: Personnel costs are for a 30% time organic chemist postdoctoral scientist. It will be essential to have a highly trained organic chemist for rapid identification and synthesis of possible attractants, to provide test materials as rapidly as possible and avoid holding up the biological/ecological parts of the project. Salary and benefit rates are mandated by the state of California, and include a 2% projected increase each year. We also request funds in each year for chemistry supplies, to include solvents, columns, reagents, disposables, and equipment maintenance costs.

OBJECTIVES

The overall objective or goal of the project is to discover and develop chemical attractants and attractant synergists for brown marmorated stink bug (BMSB) based on their host-and mate-location behavior. The experimental objectives are to:

1. Determine sex attraction responses of female BMSB, including physiological and environmental regulators of that behavior.
2. Determine host plant preferences, and female and male BMSB attraction to host plant odor.
3. Determine host plant effects on BMSB sexual pheromone behavior.
4. Isolate and identify plant kairomones that mediate or enhance BMSB attraction behavior.
5. Determine both signal and response interactions between male BMSB pheromones and host plant kairomones, to develop superior attractants.

SIGNIFICANT FINDINGS

1. A reproducing colony of BMSB was established in quarantine.
2. Arena and olfactometer systems were built and assay protocols developed for studies of BMSB responses to conspecific and host plant odors.
3. An EAD system was modified for the BMSB antenna and its effectiveness was demonstrated using BMSB antennae and sex pheromone chemicals.

METHODS (for 2014)

Sex attraction behavior. Laboratory experiments (using arena assays) to determine the importance of male age, female age, mating status, and time of day on sex attraction will be finalized. We expect those assays to be concluded in May 2014. This information will then be used to design and test a flight tunnel assay of sex attraction that can be used to evaluate sex pheromones and their interactions with host plants.

An aggregation assay will be used to look for BMSB responses to aggregating bugs, and to test the hypothesis that BMSB in aggregations produce a pheromone that recruits BMSB to aggregation sites. Small cardboard versions of field aggregation traps (called slit traps) that are used in the field are built and are ready for use in screened cages in the laboratory assays. A preliminary assessment indicated that they are suitable for aggregating BMSB in the laboratory. Experiments will determine if 1) used traps have improved bug recruitment, indicating deposition of an aggregation pheromone, and 2) if a trap with bugs is preferred over a trap without bugs, or bugs without a trap. Evidence of bug attraction to such traps will be followed with procedures to isolate the pheromone from the trap or from the airstream.

Host plant preferences. The assays that are underway to determine species of plants and which plant parts that are attractive to BMSB, and when the bugs are responsive to plant odor (time of day, age), will be concluded. To date, these assays have been conducted using a Y-tube olfactometer. In the coming months, we will look at BMSB responses to plants in a cage "arena" type assay, where bugs are released in the middle of a 0.7 x 0.7 x 0.7 m cage in which four plant bouquets are placed. This assay design will be used to determine bug choices when provided multiple plants, where the olfactometer assays underway are looking at single plant choices.

Field assessments will continue to be made to look for plants with numbers of BMSB, to determine patterns in the field that suggest host plant preferences. This information has and will generate hypotheses on which plants to evaluate in the laboratory, and considering plant phenology.

Interaction of sex attraction and host plants. Both Y-tube olfactometers and large arena assays will be used to look for preferences of host plant seeking BMSB for plants with stink bugs on them, and similarly for preferences of mate-seeking BMSB for stink bugs with plants. This work will

commence when the preliminary work with plants is concluded, so that the best plants for the experiment are predetermined.

Pheromone Chemistry. We will conduct preliminary characterizations of BMSB volatile chemical production to gain background information that we will need when determining the chemistry of aggregation pheromone, or sex pheromone interaction with host plant chemicals. These characterizations will at the least involve: 1) characterization of the defensive secretions produced by bugs when disturbed, 2) Comparison of immature and mature female volatile chemicals, and 3) Comparison of the odor chemistry of male and female overwintering BMSB. Isolation and identification of pheromones and kairomones will involve a combination of behavioral assays, volatile collection techniques, GC-EAD (see below) and GC-MS to determine chemical structures.

RESULTS AND DISCUSSION

Colony establishment. Adult BMSB were first housed in the quarantine facility at the ARS Wapato laboratory in late May 2013. The methods of Nielsen et al. (2008) and Medal et al. (2012) were used to maintain bugs and obtain eggs with which to expand and continue the colony. We confirmed that it takes about 50 days to get new adults from hatched eggs (we obtained colony adults first in early August 2013) and about one week for these adults to become reproductively mature (mating and egg laying). The colony has since been maintained and expanded under controlled laboratory conditions that mirror early summer, although batches of adults are also held at conditions that mirror late summer and early autumn.

The establishment and maintenance of the colony is necessary for the completion of any of the laboratory studies of bug behavior and chemistry, and so was a necessary step and investment.

Behavioral assays. A Y-tube olfactometer system that was used for earlier studies with codling moth larvae (Landolt et al. 2000), pear psylla (Guedot et al. 2009) and paper wasps (MacKenzie et al. 2009), was assembled for use in evaluating BMSB responses to plant odor . BMSB adults produced in the colony in August were sufficient to begin these laboratory assays of bug responses to different plants. These assays were continued through September until interrupted first by the government wide shutdown and then freezing temperatures that ended field collection of plant material. Roughly 50% of the field collected plants (6) on our "to do list" were evaluated during this time. We have since shifted the work to greenhouse grown plants which will continue until late spring.

It is too early to make conclusions regarding bug orientation responses to plants and preferences for plants and plant parts. When the first series of experiments is concluded using the Y-tube olfactometer, we will conduct the choice assays using the arena type design, so that we can more clearly see preferences on the part of the stink bugs. And clearly preferred plants or plant parts will be used in the experiments to look for interactions between mate-finding and host-finding behavior.

Coupled Gas Chromatographic-Electroantennographic Detection (GC-EAD) Analysis. Coupled GC-EAD analysis was performed using an Agilent 6890N gas chromatograph equipped with a DB-5 capillary column (30 m×0.25 mm ID, 0.25 μm film thickness; Agilent Technologies, Wilmington, DE, USA) in the splitless mode with 1 min sampling. The oven temperature was programmed for 5 min at 40 °C, 15 °C/min increase to 250 °C, and then held for 5 min. Injector temperature was set at 250 °C. Helium gas was the carrier at a constant flow rate of 2 ml/min. The column effluent was split 1:1 in the oven via an outlet splitter system (OSS-2, SGE Analytical Science, Austin, TX,USA) with nitrogen as a make-up gas (15ml/min). One arm of the splitter led to the flame ionization detector (FID) (260 °C) and the other to the heated EAD port (260 °C) (Syntech, www.syntech.nl) introduced

into a humidified air stream (300 ml/min) directed toward the mounted antennae of the brown marmorated stink bug.

One of the two antennae was separated from the head and it was positioned between two gold wire electrodes immersed in saline-filled (46mmol NaCl, 182mmol KCl, 3 mmol CaCl2, and 10mmol TrisHCl at pH 7.2) micropipettes in an acrylic holder. The output signal from the antenna was amplified (10×) by a customized high input impedance DC amplifier and converted to a digital signal (IDAC-232, Syntech) and recorded on a computer using a dedicated software (GC-EAD, Syntech). A total of ten antenna set-ups were prepared and each antennae preparation was tested on SPME headspace adsorption of a commercial stink bug lure (Sterling).

Consistent and significant antennal responses were achieved for 5 different female pheromone chemicals, using male BMSB antennae. This development is important because there are no good precedents in the literature for the methods or even the ability to obtain electroantennal responses to semiochemicals from stink bugs. This accomplishment provides a powerful tool for us to isolate other semiochemicals such as plant kairomones or pheromones involved in BMSB aggregation behavior. This technique for example was critical to our rapid identification of a feeding attractant lure for spotted wing drosophila, using volatile chemicals from a wine/vinegar bait (Cha et al. 2012).

Field sampling of stink bugs. About 100 separate field collections were made to assess the species makeup of stink bugs, to detect the presence and spread of BMSB, and to determine potential preferred host plants. These collections in Washington yielded nearly 1000 stink bugs, all which were identified to species. Very few were brown marmorated stink bugs; single specimens were found in Chelan and Benton Counties.

REFERENCES CITED

Cha, D. H., T. Adams, P. J. Landolt, and H. Rogg. 2012. Identification and field evaluation of wine and vinegar volatiles that mediate attraction of spotted wing drosophila, *Drosophila suzukii*. J. Chem. Ecol. 38: 1419-1431.

Guedot, C., D. R. Horton, and P. J. Landolt. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. Entomologia Experimentalis et Applicata 139: 191-197.

Landolt, P. J., J. Brumley, C. Smithhisler, L. Biddick, and R. W. Hofstetter. 2000. Apple fruit infested with codling moth are more attractive to neonate codling moth larvae and possess increased amounts of E,E-alpha farnesene. Journal of Chemical Ecology 26: 1685-1699.

MacKenzie, J. K., P. J.Landolt, and R. S. Zack. 2009. Attraction to ornamental peony, *Paonia*, Paoniceae) by *Polistes dominulus* Christ (Hymenoptera: Vespidae) demonstrated using olfactometers. J. Kansas Entomol. Soc. 79:231-238.

Medal, J., T. Smith, A. Fox, A. S. Cruz, A. Poplin and A. Hodges. 2012. Rearing the brown marmorated stink bug *Halyomorpha halys* (Heteroptera: Pentatomidae). 95: 800-802.

Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008. Develomental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). 37: 348-355.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-13-102A

Project Title: Codling moth attract-and-kill with kairomonal lures

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Cooperators: Max Suckling, Plant and Food Research, P. O. Box 51, Lincoln 7608, New Zealand and Jim Walker, Plant and Food Research, Cnr Crosses and St. Georges Roads, Havelock North, Hawkes Bay, New Zealand 4130

Total Project Request: **Year 1:** $40,000 **Year 2: $40,000** **Year 3:** $40,000

Other funding sources: **None**

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

Item	2013	2014	2015
Wages	13,000	13,000	13,000
Benefits	3,000	3,000	3,000
Supplies	5,000	4,000	4,000
Travel	1,000	1,000	1,000
Total	$22,000	**$21,000**	$21,000

Footnotes: Supplies needed are the materials to construct the A & K stations, vials, sachets, and chemicals for the kairomone lures, and for additional and replacement BL traps and batteries. Travel costs are for trips to multiple field sites.

Budget 2
Organization Name: Agriculture & Agri-Food Canada **Contract Administrator:** Kenna MacKenzie
Telephone: (250) 494-6358 **Email address:** kenna.mackenzie@agr.gc.ca

Item	2013	2014	2015
Salaries			
Benefits			
Wages	15,000		
Supplies	3,000		
Travel			
Total	$18,000 (0)	0	0

This work was not conducted and the budget not invoiced due to problems with timely scheduling, recruitment, and competing obligations. Judd has elected to continue to assist the project, but without requesting WTFRC funding.

Budget 3
Organization Name: Plant and Food Research New Zealand
Contract Administrator: Claire Hall
Telephone: 64 3 977 7340 **Email address:** claire.hall@plantandfood.co.nz

Item	2013	2014	2015
Wages		$16,000	$16,000
Supplies		$3,000	$3,000
Total	0	**$19,000**	$19,000

Footnotes: Peter Lo, of Plant and Food Research New Zealand, will conduct work originally designated for Judd in years two and three.

OBJECTIVES

The overall objective or goal of the project is to develop and demonstrate control of codling moths in orchard plots using attract and kill stations baited with a recently developed kairomone lure. The technical objectives of the work are to:

6. Determine a best A & K station density (stations per acre) to use.
7. Determine interactions between deployment of stations baited with kairomone and stations baited with pheromone lures.
8. Determine the interactions of mating disruption and A & K stations baited with kairomone lures.
9. Determine efficacy of attract and kill stations for reducing oviposition and for prevention of infestation of fruit in orchard blocks early season (early July) as well as at harvest.

SIGNIFICANT FINDINGS (for 2013 field season).

4. We developed a sachet system to replace the vials and septum, but not yet with all chemicals in the same sachet.
   a. Acetic acid and N-butyl sulfide can be dispensed from sachets to provide the same release rates as vials.
   b. Acetic acid, N-butyl sulfide and pear ester mixed in a sachet failed in field tests.
5. Prototype lures that put acetic acid and N-butyl sulfide in sachets did not provide sufficient removal of females in plots with 50 stations per acre due to failures with the sachets. We think that this is now corrected.
6. We did not find any efficacy problems with the adhesives used in Alpha Scents or Trece trap liners or with Tanglefoot. However, there was a problem with Tanglefoot spray.
7. New Zealand field tests confirmed synergy among acetic acid, pear ester, and N-butyl sulfide for male and female codling moths.

METHODS (for 2014 field season).

1. Kairomone dispenser development. We continue laboratory work to develop sachets that will release the three chemicals at rates similar to that produced in vials. This involves measuring chemical release from sachets made of a range of materials and thicknesses and dimensions. We will conduct field experiments to test the attractiveness of two-sachet and three-sachet systems in comparison to prior published lures. These experiments build on work conducted in 2013. The experiments will be conducted in New Zealand in January and February and in Washington in May/Hune. These results should give us a clear set of specifications for a dispenser to use in a return to use in attract and kill field plots in Washington for the 2014 field season.
   a. Laboratory studies will determine release rates of acetic acid and N-butyl sulfide from single chemical dispensers as weight loss over time, measured as changes in weight. Release rates of pear ester from dispensers will be determined by trapping the chemical from an airstream passed over the dispenser, extracting the pear ester from the trap with a solvent, and then quantitative analysis by gas chromatography. Release of chemicals from mixtures in dispensers will be analyzed with the volatile collection method
   b. Determination of the attractiveness of kairomones in dispensers will be done in the field, using the Delta trap, and in comparison to the chemicals released from vials as a positive control.
2. Attract-and-kill station applications. A series of field plot tests will evaluate the impact of the attract-and-kill stations on codling moth in apple orchards.
   a. Five replicates of one acre plots treated with attract and kill stations will be set up and evaluated by the first of June, with treated plots receiving 50 stations per acre along with 2 pheromone, 2 kairomone, and 1 blacklight monitoring traps. Control plots will receive

pheromone, kairomone, and blacklight monitoring traps, but no stations. Traps will be monitored for three days before and 7 days after the deployment of stations. Treatment and control plots will be separated by a buffer equivalent to another one acre plot, and treatment and control plots will be paired within orchards to ensure the same tree variety, age, and planting pattern within treatment pairs.

b. The same experimental design will be used to evaluate the impact of a combination of 50 kairomone and 50 sex pheromone attract-and-kill stations. Monitoring traps will remain the same as in the preceding test. Again, plots will be monitored for 3 days preceding and 7 days following the deployment of stations. This experiment will be conducted at the same time as the preceding, or order to make direct comparisons of the experimental results.
c. A third experiment will be conducted, in July and August, to compare the impact of station density. The station densities tested will depend in part on the results of the tests in June. If good impact is seen at 50 stations per acre (or 100 with kairomone plus pheromone stations), then the comparison will include reduced numbers of stations. If a greater impact is needed, then an increased range of stations densities will be tested.

RESULTS AND DISCUSSION

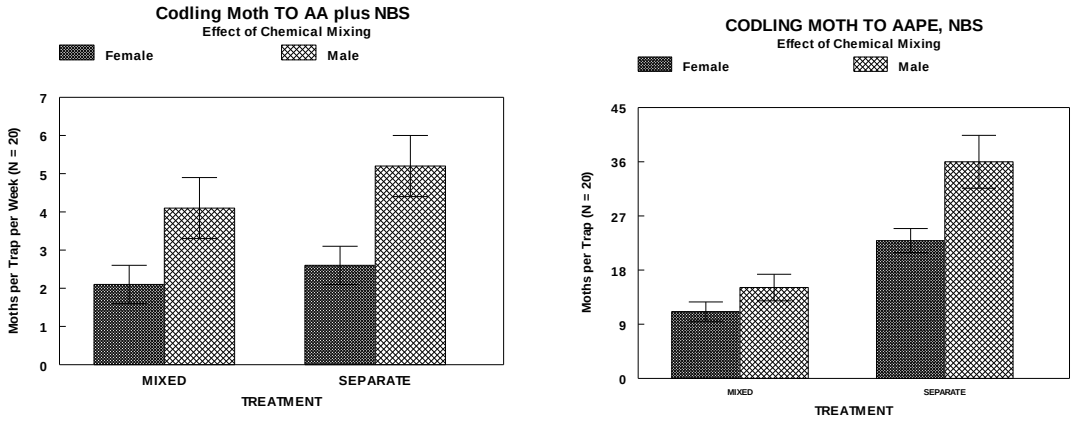
Our prior work showed the superior attractiveness of the combination of acetic acid, pear ester and N-butyl sulfide (Landolt et al. in press), and the advantages of using an adhesive-coated surface in place of a pesticide-treated surface as a station target. A critical aspect of the design and development of the attract-and-kill station that remained was the development of a cheaper means of formulating or dispensing the chemical attractant. As the scope of the work is scaled up, much larger numbers of lures are needed which then gets costly. We have been using a polypropylene vial dispenser which can cost over a dollar per lure, and we wish to switch to a polyethylene sachet type dispenser. The latter costs pennies in materials and can easily be manufactured in the laboratory using plastic sheeting (such as trash bags) and a food grade heat sealer. Polyethylene is often used for this purpose and much of our work has found various thicknesses of polyethylene sheeting to be good for many types of kairomone attractants.

Release rate measurements. Numerous laboratory experiments determined patterns of release of acetic acid, pear ester, and N-butyl sulfide from sachets, and how that varied with the thickness of the polyethylene, the dimensions of the sachet, and the load of the chemical. We found that a rectangular sachet that is 2 x 6 cm and made of 4 mil thick polyethylene, releases the same amount of acetic acid as an 8 ml vial with a 3 mm hole (our prior standard lure, as in Landolt et al. 2007, and Landolt et al. in press). We found that N-butyl sulfide diffusion across polyethylene is much faster, making it difficult to make a long lasting lure. So we are conducting laboratory evaluations at this time of the use of an"odor proof" plastic membrane for part of the sachet design. We do not yet have measurements of pear ester released from sachets, and will conduct that work during the spring of 2014.

Testing of lure component mixtures. Our original demonstrations of positive interaction or synergy among acetic acid, pear ester (Landolt et al 2007), and then with N-butyl sulfide (Landolt et al. in press) as lures for CM traps, were accomplished with each chemical released from a separate dispenser. A subsequent goal was to be able to mix attractant chemicals within a dispenser, to cut down on cost and complexity. So, subsequent field work with the pear ester showed that acetic acid and pear ester could be mixed and released from the same vial type dispenser while keeping the same attractiveness to codling moth. In May 2013, we showed similarly that acetic acid and N-butyl sulfide can be mixed and dispensed from the same vial (Figure 1). A first attempt at combining acetic acid, N-butyl sulfide and pear ester in a vial however, resulted in a loss of lure attractiveness (Figure 2).

We also evaluated the use of the sachet in place of the vial in the field. Although these results are somewhat preliminary, a problem occurred with the switch from vials to sachets. We showed first that acetic acid and N-butyl sulfide can indeed be used in traps in sachets rather than vials, without losing attractiveness (Figure 3). In an expanded comparison of dispenser types for the acetic acid, pear ester, N-butyl sulfide lure (Figure 4), best results were obtained with acetic acid and NBS dispensed from separate vials and pear ester in a rubber septum. When acetic acid and NBS were dispensed from polyethylene and polyester sachets respectively and pear ester from septa, trap results were good for the Delta trap and not the tube trap (Figure 3).

Comparison of adhesives. The purpose of this test was not to provide an exhaustive assessment of adhesives, but rather to confirm that there is no problem with the trap liners that we are and will be using for attract-and-kill; the Alpha Scents Delta trap liner. Our results indicated no difference among the two commercial trap liners and our application of Tanglefoot. However, our application of a Tanglefoot spray resulted in a significant reduction of captures of codling moth in the Delta trap (Figure 6).



Figures 1 and 2. Mean numbers of codling moths captured in Delta traps baited with vial dispensers loaded with acetic acid (AA), N-butyl sulfide (NBS), and pear ester (PE). Chemicals were either mixed together and placed in one vial (Mixed) or were presented in traps in separate dispensers (Separate). One test evaluated the effect of mixing AA and NBS (on left), while a second test evaluated the effect of mixing AA, NBS, and PE (on right).

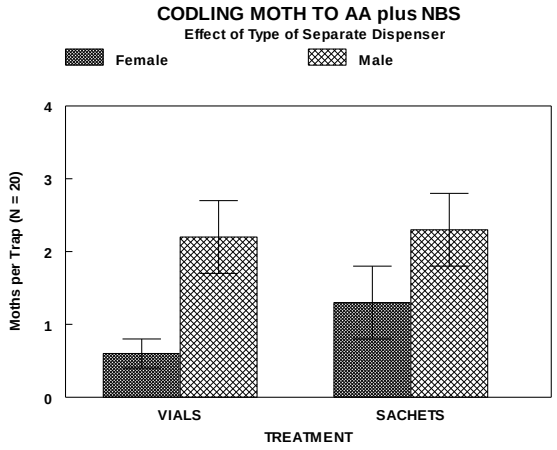


Figure 3. Codling moths captured in Delta traps baited with acetic acid (AA) and N-butyl sulfide dispensed from two vials or from two sachets.

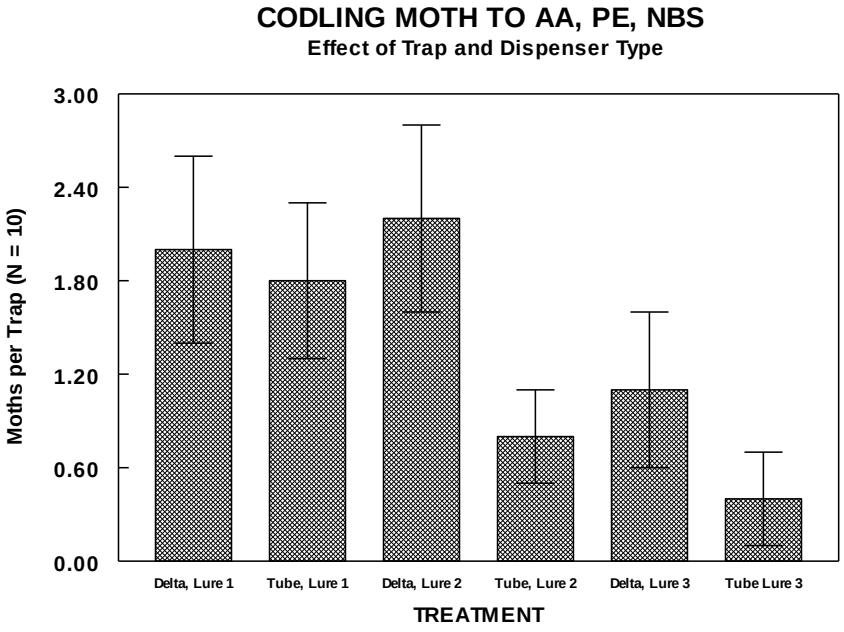


Figure 4. Codling moths captured in Delta and Tube traps baited with the combination of acetic acid (AA), pear ester (PE) and N-butyl sulfide (NBS). Lure 1 had AA and NBS in vials, and PE in a septum. Lure 2 had AA in a polyethylene sachet, NBS in a polyester sachet, and PE in a septum. Lure 3 had AA and NBS in polyester sachets and PE in a septum.

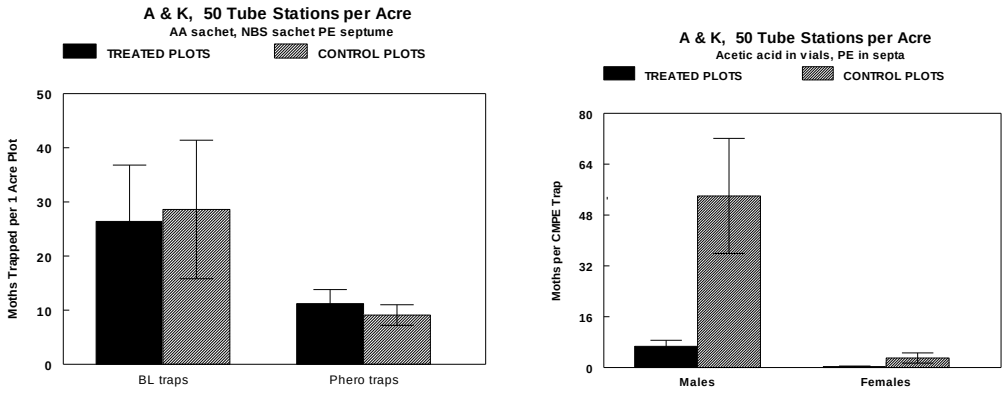


Figure 5. Mean numbers of codling moths captured in monitoring traps in attract-and kill plots. On the left, numbers of moths captured in traps were not reduced with A & K using the sachet system to dispense acetic acid and N-butyl sulfide. On the right, numbers of moths were greatly reduced in monitoring traps in plots with A & K stations baited with acetic acid plus pear ester lures, using vials and septa.

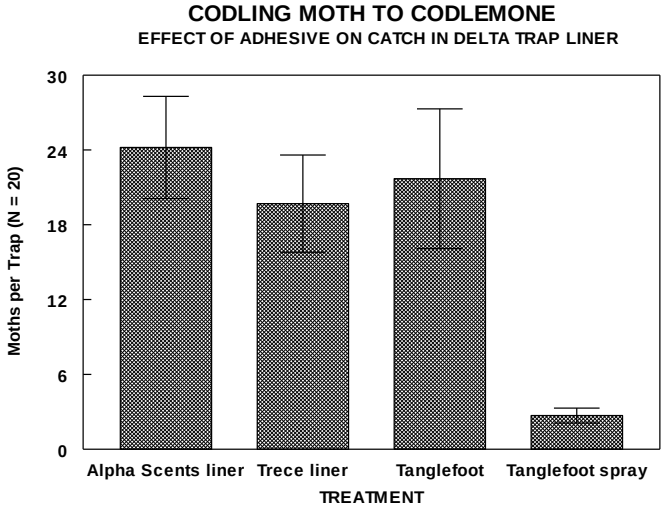


Figure 6. Mean numbers of codling moths captured in traps with liners made with different adhesives.

We made progress towards developing and demonstrating an inexpensive dispenser system for the kairomone lure, but have more work to do to formulate N-butyl sulfide and determine how pear ester can be mixed with acetic acid in a sachet. It is important to be certain that a new sachet system is as attractive to female CM as the old vial system. Field testing is being set up at this time in New Zealand to use their second CM flight to determine if newly modified sachets for acetic acid and N-butyl sulfide provide the same lure power as these two chemicals in vials. Using the sachets (Figure 4) in place of vials is a tremendous cost savings, which is necessary for the scaling up of field studies. In early 2013, a prototype sachet system was evaluated in attract and kill stations in field plots before adequate field testing of lure efficacy and that test was unsuccessful. Subsequent tests showed that attract and kill test of 2013 was conducted with a lure that greatly reduced in effectiveness. That is the lure 3 of Figure 3 in the tube type trap.

Field work was not conducted in Summerland, British Columbia, and that part of the budget was not accessed and is returned. This did result in a slowdown in progress made in assessing the kairomone

lures in the field. We are working at this time to expand the collaboration with researchers in New Zealand to compensate.

REFERENCES CITED

Landolt, P. J., D. M. Suckling, and G. Judd. 2007. Synergism of a feeding attractant and a host kairomone for the codling moth (Lepidoptera: Tortricidae). Journal of Chemical Ecology. 33: 2236-2244

Landolt, P. J., T. S. Davis, B. Oehler, D. Cha, and J. Brunner. (In press). N-butyl sulfide as a co-attractant with kairomones for male and female codling moths, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Environ. Entomol. (Accepted November 2013).

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-12-101

Project Title: Olfactory proteins as targets for enhanced codling moth control

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Cooperators: Pete Landolt, Tom Unruh, Alan Knight (USDA, Wapato WA), Jocelyn Millar (University of California, Riverside), Walter Leal (University of California, Davis)

Total Project Request: **Year 1**: $ 44,250 **Year 2**: $40,047 **Year 3**: **$ 40,837**

Other funding sources

None

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

Item	2012	2013	2014
Salaries	$24,958	$25,714	$26,470
Benefits	$ 4,292	$ 4,333	$ 4,367
Wages			
Benefits			
Equipment			
Supplies	$10,000	$10,000	$10,000
Travel			
Plot Fees			
Miscellaneous	$ 5,000		
Total	$44,250	$40,047	**$40,837**

Footnotes: [1]**For part of a GS-6 Technician**
[2]**First year miscellaneous request is for antibody production**

OBJECTIVES

1) Express and characterize proteins involved in codlemone detection. This will include odorant binding proteins, nerve membrane receptors, and odorant degrading enzymes. In my previous project, CP-09-903 – "Identification of critical physiological targets in codling moth", gene transcripts encoding several important protein families involved in odorant detection were identified. These protein families include odorant binding proteins (OBPs), sensory neuron membrane proteins (SNMPs), odorant receptors (ORs) and odorant degrading enzymes (ODEs). Based on homology to previously characterized proteins from other lepidopteran pests, we have six candidate OBPs, three ORs, two SNMPs and four ODEs that may be involved in codlemone signaling. As a first step, we need to clone the gene transcripts encoding these candidate proteins to verify their presence in codling moth antennae, as well as to use in protein expression systems so that we can use them in Objective 2.

2) Determine which odorant binding proteins, nerve membrane receptors, and odorant degrading enzymes are involved in the codlemone signaling pathway using in vitro protein expression and binding assays. Proteins (OBPs, SNMPs, ORs, and ODEs) generated in Objective 1 will be expressed and purified, and then used in assays to determine those which interact with codlemone. Procedures for monitoring the interactions of codlemone with these proteins are available in the literature, and routinely used by my collaborators at other institutions. Once we determine which proteins interact with codlemone, they will be used to generate antibodies that will be used to detect these proteins in codling moth antennae.

3) Determine where codlemone reactive proteins are expressed in antennae using fluorescent in situ hybridization and immunofluorescent detection methods. Gene transcript expression does not necessarily mean that a protein is produced. The purpose of this objective is to monitor mRNA production and then determine how much of that transcript is being converted into protein. One hypothesis is that OBPs are translated in response to codlemone exposure, and act as determinants of sex pheromone concentration while regulating the amount of pheromone that makes it to the nerve surface to activate codlemone ORs. We will monitor the amounts of gene transcript using quantitative PCR and we will determine protein amounts using antibodies that bind to the corresponding OBP that are present in the cell. We will also use these same techniques to determine if transcript and protein amounts change in response to codlemone exposure.

4) Determine if codlemone signaling can be disrupted using various odorant degrading enzyme inhibitors and parapheromones in flight tunnel studies. To determine the importance of the various classes of proteins involved in pheromone reception, flight tunnel studies will be used to assess protein functions. Many inhibitors are commercially available for ODEs, and other ODE inhibitors will be obtained from other laboratories. Parapheromones, compounds structurally related to natural pheromone components, are semiochemicals which have a large variety of effects on a target organism, and have been called agonists, pheromone mimics, synergists and hyperagonists, or pheromone antagonists, antipheromones and inhibitors. Through a collaborative research project studying navel orangeworm semiochemicals, it was discovered that a pheromone derivative binds more strongly to the sex pheromone OR and is a more potent agonist. We will produce a codlemone derivative using the structural features of parapheromones designed against the navel orangeworm to determine if this modified semiochemical is more attractive to codling moth males.

SPECIFIC OBJECTIVES FOR YEAR 3

1) Determine codlemone interaction with OBPs and ODEs
2) Flight tunnel behavioral studies with codlemone based parapheromone and ODE inhibitors
3) Studies to determine if parapheromone disrupts mating
4) Improve cell-based assays to identify odorant receptors that bind codlemone
5) Quantitative PCR studies to determine relative abundance of proteins that interact with codlemone

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

- Additional olfactory proteins were identified from a transcriptome
  - 54 transcripts encoding putative odorant receptors
  - 48 transcripts encoding putative odorant binding proteins
  - 22 transcripts encoding putative odorant degrading enzymes
  - 25 chemosensory binding proteins
- Cloned gene transcripts encoding two additional candidate odorant degrading enzymes
- Cloned one additional candidate pheromone family receptors
- Identified one pheromone receptor that responds to codlemone in a cell-based assay

METHODS (PROJECT APPROACH)

The overall goal of this project is to provide a more thorough characterization of the codling moth olfactory system, especially as it relates to the detection of pheromones. Through prior Commission support, we have identified proteins that by homology are thought to play critical roles in codling moth detection of codlemone. In this project, we propose to determine which proteins are important for codlemone signaling. To achieve our goal, the following approach and methods will be used.

1) Cloning and expression of proteins involved in codlemone signaling. The critical proteins in pheromone detection and signaling are odorant binding proteins (OBPs), sensory neuron membrane proteins (SNMPs), odorant receptors (ORs), and odorant degrading enzymes (ODEs). For the codling moth, orthologs of these proteins were identified through analysis of transcriptomes generated from antennae, the site of codlemone detection. Specific transcript sequences were determined, and we will use these nucleotide sequences to design gene specific primers to clone full-length mRNA molecules. We will express these proteins in bacterial cells and purify them using standard chromatography techniques.

2) Codlemone interaction assays. Once proteins are expressed, we will use assays to determine their interaction with codlemone. For OBPs, codlemone is incubated with individual proteins at a neutral pH. The proteins are then precipitated and resuspended in an acidic buffer which releases codlemone into solution. The solution will be analyzed by gas chromatography (GC) to detect the bound codlemone. Similarly, codlemone will be incubated with ODEs and after the incubation the codlemone will be extracted and analyzed by GC to detect degraded pheromone products. We have already identified an OR which binds codlemone, but will confirm this using microplate assays developed in my laboratory.

3) Determination of gene transcript expression and protein detection. Quantitative PCR (qPCR) will be used to determine the relative amount of gene transcripts expressed in antennae. Sequence specific primers will be designed for each gene transcript of interest, and the amount of transcript will be determined for each transcript using qPCR detection. To detect proteins, specific antibodies will first be generated for each of the proteins of interest (OBP, OR, SNMP or ODE) and obtained through a commercial production facility. The antibodies will be used in a protein blot procedure where antennal proteins will be separated on an acrylamide gel and transferred to a nitrocellulose filter. The filter containing the separated proteins will be incubated with the antibodies corresponding to the proteins of interest. The bound antibodies will be detected using immunofluorescent detection methods. This "Western" Blotting procedure has been performed routinely in my laboratory. Protein fluorescence will be detected and quantified using computer software supplied with our fluorescent gel reader.

4) Flight tunnel studies using ODE inhibitors and parapheromones. A variety of chemical inhibitors of ODEs are commercially available. We will treat codling moth with these inhibitors to disrupt ODE function, and then use flight tunnel studies to determine the effects on codling moth attraction to codlemone. We will score moth response to codlemone with and without inhibitor treatments and positive effects will be either disruption of codling moth attraction or enhanced responses to the pheromone source. In a recent collaboration, a chemical modification of the navel orangeworm sex pheromone made this new semiochemical more potent than the original pheromone. We will have a modified codlemone parapheromone synthesized to determine if this modification will produce a more potent attractant. To test the potency, flight tunnel studies will be used as above. If this parapheromone is attractive to codling moth, we will proceed with a limited field trial to determine if it is more effective than codlemone for attracting males.

RESULTS AND DISCUSSION

The overall goal of this project is to identify and characterize proteins expressed in codling moth antenna that play critical roles in codlemone signaling and behavioral response. Our studies are focused on three classes of proteins (odorant binding proteins, odorant receptors and odorant degrading enzymes) that in other moths are thought to play roles in the regulation of sex pheromone signaling.

Identification of olfactory proteins expressed in codling moth

When this proposal was originally submitted we had identified 6 candidate OBPs, 3 ORs, 2 SNMPs and 4 ODEs that may be involved in codlemone signaling. Through data mining of our most current transcriptome, we have added several more candidates including 5 OBPs, 2 ORs and 22 ODEs. In addition, we have identified 25 chemosensory proteins, some of which may interact with codlemone. Chemosensory proteins are structurally related to OBPs, and in other moths have been reported to interact with sex pheromones. Data mining of the transcriptome has also provided an almost complete set of olfactory proteins expressed by codling moth. We now have sequence information for 54 ORs and 48 OBPs. This information can be used in the future to gain a more thorough understanding of the codling moth olfactory system. Furthermore, cell based assays can be used to identify ORs which interact with future odorants of interest. Such work could include determining how larvae find apples or how females find suitable sites for laying eggs.

Identification of an OR that interacts with codlemone

We have determined the relative expression for candidate codlemone receptors (Figure 1), and one receptor, CpOR11 (labeled C11 in figure), was chosen for use in cell-based assays. My original plan was to express this receptor in a cell line along with the required co-receptor (OR2 in figure) and then monitor cell response when codlemone is added. Unfortunately, the microplate detector system in my lab was not able to monitor this cell response. To overcome this problem, we engineered the cell lines to express a protein which forms a product that we could monitor for, namely cyclicAMP (cAMP). When codlemone was added to the cell line expressing CpOR11, we were able to detect an increase in cAMP accumulation (Figure 2), indicating that this receptor is responding to the addition of codlemone. Limitations of this cell based assay system are expense, difficulty to perform and its lack of sensitivity. In September 2013, we acquired a new microplate reader capable of monitoring cell response with reduced expense and higher sensitivity. We are currently optimizing the cell based assays on our new microplate reader. In addition, we are generating a new cell line which when completed will allow for more rapid and inexpensive screening of ORs.

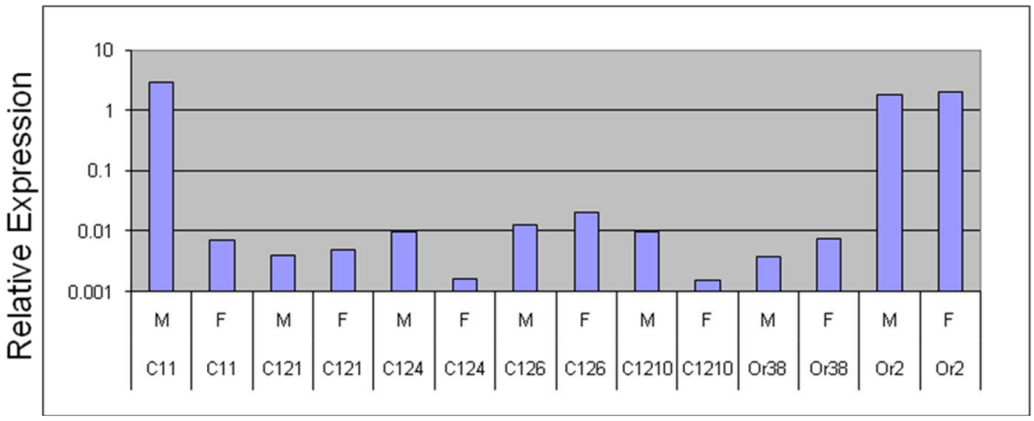

Figure 1. Codling moth odorant receptor gene expression levels in female and male antennae determined by quantitative real-time PCR. Expression levels relative to the control gene CpRPS3 and reported on a log10 scale. Relative expression levels in male and female antennae were determined for seven ORs. ORs C11, C121, C124 and C1210 are members of the pheromone receptor family, OR2 is a co-receptor and forms heterodimers with other ORs and is required for function, and OR38 is a non-pheromone receptor used as a control.

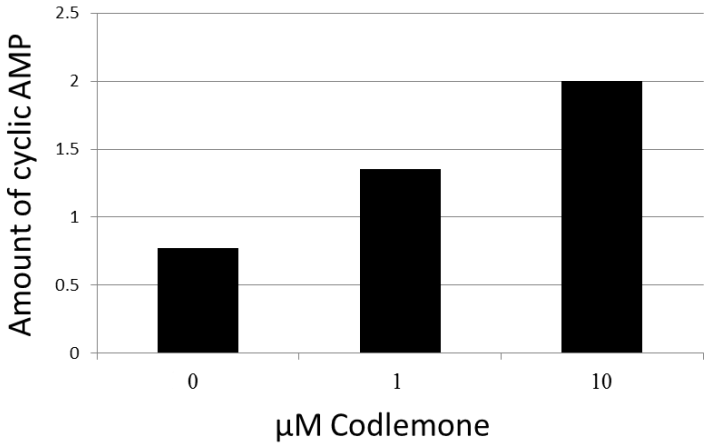

Figure 2. Response of CHO cells expressing CpOR11 to various concentrations of codlemone. CHO cells expressing CpOR11, CpOR2 and a G protein which converts calcium signaling to cAMP were seeded into 96 well microplates and the cells were cultured overnight. To the cultured cells, control buffer (to determine basal levels of cAMP) or buffer containing 1 or 10 µM codlemone was added, and the cells were incubated for 4 hrs. After the incubation, cells were assayed to determine the amount of accumulated cAMP.

Flight tunnel and disruption of mating studies

Two of our goals for this year were to perform flight tunnel behavioral studies with codlemone based parapheromone and ODE inhibitors, and to determine if parapheromone disrupts mating. We had planned these studies for this fall but were unable to complete them due to the government shutdown.

During our 16 day furlough, we lost 90% of our codling moth colony and did not have enough insects to perform the flight tunnel and disruption of mating studies. Our colony is now back up and insects will be available soon. We plan on performing these studies February and March 2014.

SUMMARY

The overall goal of this project is to provide a more thorough characterization of the codling moth olfactory system, especially as it relates to the detection of pheromones. In year 2 of this project we have produced an expanded list of olfactory proteins (ORs, OBPs, ODEs and CSPs) to the point that we are nearing theoretical maximums. We have also identified a putative codlemone receptor (CpOR11) that is highly expressed in male antennae and when assayed using a cell based system responds to codlemone. During the next year we will work on completing flight tunnel and disruption of mating studies, as well as performing codlemone interaction studies with OBPs and ODEs. The information we have generated thus far will provide us with years of studies to determine the ligands for each of the 54 ORs expressed by codling moth.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-13-101

Project Title: Study of molecular mechanisms to preserve codling moth control agents

PI: Stephen F. Garczynski
Organization: USDA-ARS YARL
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Cooperators: Tom Unruh, YARL; Rodney Cooper, YARL; Ron Nachman, USDA-ARS, Texas

Total Project Request: **Year 1**: $39,000 **Year 2: $42,000** **Year 3**: $45,000

Other funding sources
NONE

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

Item	2013	2014	2015
Salaries[1]	26,100	27,000	28,000
Benefits	1,900	9,000	9,000
Wages			
Benefits			
Equipment			
Supplies	11,000	6,000	8,000
Travel			
Miscellaneous			
Plot Fees			
Total	39,000	**42,000**	45,000

Footnotes: [1]Salaries are to support one part time GS-6 level technician (30 hrs / wk)

OBJECTIVES

1) Determine the effects of Altacor, Delegate, Calypso and granulosis virus on gene expression levels of codling moth heat shock (stress response) proteins and detoxification enzymes. The purpose of this objective is to identify gene transcripts that are elevated in response to sublethal doses of Altacor, Delegate, Calypso, and granulosis virus. We will focus on the most likely candidates based on studies performed in other moths, which include transcripts encoding detoxification enzymes (cytochrome P450s, esterases, and glutathione *S*-transferases), or increased expression of stress response proteins (heat shock proteins). Induced expression of gene transcripts encoding these proteins has been correlated with their potential as "insecticide resistance" factors. To complete this objective we will first clone gene transcripts encoding codling moth heat shock proteins and detoxification enzymes. From previous codling moth transcriptome data we have identified gene transcripts encoding 24 different heat shock (stress response) proteins, 20 cytochrome P450s, seven esterases and 10 glutathione *S*-transferases. Once these transcripts are cloned and their nucleotide sequences verified, we will design oligonucleotide primers for use in quantitative PCR (qPCR). Once qPCR conditions are established, we will then quantify the expression levels of gene transcripts encoding heat shock proteins and detoxification enzymes from untreated eggs, neonates, and adults or those exposed to heat, cold, or sublethal doses of Altacor, Delegate, Calypso and granulosis virus. This will allow us to determine if any of these proteins have a potential role in resistance.

2) Determine the effectiveness of PBAN antagonists to inhibit codlemone production by codling moth females. Another way to prevent insecticide resistance is to use control agents that utilize a different mode of action to help control insect pests. Dr. Ron Nachman (USDA-ARS, Texas) has developed and synthesized several PBAN antagonists that reduce or eliminate pheromone biosynthesis in the tobacco budworm, *Heliothis virescens*. Because of the amino acid diversity of codling moth PBAN vs tobacco budworm PBAN (only 45 % similarity), it will be prudent to determine the effectiveness of the PBAN antagonists **before** they are fully developed into commercial products. This collaboration provides us the unique opportunity to test the PBAN antagonists on codling moth; first to determine if they work, then to determine dosage and timing of applications. To complete this objective, we will clone gene transcripts encoding codling moth PBAN receptors (PBANR) and then to express the cloned receptors in mammalian cell lines. We will then use cell based assays to verify PBANR activity and determine if PBAN antagonists block receptor activity. We will also test PBAN antagonists on female codling moth to determine biological activity in pheromone biosynthesis inhibition and to see if these compounds disrupt mating.

SPECIFIC OBJECTIVES FOR YEAR 2

1) Continue cloning transcripts encoding detoxification enzymes, heat shock proteins and targets
2) Complete design of oligonucleotide primers for qPCR and optimize reaction conditions
3) Select mammalian cell lines stably expressing codling moth PBANR and begin cell based assays with synthetic PBAN and PBAN antagonists
4) Perform pheromone production assays with PBAN antagonists
5) Perform flight tunnel and mating bioassays with PBAN antagonists

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

- Additional detoxification enzymes/heat shock proteins were identified from a transcriptome
  - 50 transcripts encoding putative esterases
  - 80 transcripts encoding putative cytochrome P450 monooxygenases
  - 22 transcripts encoding putative glutathione *S*-transferases
  - 45 transcripts encoding putative heat shock proteins
- Protein targets for most classes of insecticides, past and present, were identified

- Nicotinic acetylcholine receptor subunits (targets of neonicotinoids and spinosads)
- Ryanodine receptor (target of rynaxypyr – Altacor)
- Acetylcholinesterases (targets of Carbamates and Organophosphates)
- GABA-gated chloride channels (targets of Organochlorines and phenylpyrazoles)
- Sodium channels (targets of Pyrethroids and Indoxacarb)
- Glutamate-gated chloride channels (targets of Avermectins and Milbemycins)

- A putative PBAN receptor has been cloned

METHODS (PROJECT APPROACH)

Methods for Objective 1

1) Cloning gene transcripts that encode codling moth heat shock (stress response) proteins and detoxification enzymes. Through prior WTFRC funding, two codling moth transcriptomes have been generated. Using bioinformatics approaches, we will identify gene transcripts encoding heat shock (stress response) proteins, cytochrome P450 monooxygenases, carboxylesterases, glutathione *S*-transferases and protein targets of insecticides. We will use the nucleotide sequences determined from the codling moth transcriptomes to design gene specific primers that can be used in PCR reactions to clone full length mRNA molecules that encode each protein of interest.

2) Determination of gene transcript expression by quantitative PCR. Quantitative PCR (qPCR) will be used to determine the relative amount of gene transcripts expressed in codling moth. Sequence specific primers will be designed for each gene transcript of interest, and the amount of transcript will be determined for each transcript using real time qPCR detection. This technique has been developed in my lab for analysis of heat shock protein expression differences in untreated and heat treated insects. First, we will optimize qPCR conditions for each gene transcript of interest. After optimization, we will determine basal levels of gene transcripts expressed in codling moth eggs, neonates, and adults. Once the baseline expression levels are determined, we will perform the qPCR analysis to untreated or treated (sublethal doses of Altacor, Delegate, Calypso, and granulosis virus) codling moth to determine the effects on gene transcript levels. Of particular interest will be gene transcripts in which expression levels are significantly increased.

Methods for Objective 2

1) Cloning and expression of the codling moth PBAN receptor (PBANR). Oligonucleotide primers will be designed against the nucleotide sequence we have previously obtained for the codling moth PBANR and PCR will be used to amplify the full-length transcript. Once the full-length transcript is obtained, we will clone the protein encoding portion into a mammalian expression vector and incorporate this clone into a mammalian cell line.

2) Determine if the codling moth PBANR is biologically active. Cell based assays will be used to confirm the identity of the codling moth PBANR. Mammalian cell lines expressing PBANR will be exposed to synthetic PBAN (based on the amino acid sequence of codling moth PBAN) and will be monitored for activation of the cells' second messenger pathways. Once we confirm that the synthetic PBAN activates PBANR signaling, we will use the cell based assay to determine the effectiveness of the various PBAN antagonists (provided by Dr. Ron Nachman, USDA-ARS, Texas).

3) Biological assays to determine the effectiveness of PBAN antagonists. We will use pheromone production and mating assays to determine if the PBAN antagonists inhibit codlemone production and reduce female attractiveness to codling moth males. Pheromone production will be determined by injecting females at the 2^{nd}-4^{th} hour of photophase with either synthetic PBAN or with the

antagonists. After treatment, pheromone glands will be dissected and placed in hexanes and pheromone quantified by gas chromatography. Mating bioassays will be performed in two ways; first we will use flight tunnels to determine the attractiveness of females, untreated controls or treated with PBAN antagonists, to codling moth males. Secondly, we will set up mating bags with untreated or antagonist treated females and codling moth males. We will then determine egg production and viable offspring to determine the effectiveness of the antagonists in close quarter matings.

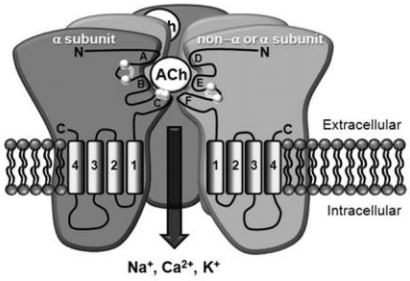
RESULTS AND DISCUSSION

Identification of detoxification enzymes, heat shock proteins and insecticide protein targets

In the original proposal for this project, we had already identified gene transcripts encoding 24 heat shock (stress response) proteins, 20 cytochrome P450s, seven esterases and 10 glutathione *S*-transferases. While this information provided a starting point, it was in no way complete. To obtain a more complete set of gene transcripts that may play roles in codling moth resistance to pesticidal agents, we data mined our recently completed codling moth head transcriptome (generated with previous WTFRC funding – CP-11-100A and CP-11-100B). Results of our data mining efforts allowed us to identify additional gene transcripts encoding for proteins that may be involved in insecticide resistance. Transcripts identified included 50 putative esterases, 80 cytochrome P450 monooxygenases, 22 glutathione *S*-transferases, 45 heat shock proteins as well as those that encode the protein targets of the major insecticides used for insect control (data not shown). In the last six months, we have cloned transcripts encoding four esterases, 10 heat shock proteins and 14 nicotinic acetylcholine receptor subunits (the targets for neonicotinoids and spinosyns). In the next year we will be using the sequence information we have to design the oligonucleotide primers for use in quantitative PCR experiments to determine basal expression levels of transcripts encoding detoxification enzymes. This information will allow us to proceed with experiments to determine which transcripts may be elevated in response to insecticide treatment, providing us with candidates to test for in future field resistance studies.

Expression patterns of nicotinic acetylcholine receptor subunits

Nicotinic acetylcholine receptors (nAChR) are the protein targets of neonicotinoids and spinosyns. nAChRs are nerve membrane proteins composed of a combination of 5 alpha and beta subunits (see diagram at left), and the subunits can be all the same (homomeric) or any combination of multiple subunits (heteromeric). For codling moth, we have identified 9 nAChR alpha subunits and 4 nAChR beta subunits. With this many subunits, codling moth (and other insects) can produce a large number of protein combinations. Recently, mutations in two of the nAChR alpha subunits (alpha 5 and alpha 7) has been shown to confer resistance to neonicotinoids. We have identified a similar region in codling moth and are currently designing PCR primers that can be used to test for target site resistance in future work.



The transcriptome used to identify nAChR subunits was prepared from heads representative of all codling life stages. To determine which stages express the various nAChR subunits, we performed a stage expression profile (Figure 1). PCR reactions were done to determine the expression of 8 nAChR subunits (6 α subunits and 2 β subunits) in the various codling moth life stages (larval, pupal and adult). For each stage (except 1st, 2nd and 3rd instar), we further separated males (♂) from females (♀), and heads (H) from bodies (B). Some points of interest:1) nAChRα1 is expressed in heads all larval and pupal stages, and appears to have a male biased expression in the bodies of 4th and 5th instar and adult males. 2) nAChRα4 is not expressed in adults. 3) nAChRα8 expression appears to be limited to heads. 4) nAChRβ2 and nAChRβ3 appear to be expressed in both heads and bodies.

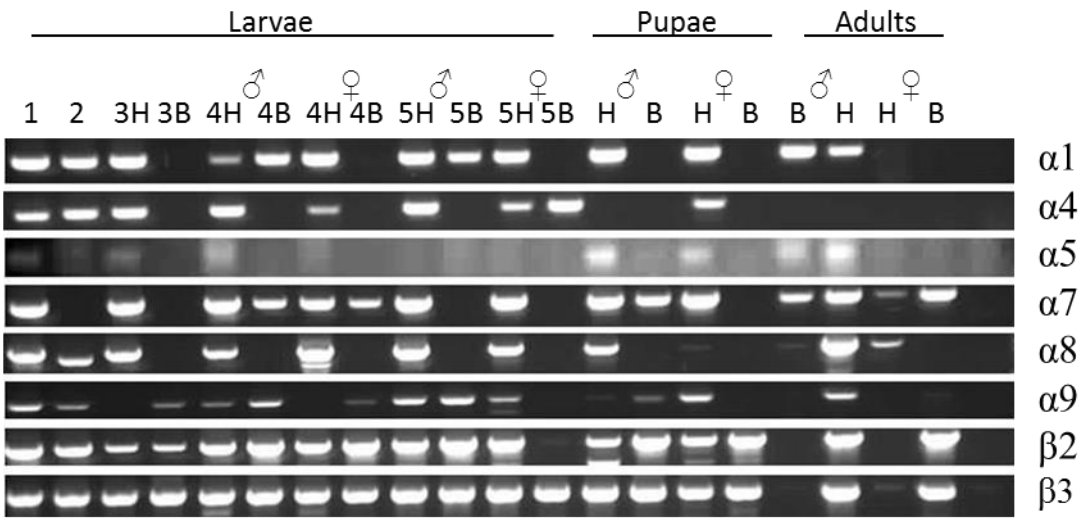


Figure 1. Stage expression profiles of eight nicotinic acetylcholine receptor subunits in codling moth. PCR reactions were done to determine nAChR expression in the various codling moth life stages (larval, pupal and adult). For each stage (except 1st, 2nd and 3rd instar), we separated males (♂) from females (♀), and heads (H) from bodies (B). nAChR subunits tested are listed to the right of each panel. Expression is deemed positive if amplification products are present (the white bands in each panel).

SUMMARY

The overall goal of this project is provide information to help prolong the use of Altacor, Delegate, Calypso and granulosis virus in the orchard by identifying potential resistance factors for each insecticide. Most resistance is due to detoxification, mainly by esterases, cytochrome P450 monooxygenases or glutathione *S*-transferases. Resistance can also be caused by modification of the insecticide target proteins. In this first year, we have identified the protein targets of insecticides and potential detoxification enzymes that may play a role in the development of resistance to Altacor, Delegate and Calypso. We will use this information to monitor detoxification expression in response to sub-lethal doses of each of the insecticides of interest. Results from this project should provide a means to determine potential field resistance based on detoxification enzyme expression.

CONTINUING PROJECT REPORT **Year** 2 of 3
WTFRC Project number: CP-12-102

Project Title: Models to assess pesticide impacts on CM, OBLR and *C. nigricornis*

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Co-PI(3): Jay Brunner
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City/State/Zip: Wenatchee, WA 98801

Total Project Request: **Year 1:** $76,566 **Year 2:** $81,679 **Year 3: $82,956**

Other funding sources

Agency Name: Washington State Commission on Pesticide Registration
Amt. awarded: $21,438, one year
Notes: We have submitted and received a new grant to leverage some of the work being done on this grant. That grant is "Evaluating low dose insecticide residues on codling moth flight and behavior".

WTFRC Collaborative expenses:

Item	2012	2013	2014
Salaries	0	0	0
Benefits	0	0	0
Wages	0	0	0
Benefits	0	0	0
RCA Room Rental	0	0	0
Shipping	0	0	0
Supplies	0	0	0
Travel	0	0	0
Miscellaneous[1]	2300	2392	2488
Total	2300	2392	**2488**

Footnotes: [1]WTFRC Collaborative expenses for spraying plots

Budget 1
Organization: WSU-TFREC **Contract Administrator**: Carrie Johnston/Joni Cartwright
Telephone: 509-335-4564/509-663-8181 x221 **Email:** carriej@wsu.edu / joni_cartwright@wsu.edu

Item	2012	2013	2014
Salaries [1]	46,783	49,233	51,889
Benefits [2]	18,429	19,317	19,494
Wages	3,200	4,500	4,680
Benefits [3]	554	437	454
Equipment	0	0	0
Supplies [4]	2,500	2,600	2,704
Travel [5]	800	1,200	1,248
Miscellaneous (plot charges at WSU Sunrise: 2 acres@$1,000/acre)	2,000	2,000	0
Total	74,266	79,287	**80,468**

Footnotes:
[1] Tawnee Melton (0.7 FTE for 7 months), Angela Gadino (1.0 FTE for 7 months).
[2] Tawnee Melton (49.2%), Angela Gadino (33.7%).
[3] 9.7%
[4] Includes bioassay and field supplies needed for objectives 2 & 3.
[5] Within State Travel.

Objectives:

1. Develop life history information needed for the *Chrysopa nigricornis* model.
2. Develop mortality versus residue age curves for the three species to six commonly used pesticides.
3. Develop and validate demographic models that will estimate the pesticide effects on *C. nigricornis*, OBLR, and CM.

Significant Progress:

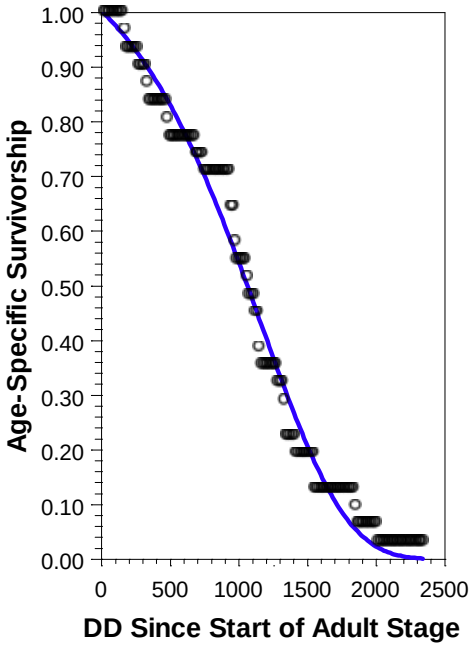
- A laboratory colony of *C. nigricornis* has been successfully established from field-collected adult lacewings and is being maintained to provide lacewing larvae and adults needed for experiments.
- Newly emerged adult lacewings were selected from the colony and used to gather life history data needed for the demographic models.
- We have developed a successful methodology to test the effect of leaf residues from the six pesticides on 1st instar lacewing larvae. These experiments are currently in progress and will continue through the winter of 2013-2014.
- Residue mortality bioassays for OBLR were conducted for a second season using leaves from a non-bearing apple orchard treated with six pesticides. Leaves were collected and bioassays conducted twice during this season. The data will be used this winter in the demographic models for this species.
- Extra leaves from field applications for this season were collected, sealed and frozen for use in lacewing and codling moth bioassays.
- Shoot growth was measured throughout the season and used to estimate how much of the plant was not covered by pesticide residues over time.
- The model for codling moth has been completed and only needs the residue data.
- The OBLR model will be considerably more complicated than those for CM or the lacewings because OBLR has some individuals that go through five larval instars and some that go through six. This requires two models that run independently and then combine their data after the simulations are done. We have finished the simulation of phenology but have not yet incorporated the residue data and needs the modifications to allow simulation of pesticide effects.
- Demographic models for the lacewings *Chrysoperla carnea* and *Chrysopa nigricornis* are complete for the control populations and matches phenology observed in the field. The models still need the module that allows simulation of pesticide effects.

Results and Discussion:

Objective 1. Newly emerged adult *C. nigricornis* were selected from the colony and used in experiments to gather life history information such as adult female longevity and oviposition rates. A single male and female lacewing were paired together in a large plastic deli container covered with mesh netting for aeration and containing a 2-ounce deli cup with 10% honey water. Each pair was fed weekly with Mediterranean flour moth eggs and three times per week with frozen wooly apple aphids collected from low input or non-sprayed orchards. Each pair was held in a growth chamber with 16L:8D photoperiod, at 77°F and 50-60% relative humidity. Containers were checked daily for egg clusters, which were counted and removed once recorded. A subset from the removed eggs were placed in a 4-ounce deli cup, given *E. kuehniella* eggs and placed in a growth chamber to evaluate progeny survival rates.

Results: Life history data has been collected for 31 *C. nigricornis* mating pairs and has been analyzed and used as the basis for the demographic models (obj. 3). The data shows that this species (which is predaceous as an adult) is extremely long lived (Fig.1) and has an extremely long oviposition period (95% egg production does not occur until 79 days (2120 DD) after emergence (Fig. 2)). The long adult life span and a high reproductive rate results in an average egg production of 739 female eggs/female. Comparing these results to *Chrysoperla carnea* (that is not predaceous as an adult), the adult longevity of *C. nigricornis* is much longer (nearly 2x as long) and 95% of the total egg production occurs nearly 1.5 fold later. Even without the initial demographic model, it is clear that overlapping generations are the rule (even if we assume the mortality rate in the field is 2-3 x what happens in the lab), and that the phenology models are primarily telling us when the next generation's reproduction starts to increase. If adult lacewings do have the sort of mortality rates found in the lab (very unlikely), some of the overwintering generation of adults could last until late July or early August.

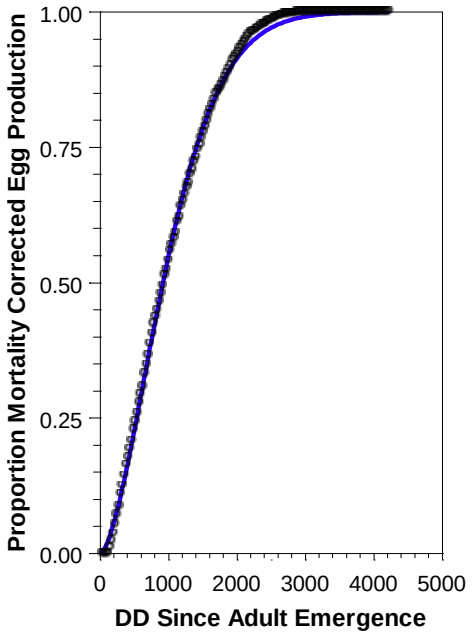
Fig. 1. Age-specific survivorship of adult female *C. nigricornis* on a DD scale.



Plans for 2014: This section has been completed, the analysis and modeling moves to objective 3.

Objective 2. Bioassays were conducted for a second season during early and late summer to obtain the mortality versus residue age curves needed for the demographic models. The maximum recommended field rate was applied to a non-bearing Red Delicious orchard (Quincy, WA) for Altacor, Assail, Delegate, Entrust, Proclaim, and Warrior. Materials were applied on 31 May (spring) for the first set of bioassays and on 19 July (summer) for the second set. Control plots remained untreated for all experiments. Leaves were sampled every 4-7 days up to 45 days (early treatment) or 67 days (July treatment) after pesticide applications. We marked the youngest nodes completely expanded at the time of pesticide application, so that we would always pick leaves that had been treated for the bioassay treatments. Similar to 2012, extra leaves were collected for each treatment on all sample dates and brought back to the laboratory where they were vacuum packed and frozen for use in future bioassays.

Fig. 2. Cumulative proportion of total egg production by female *C. nigricornis* on a DD scale.



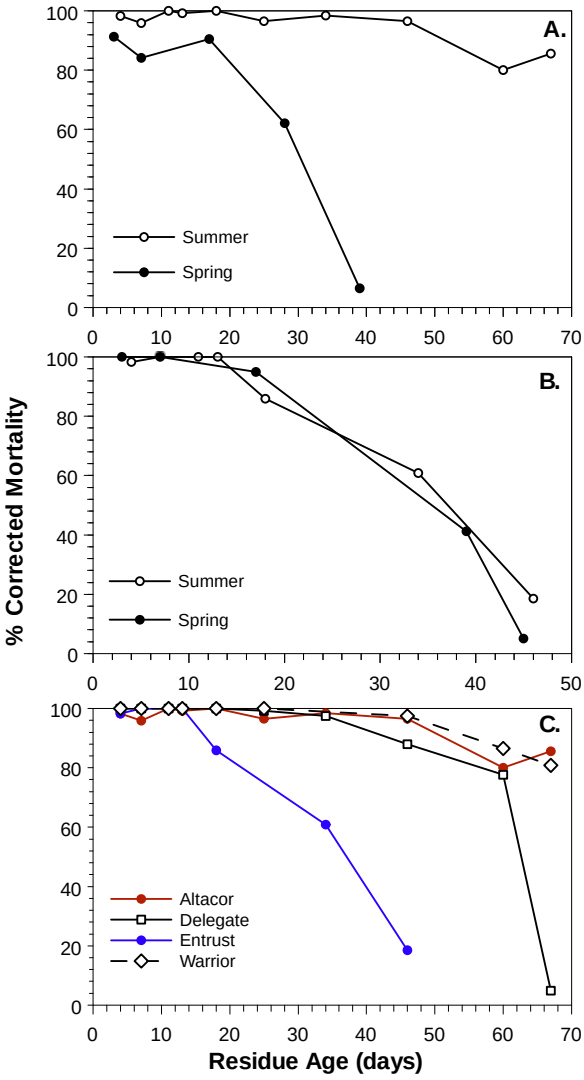
Residue bioassays were conducted for OBLR and CM in the laboratory using fresh leaves containing the field-aged residue for each sample day. First instar OBLR larvae (0-4 days old) or neonate (<24h old) CM larvae were confined to four leaf discs (2cm diameter) in a plastic petri dish. A total of 125 CM or OBLR larvae were evaluated for each pesticide and residue sample day. Larvae were checked for

mortality at 7 days after exposure to each residue sample. These data were then used to develop the mortality versus residue age curves.

Residue bioassays were started for lacewing larvae in the spring of 2013 using frozen leaves from the 2012 applications. A series of preliminary experiments were conducted to develop a successful method for evaluating the effects of field-aged residues on first instar lacewing larvae. Lacewing larvae are cannibalistic and therefore each test unit could only contain one larva. Leaves containing a known aged residue were thawed under a fume hood and then used to line a 5 mL plastic test tube. A single first instar larva (0-2 days old) was transferred to the test tube and fed *E. kuehniella* eggs. Leaf discs approximately 0.5cm in diameter were used at the top and bottom of the test tube to insure larvae were in contact with leaf residues at all times. Test units were held in a growth chamber at 22 ± 2°C, 16:8D photoperiod with 50-60% relative humidity and checked after 7 days for larval mortality.

Results: Residue bioassays for CM larvae using fresh leaves did not yield the expected data due to high mortality (>20%) in the control treatment. Reasons for this failure are not clear as successful preliminary assays were conducted on previously frozen leaf discs. We investigated possible causes for this issue such as increasing humidity and neonate robustness in the fresh leaf assays but are still working to solve the problem. Our next step is to run additional assays using frozen leaves to determine if the difference in results is due to using fresh versus previously frozen leaves.

Fig. 3. Efficacy of field-aged insecticide residues against OBLR larvae in 2013. **A**. Comparison of Altacor efficacy in the spring and summer tests. **B**. Comparison of Entrust in spring and summer tests. **C**. Summer trial showing residual activity of four insecticides. Notice residue age scale is different in the middle figure.



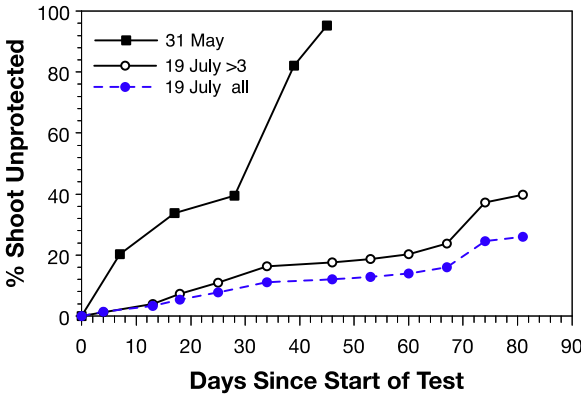
OBLR mortality was obtained for the six pesticides for both the spring and summer applications. Results from the spring applications were less consistent compared to summer applications and typically showed lower mortality at earlier residue days. For example, larval mortality for Altacor residue was below 80% by day 25 in the spring bioassay, but mortality levels remained above 80% until 67 days after application in the summer assay (Fig. 3A). This trend was also observed for Assail, Delegate, Warrior and Proclaim treatments; however Entrust bioassays showed virtually no difference between spring and summer (Fig. 3B). Several rain events in the spring may be responsible for degradation of the residue on the leaves, so differences between the times may indicate stability of the formulation to rain events.

In general, data from the summer bioassays was similar to results found from 2012 assays. After correcting for the natural mortality occurring in the control assays, we found that 60 day-old residues

of Altacor, Delegate and Warrior continued to show activity with >80% OBLR larval mortality (Fig. 3C). Residue activity decreased earlier for Proclaim and Entrust where OBLR mortality was <60% by day 20, then <20% by day 46. Assail showed higher mortality in this seasons bioassays compared to last year with >70% mortality with 4 day old residue, however mortality was below 30% by residue day 7 and consistent with last years findings.

We have started residue bioassays on lacewing larvae for Warrior, Altacor, Proclaim, and Delegate, but have only completed them for the four-day-old residues. However, the colony is in good shape and we are working to catch up on these this winter.

Fig. 4. Average percent of leaf nodes generated by different days early and mid-summer in our residue trials 2013. Growth is average of 35 shoots examined in non-treated areas.



In addition to the residue data, we also collected shoot growth data (Red Delicious) in the plots over the entire experiment. Shoot growth was extreme on the early season trial (31 May in Fig. 4), with >40% shoot growth within the first month. Given the relatively short residual effectiveness of most insecticides for OBLR (compared to the summer), growth makes treatments at this time more of a concern and re-treatment intervals are shorter than residue data alone would indicate. In the summer, growth was much lower and out to about 2 months, there was <20% shoot growth that would have been unprotected. In fact, 14 of the 34 shoots measured grew less than three nodes over the summer trial, so we provided the average growth data considering both groups together and those that only grew more than three nodes (Fig. 4).

Plans for 2014: In the remaining year, we will complete the residue bioassays for the lacewing larva and use the data to complete the demographic model. We also will work to develop a successful method for the CM residue bioassays. We will start to run additional assays on CM neonates using previously frozen leaves to determine if this method can produce usable data. We also plan to run CM residue bioassays on treated apples. For this we will need to apply mating disruption to the WSU Columbia View research orchard in order to obtain clean fruit without additional pesticide applications that could obstruct the treatment residues.

Objective 3. Develop and validate demographic models that will estimate the pesticide effects on *C. nigricornis*, OBLR, and CM.

Because we did not have the life history data for the *C. nigricornis* model until late November, we started on a demographic model for the lacewing *Chrysoperla carnea*, which is the second most common lacewing found in our surveys of the natural enemy fauna in apple, cherry, and pear orchards. The idea was to work out most of the issues with the model and then use that model as the basis for our *C. nigricornis* model when the life history data became available. For *C. carnea*, life history data were taken from multiple literature sources, synthesized, and used to create the model and the model output was checked against the preliminary phenology model developed using data from the SCRI grant and projects currently or recently funded by WTFRC. *C. carnea* overwinters as a mated adult under the bark or in leaf litter under the tree, so while we can accumulate trap catch of the overwintering adults, we do not actually track the phenology of the overwintering individuals (since they are all adults). The model shows that adults are very long lived, and the first and second summer generations overlap considerably, making the phenology tracked by traps alone difficult to

interpret. The model shows that there is very little overlap between the overwintering adults and the emerging of the first summer generation, and the demographic model tracks the observed emergence estimated from trap catch (Fig. 5). However, the second summer generation is predicted by the demographic model to occur about 144 DD later than what is observed in trap catch. The discrepancy is caused by the overlap of the generations that occur at that time – because our demographic model provides the age of individuals, we were able to show that there are more old adults (from the previous generation) still alive when the first individuals of the second summer generation occur and only ≈150 DD later does the ratio of new to old adults present shift towards greater numbers of new adults. Thus, the trap catch is a poor indictor of exact phenology in situations where significant overlap of generations can occur, unless we have an accurate way to determine whether age of individuals caught in the traps. The overlap of generations was something that we were very concerned with in the *C. nigricornis* model because females live longer, and developmental data suggests that there are more generations possible per year. In *C. carnea*, we occasionally see a partial third summer generation start, but even in California walnut orchards, our data suggest that a third summer generation would be unlikely to be completed.

Fig. 5. Comparison of the phenology of *C. carnea* adults from trap catch and the demographic model. The differences in trap catch and model output in the second summer generation is caused by traps catching a mixture of adults from the first and second summer generations.

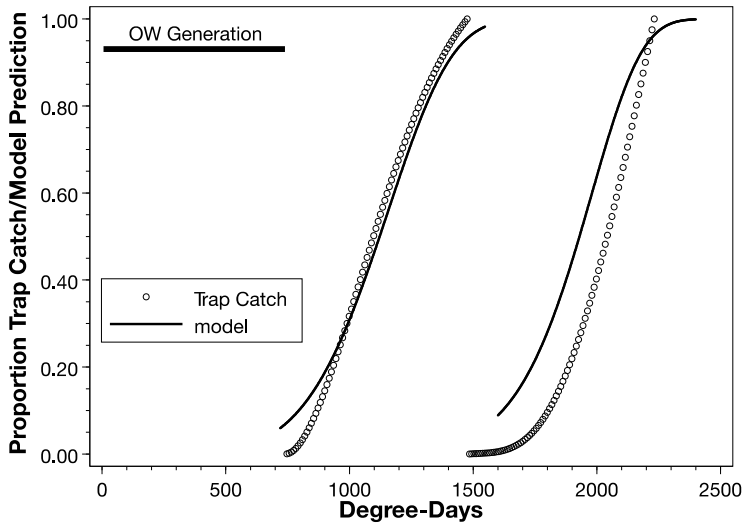
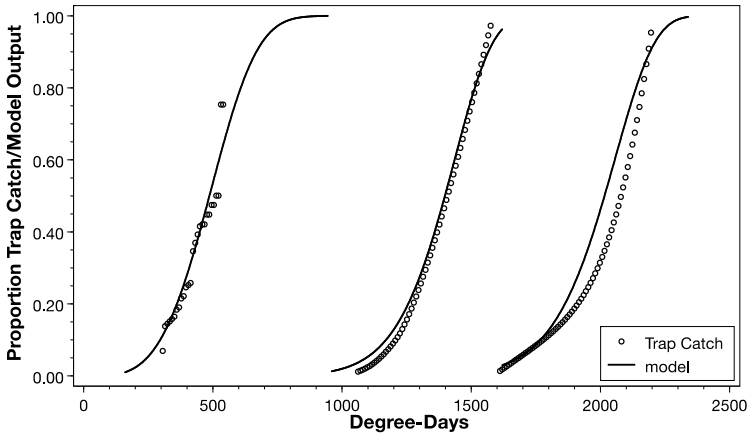


Fig. 6. Comparison of *C. nigricornis* adults from trap catch and the demographic model. Deviation in the third generation is caused by overlap of generations 1-3 all contributing to the newly emerged adults.



The modification of the *C. carnea* model to predict *C. nigricornis* was relatively simple. The big changes were that *C. nigricornis* overwinters as a pre-pupa (instead of as an adult), the length of the different stages was shorter, the adult stage was longer, and oviposition curves were quite different. We were surprised that the overlap of generations were not a greater issue than for *C. nigricornis* (Fig. 6), but this is likely because the adult stage and the egg laying is so much more protracted in *C. nigricornis* that young individuals are found at all points after the initial generation occurs. There is some variation in the second summer generation as the ratio of young to old

changes, but the phonological predictions are actually quite close, so that pesticide effects can be estimated with no issues.

At this point, the demographic model for *C. carnea* and *C. nigricornis* are both done for the control treatment and we need to develop the different pesticide effects modules (which should be done by spring). The full models will require the bioassay data, but the residue decay curve will be easy (<1 day) to include into the pesticide module. The OBLR model for the control treatment is nearly complete, the pesticide effects models are relatively straightforward at this point and will be finished in the spring. The codling moth model is finished, but doesn't include the residue effects because we have no data at this point.

Plans for next year: We clearly will not be in a position to test the models in the field this year. The residue data is complete for OBLR, but has just started for the lacewings and 2014 will be required to get the codling moth data as well. Our plan is to get all the models finished this year, incorporate the residue data, test the models, and decide how field validation is best accomplished.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-12-104A/B

Project Title: Incorporating fire blight resistance into Washington apple cultivars

PI:	Jay Norelli	**Co-PI (2):**	Kate Evans
Organization:	USDA-ARS-Appalachian Fruit Research Station	**Organization:**	WSU Tree Fruit Research and Extension Center
Telephone:	304-725-3451 x264	**Telephone:**	509-663-8181 x245
Email:	jay.norelli@ars.usda.gov	**Email:**	kate_evans@wsu.edu
Address:	2217 Wiltshire Road	**Address:**	1100 N. Western Ave
City:	Kearneysville	**City:**	Wenatchee
State/Zip:	WV 25430	**State/Zip:**	WA 98801

Cooperators: Cameron Nursery, LLC, Eltopia, WA donated 4,000 MM.111 EMLA rootstocks to project for tree propagation.

Total Project Request: **Year 1:** $3,200 **Year 2:** $19,679 **Year 3: $62,600**

Other funding sources:

Agency Name: USDA-ARS-National Plant Germplasm System
Amt. awarded: $10,000
Notes: *Title:* 'Genotyping By Sequencing (GBS) *Malus sieversii* accessions to identify and characterize new sources of resistance to *Erwinia amylovora'.* Funds were provided to identify genetic markers in the accessions of *M. sieversii* used in this WTFRC project. This "raw" genetic information will be combined with the results of this project to identify genetic markers for the fire blight resistance identified. This will make the information obtained from the project directly applicable and usable in the WSU apple breeding program.

WTFRC Collaborative expenses: None

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

Item	2012	2013	2014
Salaries		$6,000 [1] (1)[2]	$7,000[1] (1)[2]
Benefits		$480 (1)	$560 (1)
Supplies		$760[3] (1)	$800[3] (1)
Travel		$1,850[4] (1)	$2,070[4] (1)
Plot Fees	$1,200[5] (1)[2]	$1,200[5] (1)	$1,200[5] (1)
Miscellaneous			
Total	$1,200	$10,290	**$11,630**

Footnotes: : 1: summer student to assist with fire blight inoculation, recording data and plant maintenance; **2:** (#) = Objective # associated with expense; **3:** supplies to grow bacteria, inoculation process and plant labeling; **4:** travel to Wenatchee, WA to work with Kate Evans on fire blight disease evaluation; **5:** maintenance of existing planting of *Malus sieversii* previously established with WTFRC support.

Budget 2

Organization Name: WSU-TFREC **Contract Administrator:** Carrie Johnson & Joni Cartwright

Telephone: 509-335-7667, 509-663-8181, respectively **Email address:** carriej@wsu.edu, joni.cartwright@wsu.edu, respectively

Item	2012	2013	2014
Salaries		$5,990[1] (1)[2]	$6,760[1] (1)[2]
Benefits		$599 (1)	$656 (1)
Wages			
Benefits			
Equipment			
Supplies		$1,000[3] (1)	$1,000[3] (1)
Travel	$500[4] (1&2)[1]	$800[4] (1)	$1,000[4] (1)
Plot Fees	$1,000[5] (1)	$1,000[5] (1)	$1,000[5] (1)
Plot Fees	[2]		$13,400[6] (2)[2]
Miscellaneous	$500[7] (2)		
Total	$2,000	$9,389	**$23,816**

Footnotes: 1: summer student to assist with fire blight inoculation, recording data and plant maintenance, ; **2:** (#) = Objective # associated with expense; **3:** supplies to grow bacteria, inoculation process and plant labeling; **4:** travel to field; **5:** maintenance of existing planting of *Malus sieversii* previously established with WTFRC support; **6:** planting of RosBREED Crop Reference Set for fire blight evaluation, new orchard planting $6,000/acre, fumigation $700/acre, 2 acre Obj. 2; **7**: source and send budwood for tree propagation.

Budget 3

Organization Name: Willow Drive Nursery **Contract Administrator:** Roger Adams

Telephone: 509-787-1555 **Email address:** roger@willowdrive.com

Item	2012	2013	2014
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Plot Fees			
Miscellaneous:			ca.[1] $27,154[2] (2)[3]
Total	$0	$0	**$27,154**

Footnotes: 1: ca.= estimated cost, exact number of trees will not be known until trees are dug, actual cost should range between $24,784 - $27,154; **2:** $27,154 tree propagation ($6.41/tree x ca. 3,850 trees), $1,975 sales tax (0.065 State, 0.015 Local), and ca. $500 shipping;); **3:** (#) = Objective # associated with expense.

OBJECTIVES

1. Identify the best *M. sieversii* (wild apple) accessions to be used as sources of fire blight resistance in the WSU apple breeding program. ($22,046)

2. Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set in Wenatchee, WA for future fire blight evaluation. ($40,554)

SIGNIFICANT FINDINGS

- Field plantings of 194 wild apple accessions and 8 control cultivars were established in Wenatchee, WA (WSA-TFREC) and Kearneysville, WV (USDA-ARS-AFRS) in 2012 for the purpose of identifying the best wild apple accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

- Based upon controlled challenge with the fire blight pathogen in 2013 at both the Wenatchee, WA and Kearneysville, WV plantings, 26 wild *Malus sieversii* accessions were identified as highly resistant to fire blight (resistance equivalent to Geneva rootstocks), and another 38 were identified as resistant to fire blight (resistance equivalent to 'Delicious').

- The RosBREED apple Crop Reference Set and the Washington State Breeding Pedigree Set (approximately 600 cultivars) were budded onto MM.111 rootstock at Willow Drive Nursery in 2013 for future evaluation of their fire blight resistance (to be planted at WSU-TFREC, Wenatchee, WA in spring 2015).

METHODS

Objective 1: Identify the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

Reliable evaluation of fire blight resistance requires controlled challenge of test plants with the fire blight bacteria because fire blight is sporadic in its occurrence from year to year and in its distribution within the orchard. Due to the destructive nature of the disease, orchards established for the evaluation of fruit quality or other purposes cannot be used for fire blight evaluation and distinct orchards need to be established for evaluating fire blight resistance. Based upon preliminary evaluation of thousands of wild *Malus* (apple) *sieversii* trees grown from seed collected in Kazakhstan, 194 wild apple accessions were selected for further evaluation as possible sources of fire blight resistance for the WSU apple breeding program. What distinguishes *M. sieversii* from other wild apple species as an excellent source of disease resistance for scion breeding is its large and palatable fruit. The selected wild apple accessions were budded onto M.7 rootstock and planted in both Wenatchee, WA (3 trees per accession) and Kearneysville, WV (4 trees per accession) in 2012. Each planting in WA and WV includes the same 194 *M. sieversii* accessions and 8 control apple cultivars. Having plantings in both WA and WV will allow determination of fire blight resistance in diverse environmental conditions that will result in more reliable and precise resistance ratings. Standard cultivars are included in both orchards so that results obtained in different locations and years can be directly compared. Moderately resistant 'Delicious', intermediate 'Empire' and moderately susceptible 'Golden Delicious' are included to establish the lower limit for a "resistant" rating. Two highly susceptible cultivars ('Gala' and 'Jonathan') are included to establish the high end of the disease scale when comparing tests and to ensure that a minimum disease pressure threshold is achieved in every test. A highly resistant cultivar ('Robusta 5') is included to establish the low end of the disease scale when comparing tests. 'Goldrush' and 'Splendour', two cultivars reported to be

resistant to fire blight, are included to directly compare their resistance with that observed in *M. sieversii* accessions.

Plants were challenged with the fire blight pathogen by dipping a pair of scissors in a suspension of the fire blight bacteria (*Erwinia amylovora*) and then cutting the youngest leaves of vigorously growing shoot tips (Fig. 1). The strain of the fire blight pathogen (153N) used in the trials was originally isolated from 'Gala' apple in Milton-Freewater, OR and is considered to be a typical pacific northwest strain of the fire blight pathogen. The same freeze-dried preparation of the pathogen diluted to the same concentration was used in both the WA and WV trial, ensuring that differences observed in the resistance of the accessions grown in WA and WV was due to differences in environment rather than differences in the virulence of the pathogen. Depending upon the size and vigor of the tree, 0-5 shoots on separate branches were challenged with the fire blight pathogen. Because multiple trees of each accession were challenged there was an average of 18 shoots challenged per accession in the WV trial and 8 shoots per accession in the WA trial (number lower in WA due to significant deer damage at the Columbia View orchard, see Discussion below).

Figure 1. Procedure used to challenge *M. sieversii* trees with the fire blight pathogen. **A**: Vigorously growing shoot selected for challenge. **B**: Pair of scissors dipped in a suspension of fire blight bacteria and used to cut the youngest leaves of shoot tip. **C**: Shoot after challenge. **D**: Ryan Potts, summer student hired through WTFRC support to assist in project.

Because economic losses from fire blight are the result of the death of young trees and woody tissue, rating cultivar resistance based upon progression of disease in shoot tissue has proven a reliable method of accessing fire blight resistance. Approximately 6 weeks after challenge when all fire blight infections (cankers) ceased extension the total length of the lesion (canker) measured from the site of challenge to healthy tissue was determined, as well as the age of the wood infected (0= no disease visible, 1= infection in current season's growth, 2= infection extending into previous season's growth,

3= infection progressing into 2 yr-old wood). Several measures of resistance were then calculated including the average length of cankers, the average age of wood infected, and adjusted ratio of the canker length divided by the current season's shoot length and the proportion of the current season's shoot growth infected (see Discussion). A statistical analysis (generalized linear model) of the various measures was conducted (Statistical Analysis System [SAS] Software, version 9.2, SAS Institute, Cary, NC) and a multiple comparison procedure (Bonferroni Adjustment for Multiple Comparisons) was used to determine accessions that were significantly different from each other (Probability =0.05).

Norelli has many years of experience evaluating apple for fire blight resistance and traveled to Wenatchee, WA in June, 2013 to train Kate Evans' crew in the fire blight challenge procedure and assist Kate Evans in resistance evaluation. The above tests will be repeated in 2014 (see Discussion) and Norelli plans to again travel to Wenatchee, WA to assist is the evaluation of disease resistance.

Objective 2: Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set for future fire blight evaluation**.**

Trees are being propagated at Willow Drive Nursery in Ephrata, Washington. Budwood of the RosBREED apple Crop Reference Set and WSU Breeding Pedigree Set was collected at WSU-TFREC Wenatchee, WA or obtained from the other RosBREED core breeding programs at the University of Minnesota and Cornell University, or the USDA-ARS-Plant Genetic Resources Unit (PGRU) in Geneva and budded onto M.111 rootstock during the 2013 growing season. MM.111 rootstock was selected because of its tolerance to fire blight to prevent tree loss due to rootstock infection. Trees will be planted in Wenatchee, WA during spring 2015 with protective deer fencing.

Because fire blight resistance will be determined on shoots, the tests for resistance can be conducted on young trees and the planting is expected to be of short term duration (3 to 4 years), allowing for planting at high density. Because fire blight challenge of the planting will be required for future evaluation of fire blight resistance, the planting will be situated on the Columbia View orchard just north of Wenatchee.

RESULTS & DISCUSSION

Objective 1: Identify the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

The approach we have used in evaluating resistance to fire blight in these wild apple (*Malus sieversii*) accessions is to use a severe inoculation procedure with a high dose of the fire blight pathogen (*Erwinia amylovora*) to ensure that a fire blight infection will be initiated, if possible, and then evaluate resistance based upon how far fire blight disease progresses in the infected shoot. An alternative approach would be to evaluate the trees based on their resistance to the incidence of infection, such as determining the number of blossom infection that occurred after flowers are sprayed with a relatively low dose of the fire blight pathogen. These types of evaluation methods are appropriate when evaluating chemical or biological control treatments, but they are not appropriate when selecting sources of resistance for breeding. Although resistance to disease progression, or severity of infection, is usually correlated with resistance to the incidence of infection, there is not a one to one association between these two types of resistance. We believe that growers could tolerate a relatively high level of fire blight blossom infection if those infections do not progress systemically through the tree. Because economic losses from fire blight are the result of the death of young trees and woody tissue, we believe rating cultivar resistance based upon progression of disease in shoot tissue is the most useful and appropriate method of accessing fire blight resistance.

As expected, after challenging vigorously growing shoot tips with the fire blight pathogen we observed diverse responses among the wild apple accessions ranging from highly susceptible to highly resistant. After challenge with the fire blight pathogen, typical fire blight infections developed on many of the wild apple accessions. In some cases the infections progressed through the current season's shoot growth into the previous season's wood or into 2 year-old wood (highly susceptible response). In other cases the infections progressed through much of the current season's shoot growth but did not penetrate into the previous season's growth (intermediate response). In several cases only minor evidence of disease was observed in the challenged leaves and infections did not progress from the leaf into the shoot (highly resistant response). Initially, the wild apple accessions were quantitatively ranked based upon the average distance the fire blight infection progressed in the shoot and then assigned into categories of highly resistant, resistant, intermediate, susceptible or highly susceptible based upon comparison to known control cultivars in the trial. Those that behave similarly to Robusta 5, a source of fire blight resistance used in the Geneva rootstock breeding program, were rated highly resistant; those similar to 'Delicious' were rated resistant; those similar to 'Empire' were rating intermediate and those similar to 'Jonathan', which was severely damaged by the fire blight challenge, were rated highly susceptible.

In addition to the average distance the fire blight infection progressed in the shoot, several other measures of resistance were evaluated and the ratings of the wild apple accessions were adjusted in a conservative manner. Other measures of resistance included the average age of the oldest tissue infected and the proportion of the current season's shoot length infected. The analysis of these other measures sometimes led to the "conservative" adjustment of the resistance rating which was adjusted based on an accession's worst performance, not its best performance or average performance. For example, if an accession was rated as resistant based upon distance of disease progression but intermediate based upon age of wood infected, its rating would be adjusted down from resistant to intermediate; however if the cultivar ranked higher based upon the analysis of another measure of resistance, its rating would not increase from resistant to highly resistant. Similarly, if an accession was rated differently in the WA and WV trials, the accession would be given the lower of the two ratings. Although this conservative adjustment of resistance rating may be considered somewhat "unscientific" or unfair, it should help to ensure that the accessions selected as sources of resistance for the breeding program are in fact resistant.

Other observations of fire blight development were also considered in adjusting the resistance rating of the wild apple accessions. Because we were looking at a genetically diverse collection of wild apples we looked for the unexpected. Atypical from observations in most domesticated apple cultivars, we observed several wild accessions that appeared quite resistant to the initiation of fire blight infection, but when an infection did occur it progressed rapidly into older wood. In one case, only one of 20 challenged shoots developed into a shoot infection but that infection progressed through 2 year-old wood into the central leader, destroying the young tree (Fig. 2). Because only 1 of 20 challenged shoots were infected, the accession's average distance of progression and average age of wood infected was still quite low, however this is obviously not a useful type of resistance to incorporate into the breeding program. To eliminate this type of resistance from consideration, any accession with a single infection that progressed into 2 year-old wood was rated as highly susceptible and an accession with an infection that progressed into the previous season's growth was ranked as susceptible.

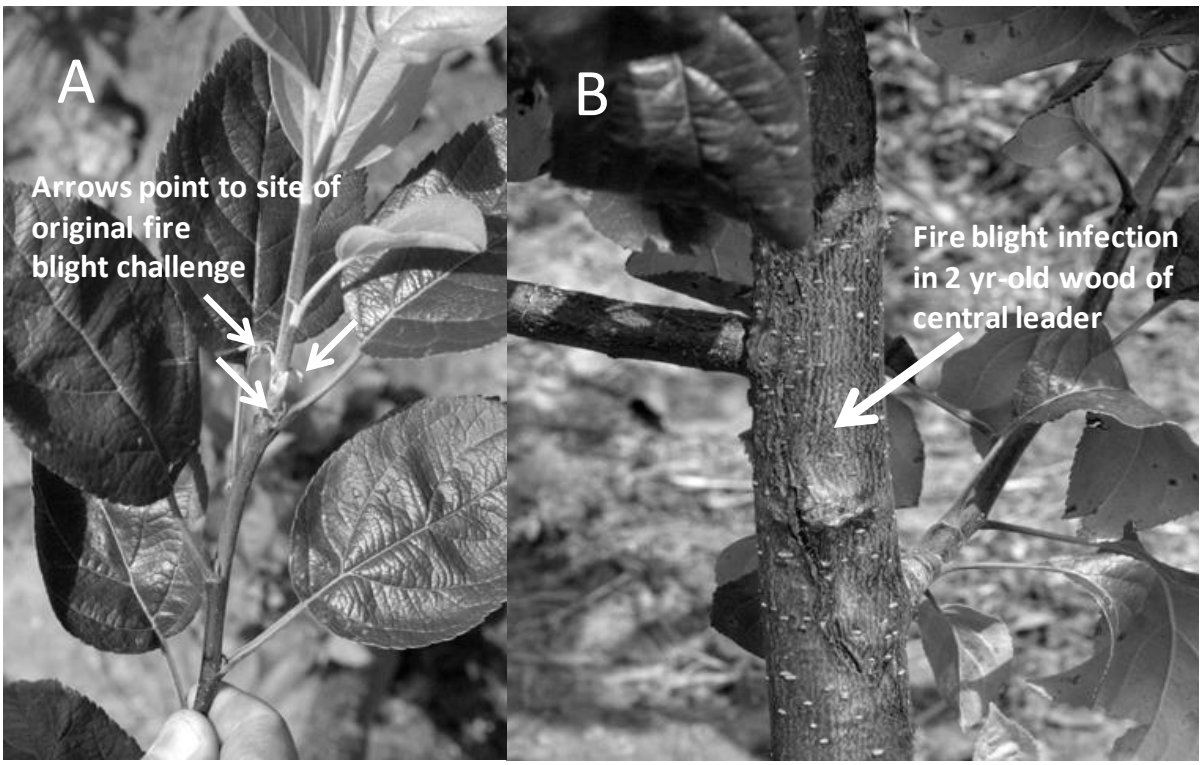


Figure 2: Evaluation of fire blight resistance of *M. sieversii* GMAL4028.h. **A**: In 19 of 20 fire blight challenged shoots no evidence of infection could be observed 6 weeks after inoculation. **B**: In 1 of 20 fire blight challenged shoots, fire blight progressed into 2 year-old wood of central leader.

In addition, naturally occurring blossom and shoot infections (infection that were not the result of our controlled fire blight challenge) were recorded and monitored, and resulted in downward adjustment to an accession's resistance rating if infection resulted in significant fire blight damage to the tree. After adjustment 26 wild apple accessions were rated as highly resistant, 38 were rated resistant and 128 were rated intermediate, susceptible or highly susceptible (Fig. 3).

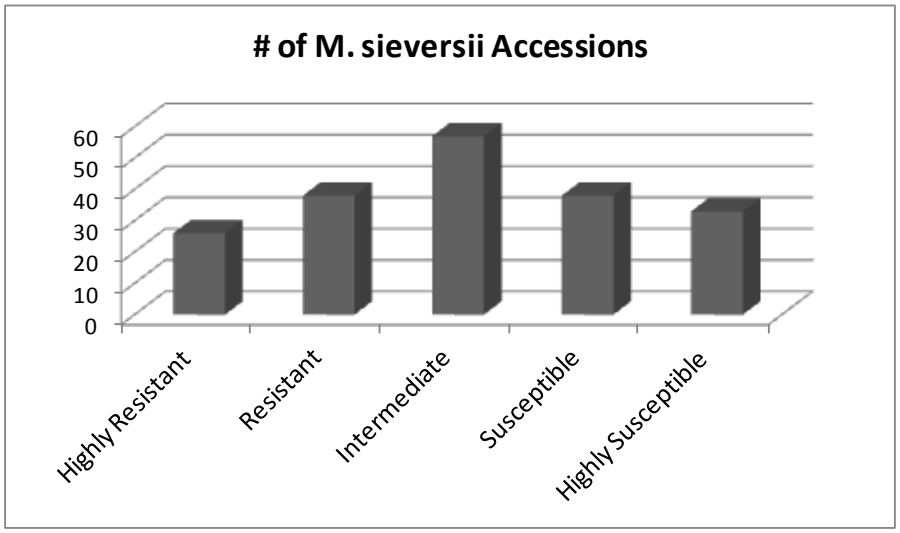


Figure 3. Number of wild apple accessions rated in different classes of resistance to fire blight.

Successful completion of this objective requires that the test be repeated next year for several reasons:

1. Fire blight disease is greatly affected by weather conditions and repeated testing under different environmental conditions is necessary for reliable evaluation of fire blight resistance.
2. The *M. sieversii* (wild apple) test orchard at WSU Columbia View orchard, Wenatchee, WA sustained heavy deer damage in the spring and early summer of 2013. This resulted in a limited number of usable shoots for fire blight challenge in 2013 and only 32 of the 194 wild apple accession had a sufficient number of shoots for reliable fire blight evaluation. The orchard has since been protected by deer fencing and the trees have had excellent growth in 2013. However, without testing in 2014 the evaluations of the wild apple accessions under WA growing conditions will be limited.
3. Because the test orchards were only in their 2nd leaf in 2013, the concentration of the fire blight pathogen was reduced to a moderately high level (ca. 1 X 10^6 colony forming units [cfu]/ wound) to prevent excessive damage to the young trees. Although we obtained sufficient disease development to evaluate the wild apple accessions and highly susceptible 'Jonathan', intermediate 'Empire' and resistant 'Delicious' developed the expected levels of fire blight, highly susceptible 'Gala' and susceptible 'Golden Delicious' both developed less disease than expected and were rated intermediate. Repeating the test in 2014 will allow for evaluation under higher disease pressure (ca. 1 X 10^7 cfu/ wound).
4. In addition, because the orchard was in its 2nd leaf the number of shoots challenged per tree was limited to 5 shoots per tree. In 2014, the larger trees will allow for a greater number of shoots to be challenged per tree (ca. 10 shoots expected to be challenged in 2014) increasing the precision of the evaluation.

Objective 2: Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set for future fire blight evaluation**.**

The goal of this objective is to determine the fire blight resistance of the RosBREED apple Crop Reference Set and the WSU Breeding Pedigree Set so that we can utilize RosBREED resources to identify markers for fire blight resistance. Although Objective 1 will identify excellent sources of fire blight resistance to be used in future crosses, it will not facilitate selection of fire blight resistance among the existing seedlings and selections of the WSU apple breeding program. Furthermore, fire blight resistance is not a trait currently targeted by the RosBREED project. Evaluating the RosBREED apple Crop Reference Set for its resistance/susceptibility to fire blight will allow us to leverage the significant financial investment of RosBREED in marker and software development to enable marker-assisted breeding of fire blight resistance in existing seedlings and selections in the WSU apple breeding program. Because fire blight disease can result in major structural damage of trees, and in some cases tree death of susceptible cultivars, existing plantings of the RosBREED apple Crop Reference Set and WSU Breeding Pedigree Set established to evaluate fruit quality traits cannot be used to evaluate fire blight resistance. In order to keep the cost of this project as low as possible, a single planting located in Wenatchee, WA will be used for the evaluation of fire blight resistance.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-12-100

Project Title: Improving the management of two critical pome fruit diseases

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

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

Total Project Request: **Year 1:** $15,155 **Year 2:** $15,737 **Year 3: $16,343**

Other funding sources

Trident: already provided in kind support (fumigation) $9000. Other necessary financial support is expected from companies supplying products tested for effect on fire blight or orchard replant during this project. I am Co-PI on the project "Development of Non-Antibiotic Programs for Fire Blight Control in Apple and Pear," from the USDA Organic Agriculture Research and Extension Initiative (OREI). My sub-award is $29,887 for 2014. This TFRC project funding helps justify the OREI grant.

Budget

Organization Name: WSU **Contract Administrator:** Maureen Bonnefin
Telephone: 509-335-7667 **Email address:** maureenb@wsu.edu

Item	2012	2013	2014
Salaries	$10,125	$10,660	$11,086
Benefits	4,305	4,477	4,656
Wages			
Benefits			
Equipment			
Supplies			
Travel	600	600	600
Plot Fees			
Miscellaneous			
Total	$15,155	15,737	**$16,343**

Footnotes: Salaries and benefits are in support of 0.25 FTE of a full time technician. Travel is to plot sites. Equipment is for a backpack mist sprayer.

Start of Project OBJECTIVES- *Fire blight of apple and pear:*

1. We will continue to test fire blight control materials in the orchard, on both apple and pear, to assess efficacy and aid registration of effective fire blight control alternatives.
2. We will further study the relationship of temperatures to fire blight infection risk.

Start of Project OBJECTIVES – *Orchard Replant Disease:*
We will demonstrate the effect on soil fumigation on the productivity and quality of apples grown on a replanted site under a very modern production system.

1. We will document apple productivity over a range of chloropicrin and 1, 3-DCP rates.
2. We will calculate the extrapolated economic impact of the various treatments.
3. We will provide this information to the fruit growers of Washington in the effort to increase the practice of pre-plant soil fumigation from its current 60% of replanted acres.
4. We will provide this information to the Northwest Hort. Council, the US EPA, the fumigant registrants, or anyone else involved in the 2013-15 re-registration of soil fumigants.

Significant Findings- Fire Blight:

1. ***Objective 1***. We continued to test fire blight control materials in the orchard, on apple only, as the pear test block is no longer usable. Innumerous past trials, the results have been quite comparable between the pear and apple trials. This year, thirteen different products were tested in 36 different combinations, series and timings (Table 1.) The treatments emphasized copper bactericides, *Bacillus subtilis* fermentation compounds ("Serenade, Optiva, Companion"), and Acibenzolar-S-Methyl (ASM, Actigard.) The streptomycin (FireWall) and oxytetracycline (FireLine) treated checks resulted respectively in 90% and 86% control, and the inoculated check developed 40% blighted flower clusters, all levels fairly typical in this series of trials. Of the 36 treatments, nineteen protected the flowers at an 80% or better level, compared to the inoculated check. This level of control in an inoculated plot indicates potential for acceptable to excellent control under orchard use conditions. Thirteen treatments gave 90% or better protection. However, researchers from several other regions report increased russet in orchards of apple and pear after the application of copper bactericides and the BP yeast, and some apple and pear cultivars seem more sensitive than others. The emphasis of this trial will shift to efficacy relative to russet induction potential, as some of the better treatments coming out of this trial are known or suspected to induce russet in regions of the world with wet, cool weather in the spring months. These products did not induce russet in the test plot, nor in a large russet trial that was carried out this season (Table 2.) Approximately 4500 -5000 acres of apple and pear were treated commercially with Blossom Protect, one of the russeting suspects. While no large-scale evaluation will be possible until most of the treated apples and pears have been graded, almost all growers contacted have expressed satisfaction with performance and no problems with increased russeting. However, in any case, we will work to develop guidelines about russet induction and avoidance, a difficult subject. Based on the differences between the spring weather in regions that have spray induced russet and those that do not, it may be advisable to direct the user to note rain and cool weather in the forecast, and avoid application to young fruit under those conditions.

2. ***Objective 2***. This was completed the first year, and is available through WSU DAS. The author continues to work with WSU DAS to make the model even more useful.

Significant Findings- Orchard Replant Disease:

1. ***Objective 1***. Production and fruit size were documented in 2013, the fifth year of growth (Table 3.) The yields in the treated replicates continued to far out-strip those in the untreated checks. Data supports the increased efficacy of the blend of 1, 3-D (Telone) and chloropicrin (the "C" in Telone C-17 and C-35) as compared to chloropicrin alone, even when the chloropicrin is applied at much higher than normal rates. The 1, 3-D part of the standard fumigant play an important role in the efficacy of the fumigants currently applied on old orchard sites, (Telone C-17 or C-35, or similar blends) indicating that a blend is necessary to the optimum treatment of replant disease. The treatment of high relative levels of chloropicrin with no 1, 3-D, while much better than the untreated areas, was the least productive of the treatments.|
   The highest yielding "treatment D" rate of 30 gallons per acre of Telone C-17 or equivalent blend, contains 51 lb/A chloropicrin and 260 lb/A 1, 3-DCP. Under current regulations, the rate of 1, 3-DCP + chloropicrin applied to a 10 acre block would require a 25 foot buffer zone; not likely to lead to application problems. The rates applied in "treatment B" would also lead to a 25 foot buffer. In contrast, the "treatment A" with the high 150 pound/acre chloropicrin rate would require 320 feet of buffer zone. "Treatment C" would require a 133 foot buffer zone. It is a positive outcome in this trial that the mixture in treatment D appears to be as effective as, or more effective than the higher more restricted fumigant rates.
2. ***Objective 2***. The gross economic differences continue to increase. The orchard was planted as a "sleeping eye in" in spring 2009. The most productive treatment has grossed about $30,000 more than the untreated check, taking into account the cost of fumigating, picking and packing. This treatment has returned over 35:1 on the cost of fumigation.
3. ***Objective 3***. These results have been presented to growers and advisors repeatedly. The data has been widely distributed by fumigant manufactures and applicators.
4. ***Objective 4***. The data from this project was submitted to the US EPA on November 14, 2013 in support of the continued registration and availability of 1, 3-dichloropropine, one of the two active ingredients in pre-planting soil treatments for orchard sites (products such as Telone C-17 and C-35.) Re. Docket ID No. EPA-HQ_OPP_2013-0154.

Results and Discussion – Fire Blight:

Note: Some of the test products reported below are not yet registered for use in orchards. They are listed only to report research results. Check the label for the crop details prior to any use. Note: A streptomycin sensitive lab strain of fire blight bacteria were used for inoculation in this trial for research purposes. Efficacy of strep. will not be similar under natural conditions.

Products	Rate/100/A	Timing	% Infection	% Control
Phyton27 (Copper product)	60 fl. oz.	80% Bloom, then 100% Bloom + 1 day	0.5	98.8a
Blossom Protect + Buffer Protect, then Provisto*	1.34 lb. 9.34 lb. 3 quarts	BP at 60% Bloom, then Provisto at 100% Bloom	1.0	97.5ab
Phyton27 (Copper product)	40 fl.oz.	80% Bloom, then 100% Bloom + 1 day	1.5	96.3abc
Blossom Protect + Buffer Protect	1.34 lb. 9.34 lb.	60% and 100% bloom open	2.0	95.0abc
Strep. (FireWall), then Actigard*	1.0 lb. 2.0 oz.	100% Bloom Petal Fall	2.0	95.0abc

Products	Rate/100/A	Timing	% Infection	% Control
Strep. (FireWall), then Actigard* + Apogee	1 lb. 2 oz.,12 oz.	Strep. 100% blm, then Actigard +Apogee PFall	3.5	91.3bcd
Actigard*, then Strep. Then Actigard*	1 oz/A, then 1 lb., then 1 oz.	50% Bloom, + 100% Bloom, + Petal Fall	4.0	90.0cd
GWN – 10276* (Copper product)	2.8 lbs.	60% then 100% bloom open	4.0	90.0cd
Companion (Bacillus subtilis GB03 liquid)	24 fl.oz.	60% then 100% bloom open	5.0	87.5d
Oxytetracycline (FireLine)	1 lb.	100% bloom open	5.5	86.3d
Provisto* (Copper product)	3 qt	80% and 100% bloom	5.5	86.3d
Cueva (Copper octanoate soap)	4 qt	100% bloom and petal fall	5.5	86.3d
Actigard* + Strep. Then Actigard*	2 oz + 1 lb. 2 oz.	Both at 100% bloom and Actigard only at petal fall	5.5	86.3d
Previsto* (Copper product)	4 qt	2 days after infection	8.5	78.8e
Previsto* then Provisto*	3 qt 4 qt	3 qt Infection day then 4 qt 3 days later	9.0	77.5ef
Optiva (B. subtilis)	24 fl. oz.	50,80 & 100% bloom	9.0	77.5ef
Product E*	Confidential	50,80 & 100% bloom	9.5	76.3ef
Previsto* (GWN10073)	4 qt	2 days after infection	11.0	72.5ef
FireLine (oxytet.) and Provisto*	1 lb. 3 qt	100% Bloom (note: a pH conflict, don't do)	11.5	71.3f
Fungicide X* (secret)	64 fl. oz.	100% bloom and P Fall	16.5	58.8g
Fungicide X*	128 fl. oz.	100% bloom and P Fall	20.5	48.8h
Product Y*	Unknown 4	100% bloom and P Fall	21.5	46.3hi
Product Y*	Unknown 7	100% bloom and P Fall	24.0	40.0i
Inoculated check	**na**	**na**	**40.0**	**0j**

Table 1. 2012 Fire Blight Control Product Efficacy on Apples: Treatments with results followed by the same letter should not be considered different**.** * Indicates the product is not registered for use as of 01/20/ 2014.

Russet Trial:

Treatment Rate per 100 Gallons per Acre	Application Timing	% of Fruit culled due to russet
Untreated, water only.	Pink, 50 & 100% bloom, Petal Fall, Petal Fall + 7 days.	9.4
Blossom Protect (*Aureobasidium pullulans*) 1.34 lb. + Buffer Protect 9.34 lb.	100% Bloom, petal fall and petal fall + 7 days	4.1
Provisto (GWN-10073) 3 qt	100% Bloom, petal fall and petal fall + 7 days	1.3
Provisto (GWN-10073) 4 qt	100% Bloom, petal fall and petal fall + 7 days	0.0
GWN-10276 (Cu, Zn, S product) 1.4 lbs	100% Bloom, petal fall and petal fall + 7 days	7.0
GWN-10276 (Cu, Zn, S product) 2.8 lbs	100% Bloom, petal fall and petal fall + 7 days	6.3
Actigard (ASM) 1 oz, then Actigard 1 oz. + Strep 1 lb., then Actigard 1 oz.	ASM 50% bloom, both at 100%, ASM at P. Fall	1.9
Actigard (ASM) 2 oz, then Actigard 2 oz. + Strep 1 lb., then Actigard 2 oz.	ASM 50% bloom, both at 100%, ASM at P. Fall	6.7
Streptomycin 17% (FireWall) 1 lb.	100% Bloom and P. Fall	3.7
Strep.1 lb, then Actigard 2 oz	100% Bloom, then P. Fall	0.0
Strep. 1 lb., then Actigard 2 oz., then strep. 1 lb 100%, then Actigard.	Pink, 50% bloom, 100% bloom, petal fall.	10.4
Strep. 1 lb, then Actigard 2 oz + Apogee 16 oz.	100% bloom, then P. Fall	9.8
Phyton27 20 oz.	100% bloom, then P. Fall	14.9
Phyton27 40 oz.	100% bloom, then P. Fall	8.7
Phyton27 60 oz.	100% bloom, then P. Fall	10.7

Table 2. Products and timings of application in the 2013 fire blight product russet potential evaluation.

This trial (Table 2) was carried out to assess russeting potential of the various test products in a larger setting than the efficacy plot, and to improve the author's experience and methods for russet induction evaluation. These treatments occurred in a block of Granny Smith and Jonagold apples that was both frosted and heavily infested with powdery mildew. No treatment showed a tendency to induce russet relative to the background russet in the plot. There was too much variation in the natural incidence of russet to sort out subtle differences. No product stood out as a consistent russet inducer. This work will continue and expand in scope next season.

Results and Discussion- Fumigation and Replant, 2013 and total 2011, 2012 and 2013 results:

Treatment A	***PicPlus (175 lbs. per ac: 150 lbs./A chloropicrin, NO 1,3-DCP)***						
Box size	% in box size 2013 crop	Acre yield (lb.)	Wt. by size group	90% pack wt.	Packed boxes	Price*	2013 yr $ by size group
72+	1	25576	256	230	6	$32	184
80/88	17	25576	4348	3913	98	31	3033
100-	82	25576	20972	18875	472	30	14156
						Total	$17,680
		**Minus costs, adjustments of:			$4567	**Adjusted:**	**$13,113**
	Total Adjusted Gross 2011 +12 + 13 crops **$34,854**						
Treatment B	***PicClor 60 (20 GPA: 144 lbs./A chloropicrin, 94 lb/A 1,3-DCP)***						
Box size	% in box size 2013 crop	Acre yield	Wt. by size group	90% pack wt.	Packed boxes	Price*	2013 yr $ by size group
72+	3	32862	986	887	22	$32	710
80/88	32	32862	10516	9464	237	31	7335
100-	55	32862	18074	16267	407	30	12200
						Total:	$20,639
		**Minus costs, adjustments of:			$5,350	**Adjusted:**	**$15,289**
	Total Adjusted Gross 2011 + 12 + 13 crops: **$40,596**						
Treatment C	***Telone C-35 (25 GPA: 98 lb/A chloropicrin, 178 lb/A DC)***						
Box size	% in box size 2013 crop	Acre yield	Wt. by size group	90% pack wt.	Packed boxes	Price*	2013 yr $ by size group
80/88	30	30734	9220	8298	207	31	6431
100-	70	30734	21514	19362	484	30	14522
						Total:	$21,322
		**Minus costs, adjustments of:			$5488	**Adjusted:**	**$15,284**
	Total Adjusted Gross 2011 +12 + 13 crops**: $41,182**						
Treatment D	***Telone C-17 (30 GPA, 51 lb/A chloropicrin 260 lb/A DCP)***						
	% in box size 2013	Acre yield	Wt. by size group	90% pack wt.	Packed boxes	Price*	2013 yr $ by size group
80/88	20	36591	7318	6586	165	31	5104
100-	80	36591	29273	26346	659	30	19759
						Total:	$25,303
		**Minus costs, adjustments of:			$6533	**Adjusted:**	**$18,769**
	Total Adjusted Gross 2011 +12 + 13 crops: **$51,311**						
Treatment E	***Untreated***						
Box size	% in box size 2013	Acre yield	Wt. by size group	90% pack wt.	Packed boxes	Price*	2013 yr $ by size group
72+	0	16792	0	0	0	32	0
80/88	17	16792	2855	2569	64	31	1991
100-	83	16792	13937	12544	314	30	9408
						Total:	$11,600
		**Minus costs, adjustments of:			$2998	**Adjusted**	**$8,602**
	Total Adjusted Gross 2011 + 12 + 13 crops: **$21,285**						

Table 3. Yield per acre, box size grouping and rough estimate of fruit gross economic value per acre. *Approximate FOB average on 11/17/2012. ****Costs, adjustments**: picking @ $20/bin, and packing @ $7 / box. Fumigation @ $650-750/acre accounted for in 2011 cost adjustments. Credit applied for 12 cents/lb. for cull fruit.