01/25/12 **APPLE CROP PROTECTION COMMITTEE**

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FINAL PROJECT REPORT

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

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

Other funding sources: None

Total Project Funding: $37,860

Budget History:

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

Footnotes:

[1]hourly worker (Tess Barlow)

[2]laboratory supplies (reagents, DNA extraction kits and associated materials, plastic ware, supplies for microscopy, collecting bags, misc)

[3]travel (7 trips from Seattle to collect research specimens in orchards, 3 nights, 4 days, per diem, est. at $614/trip)

[4]Gene sequencing (estimated 800 runs @ $10 each)

OBJECTIVES

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

Activities include:

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

SIGNIFICANT FINDINGS

This project is nearly complete, final results will be available this year once data analysis is finished..

Summary of significant findings:

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

RESULTS & DISCUSSION

We compiled data on 692 apple powdery mildew specimens collected from 61 sites. Specimens sequenced so far were selected to maximize geographic diversity. To date, only *Podosphaera leucotricha* has been detected. The following information summarizes the apple varieties collected, the number of collecting sites and the number of specimens from which variety that were collected.

Variety	# Collecting Sites	# Specimens
Braeburn	1	9
Fuji	10	119
Gala	17	193
Ginger Gold	2	23
Golden Delicious	3	34
Granny Smith	11	110
Honey Crisp	9	92
Jonathan	2	23
Pink Lady	2	26
Rubens	1	12
Variety to be confirmed	3	60

161 cherry powdery mildew samples were collected from 13 sites. Specimens sequenced were selected to maximize geographic diversity. Only *Podosphaera clandestina* was detected from samples taken in commercial orchards. Collections from a single wild *Prunus* tree yielded what might possibly be a different species; further work is under way on those specimens. The following information summarizes the cherry varieties collected, the number of collecting sites), and the number of specimens from which variety that were collected.

Variety	# Collecting Sites (# sequencing completed)	# Specimens
Bing	7	89
Rainier	1	6
Sweetheart	2	20
Van	1	2
Wild *Prunus*	1	3
Variety to be confirmed	4	41

We detected only *Podosphaera leucotricha* on apple and only *Podosphaera clandestina* on commercial cherry cultivars. Information from this study may facilitate exporting apples and cherries to other countries.

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

Results have suggested that ITS and 28S regions from strains of powdery mildews attacking apples and cherries are invariable. Results suggest that these regions are potentially very useful for PCR-based approaches to detecting these species.

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

EXECUTIVE SUMMARY

During the study a total of 692 specimens of apple powdery mildew were collected from 10 cultivars in 61 sites. A total of 522 ITS and 28S rDNA sequences were obtained as part of the characterization of the specimens. *Podosphaera leucotricha* was the only species found on apple. 161 specimens from four cultivars in 13 sites were collected from cherry. *Podosphaera clandestina* was the only species found on commercial cherry, although a different strain (and possibly species) was found on a wild cherry in a single location.

Results were somewhat surprising as older literature suggested several species might be found on these hosts. The results do suggest that at present *P. leucotricha* is the primary (and perhaps only) species to be found on apple, and *P. clandestina* is the primary (and perhaps only) species on cherry. The results did not provide any indication that other species occurred on these hosts.

Significance of the findings includes the following:

- This is the only extensive study of apple and cherry powdery mildew in Washington that included analysis of DNA sequences. The sequence data was useful in characterizing the mildews and reinforced the conclusion that apples and cherries each were attacked by a single species.

- There appeared to be no variation in ITS and 28S rDNA species in the specimens studied. This finding suggests that rDNA sequence data could be very useful in developing PCR-based detection systems for these powdery mildews.

- Findings did not support the possibility that unusual powdery mildews occur on these crops, possibly simplifying exporting them to international markets.

- Results also suggest that apple and cherry breeding programs can focus their efforts on developing cultivars with resistance to *P. leucotricha* and *P. clandestina*, respectively. While it is possible that population-level differences in virulence among powdery mildew species may exist, there is no evidence that multiple species attack these crops in Washington.

Implications for future research include the following:

- Because the apple and cherry powdery mildews behaved differently in the field (the cherry mildew populations appeared to increase more slowly), it did not prove efficient to collect species during the same collecting trips. Future research projects on these powdery mildews should take this difference into account (i.e., it is not practical to collect both species at the same time).

- It proved more difficult to amplify powdery mildew DNA as the season progressed. Many of the sequences amplified late in the season represented yeasts rather than powdery mildews. Because some yeasts are known to parasitize or suppress powdery mildews, this incidental observation suggests that interactions between powdery mildews and yeasts might deserve future study, both to provide a clearer picture of powdery mildew behavior and the possibility that naturally-occurring powdery mildew parasites might have beneficial activity in the field (such as reducing primary inoculum during the following season).

- Because of rather low incidence of cherry powdery mildew during the study period, relatively few specimens were collected. It may be useful do additional work to characterize more specimens.

FINAL PROJECT REPORT

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

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Other funding sources: None

Total Project Funding: $54,000

Budget History:

Item	Year 1: 2010	Year 2: 2011	
Salaries	$4,854[1]	$4,854[1]	
Benefits	$156	$156	
Wages	0	0	
Benefits	0	0	
Equipment	0	0	
Supplies	$10,000[2]	$10,000[2]	
Travel	0	0	
Plot Fees	0	$10,000[3]	
Miscellaneous	$14,000[4]	0	
Total	$29,000	$25,000	

Footnotes: [1]Salary is for summer undergraduate student to assist Norelli in determining fire blight resistance of GMAL4593 population (Obj. 1). [2]Supplies are to identify additional molecular markers (SNP) in *M. sieversii* (Obj. 2). [3]Plot fees were for establishment of *M. sieversii* at USDA-ARS, Kearneysville, WV ($4,000) and at WSU-TFREC, Wenatchee, WA ($6,000) (Obj. 3). [4]Miscellaneous year 1 was to propagate 10 replicates of 200 *M. sieversii* accessions at Van Well Nursery, East Wenatchee, WA (Obj. 3).

Original Objectives:

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

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

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

Significant Findings:

- Fire blight resistance in GMAL4593 mapping population was associated with molecular markers on chromosomes (linkage groups) 10 and 8 of *M. sieversii.*

- Both environment and the strain of the pathogen used for inoculation had an effect on the association of specific genetic markers to the fire blight resistance trait. However, three markers located between 20-30 cMs on linkage group 10 were consistently associated with fire blight resistance in the field in both 2010 and 2011.

- The total number of molecular markers in the *M. sieversii* GMAL4593 map increased from 107 (Wisniewski 2009 final report) to 163 markers.

- This project succeeded due to collaboration with RosBREED project to re-sequence *M. sieversii* parent of GMAL4593 and identify *M. sieversii*-specific molecular markers to fill gaps within the molecular map.

- All available field and greenhouse data on fire blight resistance of the *M. sieversii* collected by the USDA in Kazakhstan was analyzed and 191 accessions were selected for the identification of additional sources of fire blight resistance. Replicate trees of 202 selected accessions and control cultivars were propagated at Van Well Nursery, East Wenatchee, WA. Field plantings will be established this spring at WSU-TFREC, Wenatchee, WA and USDA-ARS, Kearneysville, WV.

Results & Discussion:

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

The GMAL4593 mapping population consists of seedling progeny derived from a cross between 'Royal Gala' and *M. sieversii* PI613981. To successfully map a genetic locus for fire blight resistance within this population, the population needs to segregate for resistance. In other words, when challenged with the fire blight pathogen, some individuals within the population need to be consistently resistant to the disease, and others need to be consistently susceptible to the disease. Previous (2008) greenhouse evaluation of GMAL4593 for fire blight resistance by Herb Aldwinckle (Cornell University) and Phillip Forsline (USDA-ARS) in Geneva, NY found the population to be segregating for fire blight resistance.

In 2010, approximately 2,700 vigorously growing shoots on 3-4 replicate field grown trees of the same 190 GMAL4593 individuals used in the 2008 greenhouse trial were challenged with fire blight. Similar to the 2008 trial, disease varied among individuals in the population with the percent of the current season's shoot length that became diseased ranging from 5 to 100%. However, the disease rating obtained in the 2008 greenhouse and 2010 field tests were not correlated. In other words, the individuals found resistant in the greenhouse test were not the same as those identified as resistant in the field trial. Furthermore, when fire blight resistance was mapped within the GMAL4593, fire blight resistance determined in the greenhouse was associated with markers on linkage group (LG) 8, whereas resistance determined in the field was associated with markers on LG10.

There were several possible causes for this discrepancy. The 2008 greenhouse test was conducted with a native NY fire blight strain (Ea273) whereas 2010 field test was conducted with a strain from the AFRS collection and it was possible that the genetic loci controlling fire blight resistance in GMAL4593 are specific to different strains of the fire blight bacteria. To address this possibility, the GMAL4593 population was challenged with three strains of the pathogen in 2011 from NY (Ea273 used in 2008 trial), WV (AFRS used in 2010 trial) and WA. Other possible causes for the discrepancy included: 1) differences between the greenhouse and field environments, 2) both genetic loci on LG10 and LG8 were contributing to resistance, and 3) the fire blight resistance observed within the GMAL4593 was due more to environment than genetics and reliable markers for resistance could not be determined.

In 2011, approximately 2,900 vigorously growing shoots on 3 replicate field grown trees were challenged with 3 strains of the fire blight pathogen. The percent of the current season's shoot length that became blighted (%SLB) amongst the individuals of the GMAL4593 population ranged from 3 to 100%. The AFRS strain caused the most disease with an average of 88 %SLB, followed by Ea273 with 41 %SLB and the WA strain with 33 %SLB. The results obtained with strain AFRS in 2011 were very similar to those obtained in 2010 and resistance was associated with markers on LG10 (Table 1, next page) rather than LG8 (Table 2, subsequent page). The results obtained with strain Ea273 and the WA strain were very similar to each other. Although there was some variation in the evaluation of fire blight resistance in the GMAL4593 population obtained with the Ea273 and WA strains and that obtain with AFRS, all the strains showed highly significant association with 3 markers on LG10: GDsnp00307 (21.1 cM), U50187SSR (29.7 cM) and USDA-ARS_DIP1 (30.1 cM) (Table 1). These markers were also highly associated with resistance in the 2010 trial using the AFRS strain (Table 1). Although the markers also had some association with resistance in the 2008 greenhouse trial, it was not highly significant (Table 1). In conclusion, these three markers on LG10 were the markers most consistently associated with fire blight resistance in the GMAL4593 population and will be validated in future studies.

The resistance observed when the population was challenged with strain Ea273 in the field (2011) also showed significant association with markers on LG08 (Table 2). However, only one of the LG8 markers (GDsnp1643539, 41.8 cM) that was highly associated (P=0.005) with resistance in the 2008 greenhouse trial using strain Ea273 was also associated with resistance in the 2011 trial with strain Ea273. Markers CH05a02, NH005b and GDsnp00584 which highly associated in the 2008 trial, showed no association in the 2011 trial.

The analysis of fire blight resistance at both of these loci (LG10 and LG8) is continuing, however additional funds for this aspect of the research are not being requested from WTFRC. Currently, we plan to incorporate the fire blight resistance found in GMAL4593 into genetic backgrounds with superior fruit quality using Fastrack breeding, or accelerating breeding that takes advantage of shortened juvenility of apple by transgenic expression of transcription factors controlling flowering. To validate the markers identified in this study, progeny resulting from these crosses will be screened

for resistance using both markers on LG10 and LG8 and by controlled inoculation with the fire blight pathogen.

Table 1: Association between fire blight resistance and genetic markers on **LG 10** determined by rank sum test of Krusgal-Wallis. Significance levels: -:not significant, *:0.1, **:0.05, ***:0.01, ****:0.005 and *****:0.001.

Group	Position (cM)	environment:	green-house	field	field	field
		year:	2008	2010	2011	2011
		pathogen strain:	Ea273	AFRS	AFRS	Ea273
		Locus	KW significance			
LG10	0.0	GD_SNP00260b	-	-	-	**
LG10	2.0	GD_SNP01710	***	-	**	**
LG10	2.3	CH01f12	**	*	*	****
LG10	4.5	CH03d11	-	-	-	-
LG10	8.9	GD_SNP00869	-	*	*	*****
LG10	14.2	DIP47HRM	-	-	-	**
LG10	20.1	GD_SNP00307HRM	**	****	****	****
LG10	29.7	U50187SSR	**	****	*****	****
LG10	30.1	DIP1HRMa	**	*****	****	***
LG10	34.0	GD_SNP00360b	-	***	*	-
LG10	34.1	GD_SNP00355b	-	**	-	-
LG10	38.6	GD100	-	*	-	-
LG10	38.8	Hi22a07x_202	-	-	-	-
LG10	39.7	Hi05b02	-	*	-	-
LG10	42.3	DIP5HRM	-	-	-	-

Markers consistently associated with resistance in field

Table 2: Association between fire blight resistance and genetic markers on **LG 08** determined by rank sum test of Krusgal-Wallis. Significance levels: -:not significant, *:0.1, **:0.05, ***:0.01, ****:0.005 and *****:0.001.

Group	Position (cM)	environment:	green-house	field	field	field
		year:	2008	2010	2011	2011
		pathogen strain:	Ea273	AFRS	AFRS	Ea273
		Locus	KW significance			
LG08	0.0	GD_SNP00246b	-	-	-	-
LG08	0.2	GD_SNP00299b	-	-	-	*
LG08	7.2	DIP21HRMb	**	-	-	****
LG08	8.4	GD_SNP00293a	-	-	-	*
LG08	17.2	GD_SNP1699552	-	-	-	-
LG08	28.8	CH05a02_126	****	-	-	-
LG08	29.3	NH005b	****	-	-	-
LG08	29.4	CH05a02_114	****	-	-	-
LG08	30.5	CH01f09	-	-	*	*
LG08	32.9	GD_SNP01004	-	-	-	**
LG08	41.8	GdSNP1643539	****	**	-	***
LG08	42.1	GD_SNP00584HRM	*	-	-	-
LG08	44.2	GD_SNP00584HRM	****	-	-	-
LG08	46.4	CH01e12	*	-	-	-
LG08	47.0	CH01c06	*	-	-	-
LG08	47.0	CH01c06b	**	-	-	**
LG08	47.2	CH01c06_155	**	-	-	**
LG08	50.9	DIP17HRM	-	-	-	-
LG08	61.1	GD_SNP00175HRM	**	-	-	-
LG08	70.5	DIP18HRM	-	*	-	**

The resistance observed when the GMAL4593 population was challenged with the AFRS strain did not show highly significant association with markers ('Locus' in table) on LG08 (2 middle columns under 'KW significance'). Although results with strain Ea273 did show significant association with markers on LG08, the results obtained for specific markers were generally inconsistent in that the markers most associated with resistance in 2008 greenhouse trial were not associated with resistance in 2011 field trials (except for GDsnp1699552).

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

A total of 921 molecular markers were screened in the GMAL4593 mapping population. These included simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and deletion-insertion polymorphisms (DIPs). In order for the markers to be informative in the *M. sieversii* map they must segregate either in *M. sieversii* or in both parents. Among the SSR markers, ca. 40% of the markers were informative for *M. sieversii* PI613981, whereas ca. 65% were informative for 'Royal Gala'. Among the SNP markers, only ca. 20% of markers were informative for *M. sieversii*, compared with 50% for 'Royal Gala'. The lower occurrence of SNP markers in *M. sieversii* is probably due to the broader "genetic base" or greater diversity of genes in *M. sieversii* compared to domesticated apple, making it less likely that any specific SNP markers will be present in a specific individual.

Currently, there are a total of 375 informative markers for *M. sieversii* PI613981. Of these, 252 could be assigned to specific chromosomes or "linkage groups" and 163 were mapped. All of the 17 chromosomes of apple are covered by the current map. Coverage of the individual linkage groups ranges from 50% to 95% of the 'Golden Delicious' genome reference map with an overall average of 73% coverage. Based upon the genome reference map there is on average one marker per 8.3 cM of genetic distance. Within linkage groups 8 and 10 that were associated with fire blight resistance, there is on average one marker per 3.7 and 6.3 cM, respectively.

The current map provides an excellent foundation for additional research on this population and *M. sieversii*. The map will be deposited and publicly available at the Genome Database for the Rosaceae (GDR). We are currently resolving segregation distortion amongst some of the SSR markers by reanalyzing the data using alternative software and expect to have a map completed for deposit at GRD within 3 months. In addition to this work on fire blight, resistance to apple scab (*Venturia inaequalis*), resistance to blue mold (*Penicillium expansum*) and water use efficiency is being evaluated within the GMAL4593 population at USDA-ARS Kearneysville, WV.

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

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

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

In addition to selecting accessions for a balanced fire blight resistant : susceptible ratio, additional accessions were selected based upon resistance to both fire blight and apple scab, and superior fruit quality. The remainder of the CORE collection was also included to insure full genetic and character representation of *M. sieversii* in the plantings.

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

Budwood of 202 selected accessions and control cultivars was collected at the USDA-ARS-Plant Genetic Resources Unit in Geneva, NY in late July 2010 and sent to Van Well Nursery, East Wenatchee, WA for propagation. The material was propagated on M.7 rootstock. The trees were dug and stored in fall 2011 and will be planted at sites both WSU-TFREC, Wenatchee, WA (3 reps per accession) and USDA-ARS, Kearneysville, WV (4 reps per accession). Each planting in WA and WV will include the same 202 *M. sieversii* accessions and controls.

Executive Summary

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

This project had two main goals: 1) to identify molecular markers for fire blight resistance in the *M. sieversii* GMAL4593 mapping population and 2) establish plantings of *M. sieversii* at WSU-TFREC, Wenatchee, and USDA-ARS, Kearneysville, WV to be able to identify stronger sources of fire blight resistance for scion breeding in the future.

Approximately 60 additional molecular markers were added to the *M. sieversii* GMAL4593 in order to identify markers for fire blight. All of the 17 chromosomes (linkage groups) of apple are covered by the current map. Coverage of the individual linkage groups ranges from 50% to 95% of the 'Golden Delicious' genome reference map with an overall average of 73% coverage. The current map provides an excellent foundation for additional research on this population and *M. sieversii*. The map will be deposited and publicly available at the Genome Database for the Rosaceae (GDR). In addition to this work on fire blight, resistance to apple scab (*Venturia inaequalis*), resistance to blue mold (*Penicillium expansum*) and water use efficiency is being evaluated within the GMAL4593 population at USDA-ARS Kearneysville, WV using the genetic map generated in this project.

Fire blight resistance in GMAL4593 mapping population was associated with molecular markers on chromosomes (linkage groups) 10 and 8 of *M. sieversii.* The population was evaluated for fire blight resistance in the greenhouse and in the field over multiple year using multiple strains of the pathogen to insure that the effects of both environment and genetics were taken into consideration in the genetic analysis. Although both environment and the strain of the pathogen used for inoculation had effects on fire blight resistance, three markers located between 20-30 cMs on linkage group 10 were consistently associated with fire blight resistance in the field in both 2010 and 2011. The resistance locus identified on LG8 was more greatly affected by both environment and strain of the pathogen. The analysis of fire blight resistance at both of these loci (LG10 and LG8) is continuing, however additional funds for this aspect of the research are not being requested from WTFRC. Currently, we plan to incorporate the fire blight resistance found in GMAL4593 into genetic backgrounds with superior fruit quality using Fastrack breeding, or accelerating breeding that takes advantage of shortened juvenility of apple by transgenic expression of transcription factors controlling flowering. To validate the markers identified in this study, progeny resulting from these crosses will be screened for resistance using both the markers identified on LG10 and LG8 and by controlled inoculation with the fire blight pathogen.

The GMAL4593 population was created based upon the appearance of the *M. sieversii* growing in Kazakhstan before the several thousand seed of *M. sieversii* collected in Kazakhstan were characterized and genetically analyzed. To identify better sources of fire blight resistance for future breeding, all available field and greenhouse data on fire blight resistance and fruit quality of the *M. sieversii* collected by the USDA in Kazakhstan was analyzed and 191 accessions were selected for evaluation. Replicate trees of 202 selected accessions and control cultivars were propagated at Van Well Nursery, East Wenatchee, WA. Field plantings will be established this spring at WSU-TFREC, Wenatchee, WA and USDA-ARS, Kearneysville, WV. Additional funds are being requested from WTFRC to challenge these planting with the fire blight pathogen and determine the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

FINAL PROJECT REPORT

Project Title: Apple specific issues in fire blight management

PI: Ken Johnson
Organization: Oregon State University
Telephone/email: 541-737-5249 johnsonk@science.oregonstate.edu
Address: Dept. Botany and Plant Pathology
Address 2: 2082 Cordley Hall
City: Corvallis
State/Zip OR 97331-2902

Cooperators:
Materials: Arysta Life Sciences, Croker's Fish Oil, Syngenta, Westbridge Agric. Products

Other funding sources: None

Total Project Funding: $ 39,200

Budget History:

Item	**2010**	**2011**	
Salaries FRA 3mo	10,000	10,300	
Benefits OPE 63%	6,300	6,489	
Wages			
Benefits			
Equipment			
Supplies	2,000	2,111	
Travel local	500	500	
Plot Fee	500	500	
Miscellaneous			
Total	19,300	19,900	

OJECTIVES:

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

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

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

SIGNIFICANT FINDINGS:

- The product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of apple; EPA registration is on track for 2012.
- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.
- Effective non-antibiotic strategies for fire blight control were developed. These strategies are being implemented for apples exported under the International Organic Program standard.
- A yeast material, Blossom Protect, provided excellent control of fire blight, with registration and utilization within the International Organic Program expected in 2012.
- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), slowed expansion of fire blight in inoculated shoots of potted apple rootstock cultivars ELMA 26 and Nic 29.
- ASM applied to potted 'Gala' on M26 provided a high level of protection of the rootstock after a high dose of the pathogen was inoculated directly into the graft union.

RESULTS

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards.

1. a) 2011 season. Treatments of Kasumin alone and in combination with other materials where tested in 2011, marking five years of evaluation. Trees used in the 2011 study averaged 587 flower clusters per tree. Fire blight risk as determined by the COUGARBLIGHT model was low during the bloom period but disease intensity was moderate with water treated trees averaging 78 blighted clusters per tree (14%) (Table 1). Each of the treatments significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree compared to the water-treated control. Kasumin 2L and 8L (kasugamycin), Firewall (streptomycin), and Fireline (oxytetracycline) provided excellent disease control. The integrated program of Bloomtime in early bloom followed by Fireline, or Kasumin and Fireline at full bloom also provided excellent control. The pathogen inoculum used in the study was 50% streptomycin-resistant and 50% streptomycin-sensitive *E. amylovora*. Firewall (streptomycin) suppressed disease by 85%, whereas Kasumin 2L provided 93% control.

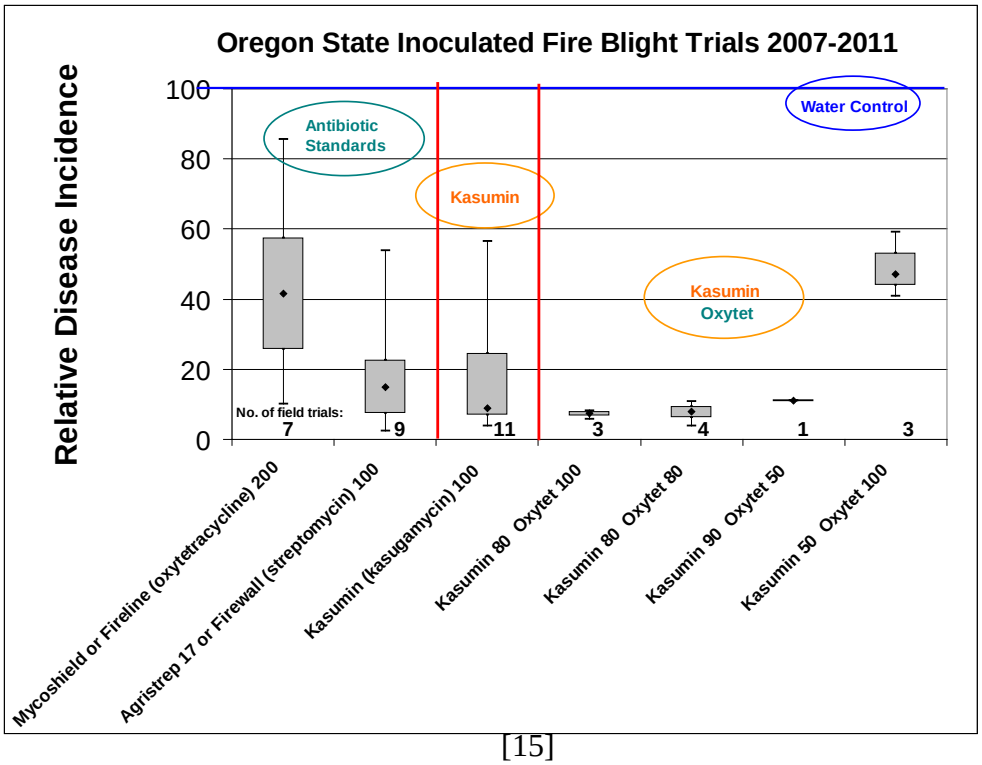
Table 1. Evaluation of Kasumin for suppression of fire bli8ght of Gala apple, 2011

Treatment	Rate per 100 gallons water	Date treatment applied* 4 May 30% bloom	6 May 70% bloom	10 May Full bloom	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	------	**X**[§]	**X**	**X**	**78**	**a**[#]	**14.1**	**a**[#]
Agri-mycin 100 ppm	8 oz.	---	---	X	13	b	2.1	b
Bloomtime then	5 oz.	X	X	---	10	bc	1.6	bc
Kasumin 2L 90 ppm	52 fl oz.	---	---	X				
plus Fireline 50 ppm	4 oz.	---	---	X				
Bloomtime then	5 oz.	X	X	---	8	bc	1.4	bc
Fireline 200 ppm	16 oz.	---	---	X				
Kasumin 8L 100 ppm	16 fl. oz.	---	---	X	8	bc	1.4	bc
Kasumin 2L 100 ppm	64 fl. oz.	---	---	X	5	c	1.0	c

* Trees inoculated on 9 May with 5 x 10^5 CFU/ml of a 50:50 mix of *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) and strain Ea153S (streptomycin-resistant). ** Transformed log (x + 1) prior to analysis of variance; non-transformed means are shown. *** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. [§] X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date. [#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at P = 0.05.

1.b) Summary Kasumin field trials from 2007 to 2011. A total of nine orchard trials in apple and pear were conducted over the period. Data were summarized in box and whiskers plots as 'Relative Disease Incidence', which for each trial is the number of fire strikes in the Kasumin (or comparative)

Fig. 1



treatment divided by the number of fire strikes in the water-treated control (expressed as a percentage) (Fig. 1). Kasumin achieved a median reduction of fire blight of > 90%, which was equivalent to streptomycin (targeted to streptomycin sensitive performed strains of the fire blight pathogen) and better than the median 58% control obtained with oxytetracycline. [Note: trials in 2007, 2008, and 2009 had two treatments of each antibiotic, and had trials in 2010 and 2011 had one treatment.]

1.c) Compatibility of Kasumin with biological control. By first treating trees with a biological agent (BlightBan C9-1 or Bloomtime Biological) followed by Kasumin, an 'integrated strategy' reduces the likelihood of selection for kasugamycin-resistance in the pathogen. The mechanisms that reduce selection pressure are suppressed pathogen populations via competition with the biological agent and limitation of Kasumin use (e.g., to one application as opposed to the two applications often typical in commercial production). Over the 2007 to 2011 period, the orchard trials showed that there was no statistical difference between integrated control with Kasumin compared to Kasumin alone (Table 1 (above) and Table 2 (below)). We also hypothesized that use of Kasumin could have a negative impact on populations of the biological agent on flowers and that this effect could be overcome by use of a kasugamycin-resistant strain of *Pantoea vagans* strain C9-1S, thereby improving the efficacy of the biological component of the integrated strategy. In the field, data collected on disease incidence and on population sizes of the biological agents on flowers (data available on request) failed to support this hypothesis. We concluded that use of Kasumin 2-3 days after a biological treatment would be expected to have minimal impacts on populations of bacterial biological control agents. In this regard, the effect of Kasumin on non-target bacteria on flowers was more like that observed with oxytetracycline than observed with streptomycin.

Table 2.

Integrated control with Kasumin and Kasumin-resistant BlightBan C9-1S		Fire blight strikes per tree
2009 Bartlett Pear	**Water**	**485 a**
	***P.v.* C91S^{kr} then Kasumin**	**38 b**
	***P.v.* C91S then Kasumin**	**42 b**
	Kasumin twice	**33 b**
2009 Gala & Golden Delicious Apple	**Water**	**132 a**
	***P.v.* C91S^{kr} then Kasumin**	**18 b**
	***P.v.* C91S then Kasumin**	**16 b**
	Kasumin twice	**8 b**
2010 Gala Apple	**Water**	**236 a**
	***P.v.* C91S^{kr} then Kasumin**	**14 b**
	***P.v.* C91S then Kasumin**	**16 b**
	Kasumin once	**20 b**

Means within cultivar and year followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Experiments conducted in orchards located near Corvallis, Oregon. *Pantoea vagans* C9-1S^{Kr} is a kasugamycin-resistant selection of *Pantoea vagans* C9-1S (the organism in BlightBan C9-1 and related to *Pantoea agglomerans* strain 325 in Bloomtime Biological).

When registered, Kasumin will enhance control and broaden the effective tool box for conventional fire blight management. Kasumin is not used in human medicine, and shows no cross resistance to streptomycin or oxytetracycline. Although not shown in our research, work by others researchers indicate Kasumin is slightly less effective than streptomycin (against sensitive strains) with tested under extreme disease pressure (very high inoculum levels). Speculating, Kasumin may not be absorbed into pear or apple tissue as readily as streptomycin, which is considered locally systemic. Analogous to oxytetracycline, sprays of Kasumin should be timed at full to late bloom beginning when moderate (as opposed to high) levels of disease risk have been forecasted.

In the years of this project, McGhee & Sundin (2011) characterized resistance in the fire blight pathogen to kasugamycin. Analogous to our results with biocontrol agent *Pantoea vagans*, selection of kasugamycin-resistant strains of the fire blight pathogen with the ability to grow at the maximum label rate of Kasumin (100 ppm) is a two-step mutation process. In contrast, a spontaneous mutation in these bacteria to resistant to the label rate of streptomycin is a one step process, and a spontaneous mutation to resistant to oxytetracycline has not been reported (even from the lab). Thus, the risk of selecting resistance in *E. amylovora* to Kasumin is apparently intermediate to the other registered antibiotics or fire blight control. A potential negative of Kasumin, reported rarely, is a rate-dependent phytoxicity to pear and apple (e.g., we have never observed a phytotoxic response in our use of Kasumin). Our results showing outstanding fire blight control with Kasumin at slightly reduced rates mixed with oxytetracycline (Fig. 1) may be a solution to a potential phytoxicity problem if it is ever observed (e.g., on a particular cultivar).

2) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets.

2.a) Frequency of treatment. In orchard trials, for both pear and apple, increasing the frequency of treatment of biological products improved blossom blight control. For example, a *Pantoea agglomerans* product (Bloomtime Biological or BlightBan C9-1) applied at 30 and 70% bloom followed by two applications of the fermentation product of *Bacillus subtilis* QST 713 (Serenade Max) at full bloom and petal fall reduced the incidence of blighted flower clusters by an average of 71% (four orchard trials) compared to 47% when each component of this product combination was used only once.

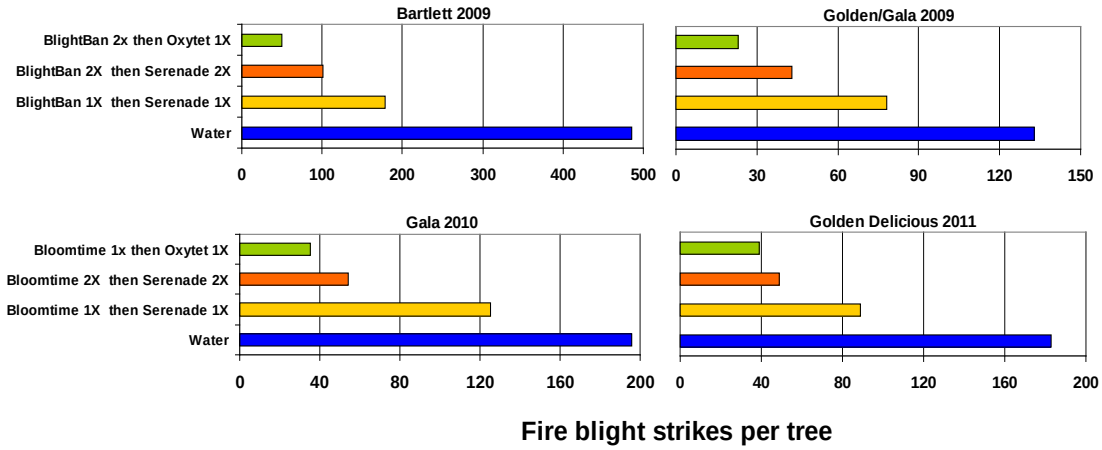


Fig. 2. Incidence of fire blight on pear and apple flower clusters as affected by integrated biological and antibiotic treatments in orchard trials conducted near Corvallis, Oregon from 2009 to 2011.

2.b) Effect of bloom thinning on fire blight . In apple, fruit load (bloom) thinning with 2% lime sulfur and 2% fish oil at 30 and 70% bloom significantly ($P \leq 0.05$) reduced the proportion of blighted flower clusters in 3 of 4 orchard trials.

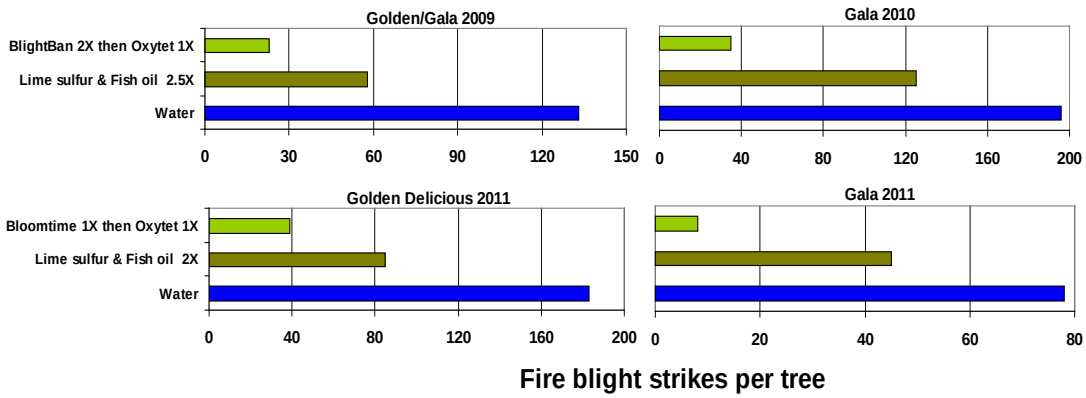


Fig. 3. Incidence of fire blight on pear and apple flower clusters as affected by as affected by the bloom thinning treatment, lime sulfur plus fish oil, in orchard trials conducted near Corvallis, Oregon from 2009 to 2011.

2.c. Integrated non-antibiotic control. Over three orchard trials, treatment with *Aureobasidium pullulans* (Blossom Protect) after LS+FO reduced the incidence of fire blight by an average of 91% blight control compared to trees treated with water only; this level of control was similar to treatment with streptomycin.

Table 3. Incidence of fire blight on apple flower clusters as affected by the bloom thinning treatment, lime sulfur plus fish oil, and subsequent biological treatments in orchard trials[x] conducted near Corvallis, Oregon from 2009 to 2011.

	Orchard cultivar and year of trial		
Treatment	Golden Delicious 2010 (%)	Golden Delicious 2011 (%)	Gala 2011 (%)
Water	32.5 a (261)	34.9 a (183)	14.1 (78)
Lime sulfur plus fish oil twice	27.3 a	15.5 b	8.9 b
Lime sulfur plus fish oil twice then Blossom Protect twice	3.2 b	3.7 c	0.9 c
Lime sulfur plus fish oil twice then Bloomtime Biological combined with Blossom Protect once then Blossom Protect once	3.1 b	1.5 c	1.7 c
Bloomtime Biological twice then Blossom Protect twice	3.6 b	-	2.5 c

Bloomtime Biological once then oxytetracycline once	6.8 b	7.4 bc	1.4 c
Streptomycin sulfate once	2.5 b	-	2.1 c

[x] Single tree plots were arranged in a complete randomized block design with three to four replications per treatment. The bloom thinning treatment, lime sulfur (2%) plus fish oil (2%), was sprayed at 30 and 70% bloom. The biological materials following LS+FO were applied at full bloom and prior to petal fall. In the comparative standard (23), Bloomtime Biological was applied at 70% bloom and oxytetracycline was applied at full bloom.

[y] Within a column, means followed by the same letter do not differ significantly according to Fischer's protected least significance difference at *P* = 0.05. The arc-sine square root transformation was applied incidence data prior to analysis of variance.

[z] Numbers in parentheses are the mean number of blighted flower cluster on water-treated trees. Incidence was computed by dividing number of blighted flower cluster by total number of cluster.

The integrated approach demonstrated by Stockwell et al. (2008) and Lindow et al. (1996) utilized a gram negative bacterial antagonist (e.g. *P. agglomerans* and/or *Pseudomonas fluorescens*) to suppress the pre-requisite epiphytic phase of *E. amylovora* on floral stigmas followed by an oxytetracycline treatment later in bloom to prevent infection in the nectary. A non-antibiotic program modeled on this strategy involved substitution of the biological product, Serenade Max, for oxytetracycline. In this role, Serenade Max proved to be inhibitory to the fire blight pathogen, but obtaining levels of fire blight control closer to that achieved with oxytetracycline after a bacterial antagonist required doubling the frequency of application of the biological products (Fig. 2). This result indicates that non-antibiotic programs for fire blight will likely be more expensive than programs utilizing antibiotics because satisfactory disease control will require more treatments in the orchard. Spraying more often also means that orchardists will need to be more preventative in their approach to fire blight control (i.e., sprays required every few days) as opposed to reactive, where a single antibiotic spray could be applied based on an imminent infection event forecasted by a disease warning model (CougarBlight).

The yeast product, Blossom Protect, provided outstanding disease control when used either as part of an integrated program following *P. agglomerans* or following bloom thinning treatments of LS+FO (Table 3). In organic pome fruit production, the former program would have the highest value in pears where bloom thinning is not practiced routinely, whereas yeast treatments after LS+FO would be more practical in apples. A negative side effect of *A. pullulans* (the yeast organism in Blossom Protect) when used in a wetter climate has been a tendency to induce skin russet on the surfaces of developing fruit (Kunz 2008, Spotts & Cervantes 2002), which we also observed in our 2011 Gala apple trial when frequent rains occurred neared the end of the bloom period. Russeting of fruit surfaces can greatly reduce crop value, and thus an improved understanding of this potential in various climates should be obtained before this product is used extensively. EPA registration for Blossom Protect is on track for spring 2012.

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

3.a). Rootstock only experiment. Drenches and paints of the a SAR material, ASM (acibenzolar-*S* methyl) slowed expansion of fire blight in one year old, potted apple rootstock cultivars, EMLA 29 and Nic 29 apple (Fig. 4). In non-treated trees, the expanding canker consumed nearly all of the current season growth, but generally did not expand into the woody tissue. Green shoots on SAR-treated trees had smaller cankers relative to the non-treated trees, but the effects of the SAR treatment on apple rootstocks was smaller than was observed previously in pear (see 2009 pear report). In pear,

the marked effect of ASM on canker expansion occurred mostly in woody tissue. For apple rootstock cultivars, we attribute the smaller effect of ASM to the fact that cankers were limited to the current season shoots. Interestingly, relative to drench and spray treatments, the ASM paint treatment (15 g a.i./L) applied at the at inoculation showed the largest reduction in canker size (top right panel).

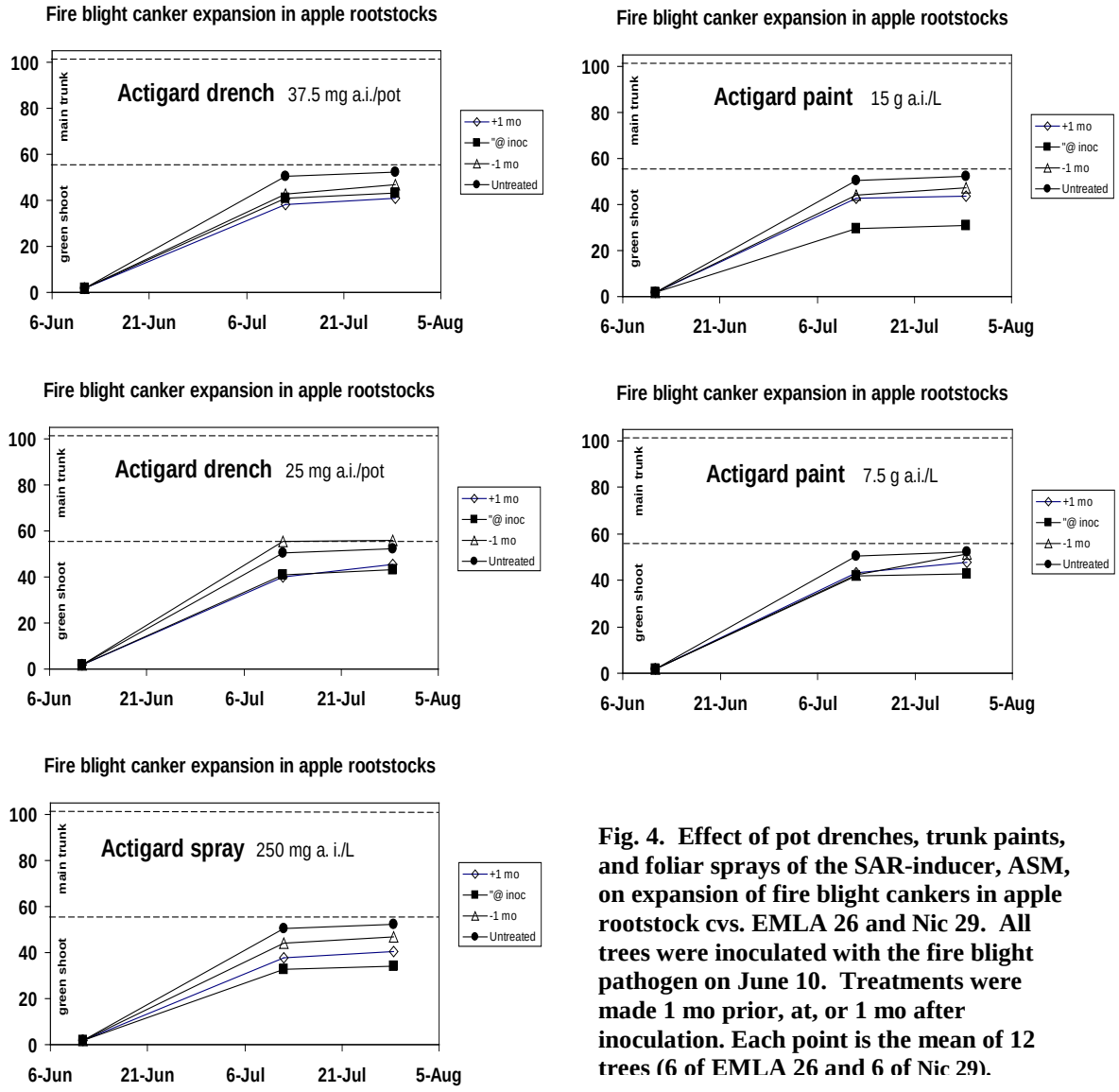
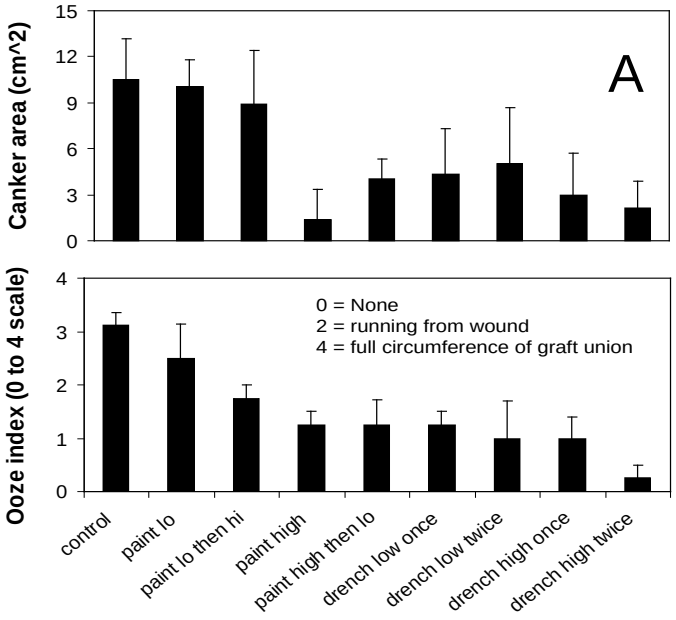


Fig. 4. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expansion of fire blight cankers in apple rootstock cvs. EMLA 26 and Nic 29. All trees were inoculated with the fire blight pathogen on June 10. Treatments were made 1 mo prior, at, or 1 mo after inoculation. Each point is the mean of 12 trees (6 of EMLA 26 and 6 of Nic 29).

3.b). Rootstock Scion experiment. Drenches of ASM, but also some ASM-paint treatments, provided significant control of canker expansion in wound-inoculated, EMLA 26 rootstock (under Gala) measured in August of 2010 (Fig. 5 A). In particular, two drenches (May 21 and July 2) of ASM (50 mg a.i. per plant) provided nearly complete suppression of canker development. By October, scions of several of the untreated trees were dead as a result of the girdling fire blight canker in the rootstock. In contrast, none of the ASM-treated trees were girdled. The re-assessment of these treatments in May 2011 showed that canker size had-increased from August 2010, and that drenches and several paint treatments still showed significant suppression of canker size (Fig. 5B). This greenhouse experiment was repeated in 2011 with EMLA 26 and Nic 29 rootstocks (under Cameo).

Preliminary observations show responses similar to what was observed for the 2010 experiment, but measurement of canker size was delayed to spring 2012. A field trial similar to the greenhouse experiment also was conducted in 2011 (EMLA 26 under Gala) but our inoculations failed, due to drying and cracking of the trunk wraps placed over the inoculation wounds. Field experiments will be initiated again in 2012.



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

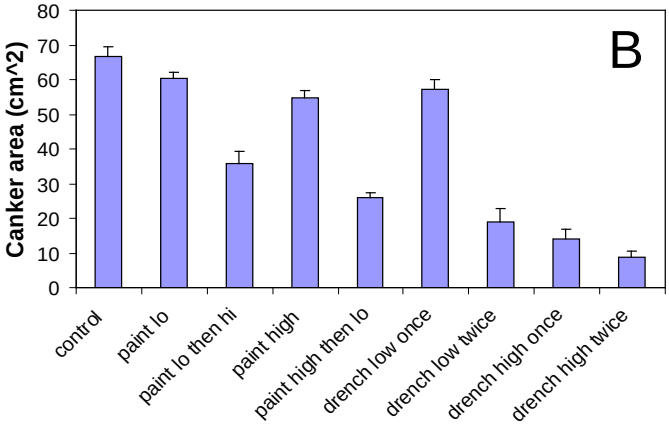


Fig. 5. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expansion of fire blight cankers in apple rootstock cv EMLA 26 (under 'Gala'). Wounds on rootstocks trees were inoculated with the fire blight pathogen on July 8. Treatments were made on May 30 and June 21. Each bar is the mean of 6 trees. A) Measurement recorded 9 August 2010; B) measurements recorded 22 May 2011.

3.c.) SAR induction of pathogenesis-related proteins. Treatment with ASM induces genes involved with host defense against pathogens, and after induction, these genes produce products collectively known as 'pathogenesis-related proteins' (PR-proteins). Whether or not a treatment with a SAR inducer has had an effect on host defenses can be evaluated by measurement of the level of expression of PR-protein genes in the treated plant (the measurements are done with reverse-transcriptase, real time-polymerase chain reaction (rt-qPCR)). In the greenhouse experiments, we measured PR-protein expression in apple leaves after application of ASM by the methods of pot drench, trunk paints and foliar sprays. Data on gene induction were not always conclusive but generally leaf samples from trees that received ASM by drench or by paint mostly showed higher levels of expression of genes PR-1, Pr-2, and PR-8 (Fig. 6). Spray treatments of ASM , however, did

not show a high level of induction and/or the induction effect was less persistent than observed with drenches and paints.

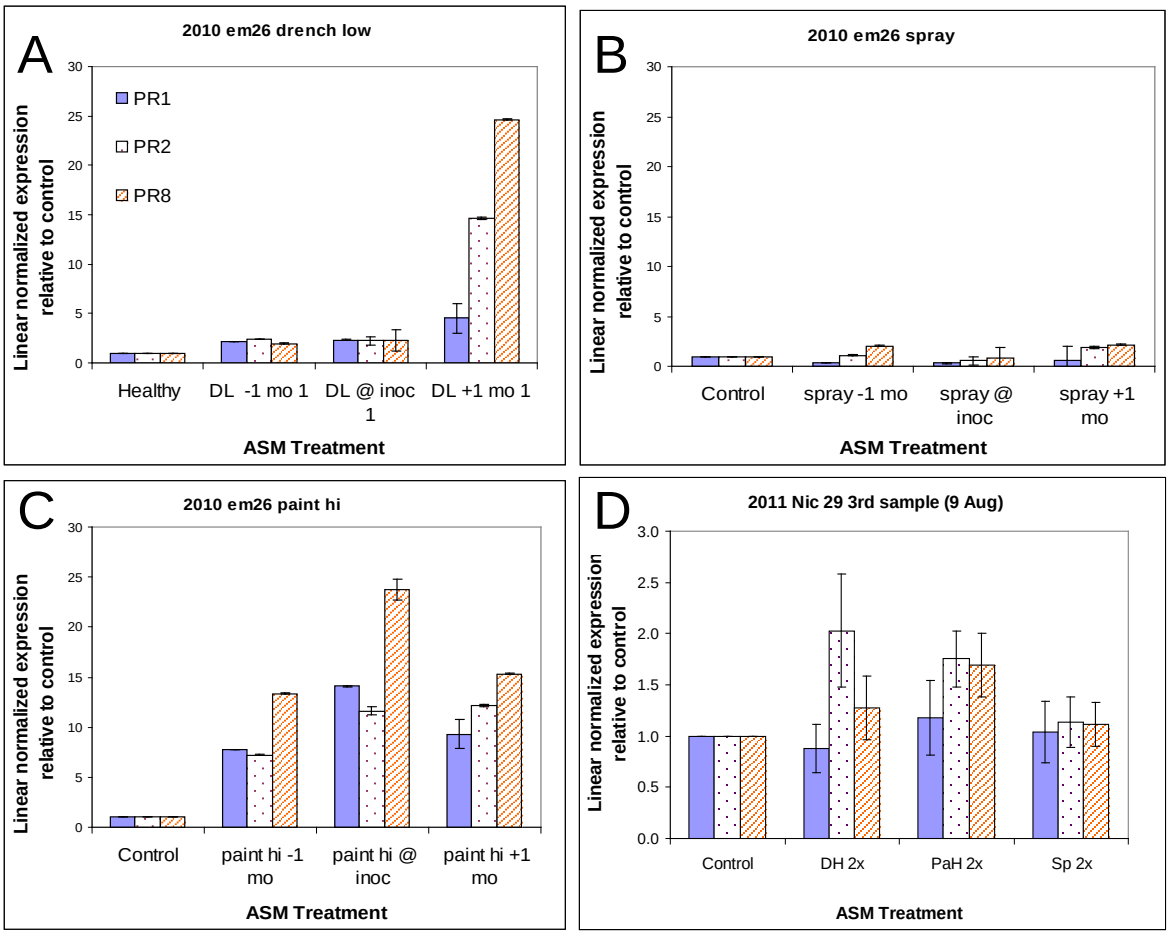


Fig. 6. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expression of pathogenesis-related proteins PR-1, PR-2 and Pr-8 in greenhouse-grown apple rootstock cvs. EMLA 26 and Nic 29. In A-C), trees were inoculated with the fire blight pathogen on 10 June; ASM treatments were made 1 mo prior, at, or 1 mo after inoculation and leaves were sampled for analysis on 29 September. In D), the trees were not inoculated with the pathogen; ASM treatments were made on 14 June and 5 July and leaves were sampled for analysis on 9 August. DL (2010) and DH (2011) = drench of 50 mg Actigard in 500 ml water; PaH = trunk paint of Actigard (30g/L) in 2% Pentrabark, and Sp = Actigard (500 mg/L) to runoff.

Discussion of SAR. Like pears, fire blight-susceptible apple rootstock cultivars respond to drenches and paints of the SAR inducer, acibenzolar-*S* methyl, resulting in slowed canker expansion in diseased trees. The effect of ASM on suppression of fire blight was most dramatic when woody tissue of EMLA 26 was inoculated with *E. amylovora* just below the graft union. We are making progress in understanding the effective rates of ASM for the various methods of application. In the future, we intend to focus on paints in rescue-type treatments as shown above, because this method of of application will likely provide the greatest responses in the field environment.

EXECUTIVE SUMMARY

Project Title: Apple specific issues in fire blight management

Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:
Kasumin:

- Over five years of testing, the product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of apple; EPA registration is on track for 2012.

- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.

Industry implications: When registered, Kasumin will enhance control and broaden the effective tool box for protection of apple flowers from fire blight in conventionally managed orchards. Kasumin is more effective than oxytetracycline, and we expect it to have a positive impact on fire blight management, particularly in high disease risk situations.

Organic fire blight control:

- Effective non-antibiotic strategies for fire blight control were developed. These strategies are being implemented for apples exported under the International Organic Program standard.

- A yeast material, Blossom Protect, provided excellent control of fire blight, with registration and utilization within the International Organic Program expected in 2012.

Industry implications: The information we have been generating has been immediately implemented by growers in the International Organic Program (IOP). Furthermore, the issue of non-antibiotic control of fire blight has increased in importance because the USDA National Organic Program (NOP) has set a 2014 sunset on use of streptomycin and oxytetracycline under the NOP standard.

Systemic acquired resistance:

- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), slowed expansion of fire blight in inoculated shoots of potted apple rootstock cultivars ELMA 26 and Nic 29.

- ASM applied to potted 'Gala' on M26 provided a high level of protection of the rootstock after a high dose of the pathogen was inoculated directly into the graft union.

Industry implications: Even with excellent products for prevention of fire blight, the disease still occurs and its clean-up can be difficult, especially in young orchards. Systemic acquired resistance (SAR) is an induced defense response in a tree, which when induced in apple and pear has the potential to slow/stop fire blight progression. Commercial products that induce SAR in apple have potential to be used as aids in cutting of fire blight to prevent re-ignition of advancing cankers, to protect graft unions from the rootstock blight phase of fire blight, and to enhance protective sprays when mixed with antibiotics.

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

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

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

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

Total Project Request: Year 1: $18,294 **Year 2:** $18,760 **Year 3:** $19,071
Total for Three Years: $56,125

Other funding sources

No other public agency provided grants to this project. Several private companies were involved financially in the testing of proprietary products. Trident deserves special recognition, as they provided substantial effort to the set-up and application of this trial, and in-kind support (fumigation) $4000 value, and a grant of $4000.

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

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

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

ORIGINAL OBJECTIVES: ***Fire blight of apple and pear:*** We will continue to test fire blight control products in the orchard, on both apple and pear, to assess efficacy of new or poorly tested substances.

1. To increase confidence in the biological organism that appeared promising in the 2008 trials, we will significantly expand our testing to include a range of alternative treatments.
2. We will further study the relationship of temperatures to fire blight infection risk.

ORIGINAL OBJECTIVES: ***Orchard Replant Disease:*** We will demonstrate the positive effect on soil fumigation on the productivity and quality of apples grown under a very modern production system.

1. We will determine apple tree growth and 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.

SUMMARY OF SIGNIFICANT FINDINGS

- Over four years and in seven separate apple and pear fire blight control material trials, a dried yeast product, *Auriobassidium pulullans*, called "Blossom Protect" in Europe, controlled fire blight as well or better than the standard and test antibiotics. Issues that remain to be resolved include potential for causing russet. No russet increase was seen in these trials.
- The antibiotic kasugamycin, usually protected blossoms as well as streptomycin (AgriStrep, Fireman), and both were slightly superior to oxytetracycline (Mycoshield, FireLine.**)** The addition of oxytetracycline to kasugamycin did not improve performance.
- Two proprietary copper compound formulations often provided blossom protection equal to antibiotics. The standard (Kocide 3000) copper compound used as a comparison in the trials did not adequately protect the flowers from infection, a result common in past trial copper treatments. The new copper compounds did not appear to russet apples, D'Anjou or Bartlett pears when applied during primary bloom. This russeting issue continues to be the main obstacle to use, and both must undergo much more fruit safety tests during the critical post bloom infection period.
- This past two season's most effective treatments in both apple and pear trials were applications of acibenzolar–s-methyl (ASM, Actigard) at 50% primary bloom, followed by an antibiotic at time of inoculation. Application of this product to the soil under the test tree reduced blight infection, but not significantly.
- The "CougarBlight" fire blight infection risk model was upgraded in 2010 by conversion of the temperature risk values to relate directly to the hourly growth rate of E. amylovora on apple stigmas. Research by Dr. Larry Pusey, USDA-ARS Wenatchee provided the basic data used for this upgrade. The model was adapted to the WSU DAS for 2011.
- Fruit production started in the 3rd season of growth in the apple replant/fumigation trial. After two seasons of marked differences in vegetative development vs. the untreated replicates, the various fumigation treatments produced profoundly more fruit than the untreated portions of the orchard. A preliminary economic analysis indicated that economic returns, adjusted to account for fumigation, picking and packing costs, were increased by $2,600 to $4,000 per acre. This was a 530% economic return over three years on the cost of the fumigation.

FIRE BLIGHT - RESULTS & DISCUSSION:

2009 & 2010 Results Summary: Standard antibiotics continued to perform very well in the material efficacy trials. The potential new alternative antibiotic, kasugamycin, proved essentially as effective as currently registered antibiotics. Certain copper containing materials performed very well when applied two or three times to blossoms prior to full bloom inoculation. Concerns remain about the potential of copper compounds inducing russet, especially when applied during the critical secondary blossom period during the first 3 weeks after primary bloom. The European yeast biological "Blossom Protect" was applied at various rates and frequency of application, and timings, with or without acid buffers. It became increasingly obvious that frequency of application during the development of flowering leading up to an infection period was a critical aspect affecting performance. Three applications were more consistently effective than two, and one application was inadequate. The search for an alternative acid buffer to replace the recommended bulky "Buffer A" was not successful. Acid buffers used alone had about a 35% suppressive effect on infection.

2011 Results: Three non-antibiotic materials performed very well in the 2011 trials. Two copper compounds, one I'm calling "copper product TS (Trade Secret)" from Gowan, and the other called "Cueva" from Certis, reduced fire blight infection as well as, or sometimes better than, standard and test antibiotics. Copper compounds have rarely performed well in past trials, and have a history of causing fruit skin marking. The "TS" copper was applied to both D'Anjou and Bartlett pears as a russet/phytotoxicity trial. There was no russet on the fruit skin observed at harvest, even on the usually russet-prone D'Anjou pears.

The biological product, to be marketed in the USA spring of 2012 as "Blossom Protect," is a mixture of two strains of *Auriobassidium pulullans*, a type of yeast, which is applied in combination with a specific pH 4-5 acidic buffer (Buffer A). This genus and species is commonly found in the Pacific Northwest as a natural colonizer of apple and pear flowers so will probably thrive and spread to newly opened blossoms under PNW conditions. It is not likely that this organism is producing its own antibiotic to achieve antibiotic-like performance in inoculated trials, as this is not typical of yeasts. It is possible that another mechanism, such as successful competition for resources on the stigma surface or within the nectary, serves as a control process. In order for control to occur, it appears that this organism must be in place soon after each flower opens so as to become well-established on the flower before the introduction of *Erwinia amylovora*, the fire blight pathogen.

Actigard (acibenzolar–s-methyl, or ASM) is a substance that has been reported to induce various plants to trigger specific disease resistance mechanisms prior to attack by a certain pathogens. This concept is called specific acquired resistance (SAR). Actigard has been tested by fire blight researchers in various countries over the last decade, and is reported to have some modest effect on the severity of host damage. In this project's 2010 trials, treatment with this product during bloom, followed by an effective antibiotic at the full bloom time of inoculation was the highest rated treatment. This triggered greatly expanded testing in 2011. Seven treatments with different rates, concentrations, timing and application methods were carried out, some with and some without an antibiotic at full Bloom. All of these various treatments involving mid-bloom application of Actigard, followed by treatment with antibiotic at the time of infection performed slightly (numerically) or significantly (statistically) superior to the antibiotic only treatment. This effect is going to be studied further in future trials with other antibiotic and non-antibiotic combinations. The Actigard was also tested for effect as a stand-alone material, sprayed prior to infection and post-infection, and lowered the degree of infection, but not enough to be encouraging.

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

2011 FIRE BLIGHT CONTROL PRODUCT EFFICACY – PEARS

Product	Rate	Timing	% Infection	% Control
Actigard Pre-bloom, Sprayed on. + Strep, 100% bloom + Act. 1 – 2" shoots *	Actigard 2 oz./A Strep. 200 ppm Actigard 2 oz./A	Actigard 50% bloom Strep. 100% Bloom Act. @ 1 - 2" shoot	0.85a	98.5
Actigard Pre-bloom, Sprayed on. + Strep 100% bloom*	Actigard 2 oz./A Strep. 200 ppm	Actigard 50% bloom Strep. 100% Bloom	2.6ab	95.5
Actigard Pre-bloom Soil drench, then Strep + Actigard @ 100% bloom *	Actigard 0.5 lb./A to soil, Strep. 200 ppm + Actigard 6.4 oz./A	Actigard drench @ 50% bloom, Strep. + Actigard @ 100% Bloom	3.4abc	94.1
Actigard Pre-bloom Soil drench and spray, then Strep @ 100% bloom *	Actigard 0.5 lb./A to soil + Actigard 2 oz./A , then Strep 200 ppm	Actigard drench and spray @ 50% bloom, Strep. @ 100% Bloom	4.5bcd	92.2
Actigard Pre-bloom drench, then Strep @ 100% bloom *	Actigard 0.5 lb./A to soil, then Strep 200 ppm	Actigard drench @ 50% bloom, Strep @ full Bloom	6.7de	88.3
Streptomycin 17%*	1 lb/A, 200 ppm	100% bloom	7.1de	87.6
"Blossom Protect" A.p. Yeast (full rate) + Buffer A (full rate)	1.34 lb/100gal/A 9.35 lb. /100/A	20 & 50% 100% bloom	8.2ef	85.7
"Blossom Protect" A.p. Yeast (3/4 rate) + Buffer A (1/2 rate)	1.0 lb/100gal/A 5.0 lb. /100/A	20 & 50% 100% bloom	8.4ef	85.4
TS Copper Product GWN-9979	64 fl.oz./A	50% and 100% bloom	10.6fg	81.5
TS Copper Product GWN-9979	48 fl.oz./A	50% and 100% bloom	10.6fg	81.5
Oxytet. (FireLine)	1 lb/A, 200 ppm	100% bloom	11.1fg	80.7
Cueva (copper soap)	1 gallon/100/A	20 & 50% 100% bloom	11.6gh	79.8

Table 1a. ***Pears***: Summary of data. Values followed by the same letter should not be considered different. **Least Significant Difference in percent infection = 2.96, (95% confidence).**
Streptomycin was effective in this trial because a streptomycin susceptible lab strain of the blight bacteria, Erwinia amylovora, was used to inoculate the flowers.

2011 FIRE BLIGHT CONTROL PRODUCT EFFICACY - PEARS, (CONTINUED)

Product	Rate	Timing	% Infection	% Control
Bacillus subtilis CX-9090 (Certis)	1 lb/100gal./A	50% and 100% bloom	13.0ghi	77.4
TS Copper Product GWN-9979	96 fl.oz./A	50% and 100% bloom	13.4ghi	76.7
Kasumin **8L** (1x)	16 fl.oz./A, 100 ppm	100% bloom	14.7ij	74.4
Bacillus subtilis QRD146 AgraQuest	1.5 lb/100gal./A	30 & 50% 100% bloom	17.2j	70.0
B. subtilis- Serenade MAX, AgraQuest	3.0 lb/100gal./A	30 & 50% 100% bloom	21.2k	63.l
Actigard Pre-bloom, Sprayed 3 times prior to inoculation	Actigard 1 oz./A each spray	20 & 50% 100% bloom	24.8l	56.5
Kasumin **2L** (1x)	64 fl.oz./A, 100 ppm	100% bloom	26.4m	54.0
Actigard, Sprayed twice after 1st symptoms seen	1.34 oz / A	Sprayed twice, three day interval, after 1st symptoms seen	35.7n	37.8
No treatment, inoculated check	**0**	**NA**	**57.4o**	**0**

Table 1b. Pears (continued): Summary of data. Values followed by the same letter should not be considered different**. Least Significant Difference in percent infection = 2.96**

Treatment	Number of Treatments	Highest Percent Control	Lowest Percent Control	Average Percent Control
Strep + ASM*	6	98.4	90.6	95.1
Copper (new forms)	9	98	76.7	85.8
Streptomycin	9	90	75	85.3
BCYP + Buffer A	13	90	72	82.6
Oxytetracycline	15	93	53	78.9
Kasugamycin	8	89	62	77.5
Gentamycin	6	88	51	74.5
Serenade	12	84	38	63.5
Copper (old forms)	7	80	26	49.5
Fungicides	6	57	33	48.6
Acid Buffers	4	39	19	30.5
SAR (Claims)	10	46	0	30.2
Nutrient minerals	3	36	5	18.8

Table 2. Summary of author's current and past fire blight control efficacy trial results. Plots all inoculated. *ASM = Actigard, BCYP = Auriobassidium pulullans, "Blossom Protect."

Year	Crop	Product	Rate / A	# Sprays	Buffer A rate / A	% Control
08	Pear	Blossom Protect	1.34 lb	4	9.35 lb/100	90
08	Apple	Blossom Protect	1.34 lb	4	9.35 lb/100	86
11	Pear	Blossom Protect	1.34 lb	3	9.35 lb/100	85.7
11	Pear	Blossom Protect	1.0 lb	3	5.0 lb /100	85.4
10	Pear	Blossom Protect	1.34 lb	3	4.7 lb /100	82.4
10	Pear	Blossom Protect	1.34 lb	3	9.35 lb/100	81.1
09	Apple	Blossom Protect	1.34 lb	4	9.35 lb/100	80.8
10	Apple	Blossom Protect	0.68 lb	3	4.7 lb /100	79.3
10	Pear	Oxytet. 17%	1.0 lb	1	na	77
09	Apple	Blossom Protect	1.34 lb	2	9.35 lb/100	73
08	Pear	Oxytet. 17%	1.0 lb	1	na	72.4
09	Pear	Oxytet. 17%	1.0 lb	1	na	72.4
10	Apple	Blossom Protect	1.34	3	9.35 lb/100	72
08	Apple	Oxytet. 17%	1.0 lb	1	na	70.5
09	Apple	Oxytet. 17%	1.0 lb	1	na	70.1
09	Apple	Blossom Protect	1.34 lb	4	No buffer	69.5
09	Pear	Blossom Protect	1.34 lb	2	9.35 lb /100	67
10	Pear	Blossom Protect	1.34 lb	3	Alternative buffer	62.8
10	Apple	Oxytet. 17%	1.0 lb	1	na	62
10	Apple	Blossom Protect	1.34 lb	3	Alternative buffer	61.8
09	Pear	Blossom Protect	1.34 lb	4	No buffer	59

Table 3. All results of treatments with A. pulullans ("Blossom Protect") since 2008, with oxytetracycline (Mycoshield, FireLine) results as a comparative standard.

Note in table 3: The best performing treatments with "Blossom Protect" were (usually): Applied at full recommended rates, applied with the recommended rate of "Buffer A," and were applied 3 or 4 times prior to inoculation. The treatments that were less effective had lower rates, alternative or no buffers and / or fewer applications. In other words, follow the label directions for best effect.

ORCHARD REPLANT DISEASE PROJECT - RESULTS & DISCUSSION:

2009 & 2010 Results: Tree growth was measured after each of the first two seasons. There were significant differences in vegetative growth of trees growing on fumigated vs. unfumigated replicates. The trunk calipers were larger and the tree height greater in replicates growing on fumigated soils, but the total shoot growth after the second season was the most different (see table 4.)

The trees produced a crop in 2011, one year prior to expectations, so tree vegetative growth was suppressed by fruit competition. In 2011 and in all further evaluation seasons, fruit yields and quality become the main evaluation criteria.

2010 (second season) tree growth data:

Treatment:	***PicPlus (150 lbs./A Chloropicrin) 0 DCP***	***PC60 (144 lbs./A Chloropicrin) 94 lb/A DCP***	***Telone C-35 (25 GPA, 98 lb/A cloropic) 178 lb/A DCP***	***Telone C-17 (30 GPA, 51 lb/A cloropic) 260 lb/A DCP***	***Untreated***
Tree Height (inches)	86a	85a	86a	88a	74b
Trunk X-sec. mm^2	249a	249a	236a	253a	139b
Total Shoots (inches)	155a	120a	139a	185a	29b

Table 4. Average inches height, cross section area of trunk 4 inches above the graft union and total current season shoot growth of second season Cripp's Pink apples planted as a "sleeping eye" on M9, planted after fumigation on a replant site.

2011 (third season from sleeping eye) fruit data:

Treatment:	***PicPlus (150 lbs./A Chloropicrin) 0 DCP***	***PC60 (144 lbs./A Chloropicrin) 94 lb/A DCP***	***Telone C-35 (25 GPA, 98 lb/A chloropic) 178 lb/A DCP***	***Telone C-17 (30 GPA, 51 lb/A chloropic) 260 lb/A DCP***	***Untreated***
Number of Fruit / tree.	16.7bc	15.5c	19.6a	18.6ab	8.5d
Weight lbs. Fruit / tree	7.5b	7.5b	9.3a	9.1a	3.7c
Weight lbs. per fruit	0.45ab	0.49a	0.48a	0.49a	0.43b
Fruit Grams average	204ab	220a	216a	222a	195b
Fruit box size average	94.1b	86.3a	89.0a	86.3a	98.3c
% size 72 & +	8.8	8.8	9.3	12.9	2.2
80	13.2	16.6	12.5	15.8	8.0
88	25.4	32.2	27.0	27.2	15.0
100	27.8	27.8	28.5	26.2	27.9
113	14.6	8.8	12.1	14.4	23.0
125	8.8	3.9	8.5	3.4	15.5
138 & -	1.4	1.9	2.1	0.1	5.4
%88+	47.4	57.6	48.8	55.9	25.2
%100-	52.6	42.4	51.2	44.1	74.8
Yield per Acre, lbs. (1708 trees)	12,808b	12,826b	15,935a	15,529a	6,286c

Table 5. Fruit production in third season Cripp's Pink apples planted as a "sleeping eye" on M9, planted after fumigation on a replant site.

Treatment A	***PicPlus (175 lbs per ac: 150 lbs./A chloropicrin, 0 1,3-DCP)***						
Box size	% in box size	Acre yield	Wt by size group	80% pack wt	Packed boxes	Price*	$ by size group
72+	8.8	12808	1127	902	21	35.41	760
80/88	38.6	12808	4944	3955	94	37.04	3488
100-	52.6	12808	6737	5390	128	27.01	3466
						Total	7714
		**Minus costs, adjustments of:			$2,495	**Adjusted:**	**$5,219**
Treatment B	***PicClor 60 (20 GPA: 144 lbs./A chloropicrin, 94 lb/A 1,3-DCP)***						
Box size	% in box size	Acre yield	Wt by size group	80% pack wt	Packed boxes	Price*	$ by size group
72+	8.8	12826	1129	903	21	35.41	761
80/88	48.8	12826	6259	5007	119	37.04	4415
100-	42.4	12826	5438	4351	104	27.01	2797
						Total	7975
		**Minus costs, adjustments of:			$2,557	**Adjusted:**	**$5,418**
Treatment C	***Telone C-35 (25 GPA: 98 lb/A chloropicrin, 178 lb/A DC)***						
Box size	% in box size	Acre yield	Wt by size group	80% pack wt	Packed boxes	Price*	$ by size group
72+	9.3	15935	1482	1186	28	35.41	1000
80/88	39.5	15935	6294	5035	120	37.04	4441
100-	51.2	15935	8159	6527	155	27.01	4198
						Total	9639
		**Minus costs, adjustments of:			$3,000	**Adjusted:**	**$6,639**
Treatment D	***Telone C-17 (30 GPA, 51 lb/A chloropicrin 260 lb/A DCP)***						
	% in box size	Acre yield	Wt by size group	80% pack wt	Packed boxes	Price*	$ by size group
72+	12.9	15529	2003	1603	38	35.41	1351
80/88	43	15529	6677	5342	127	37.04	4711
100-	44.1	15529	6848	5479	130	27.01	3523
						Total	9585
		**Minus costs, adjustments of:			$2,941	**Adjusted:**	**$6,644**
Treatment E	***Untreated***						
Box size	% in box size	Acre yield	Wt by size group	80% pack wt	Packed boxes	Price*	$ by size group
72+	2.2	6286	138	111	3	35.41	93
80/88	23	6286	1446	1157	28	37.04	1020
100-	74.8	6286	4702	3762	90	27.01	2419
						Total:	3532
		**Minus costs, adjustments of:			$898	**Adjusted**	**$2,634**

Table 6. Rough estimate of fruit gross economic value per acre. *Approximate FOB average on 11/17/2011. ****Costs, adjustments***: picking @ $!7/bin, packing @ $7 / box, and fumigation @ $650-750/Acre. Credit for 5 cents/lb. for cull fruit. Fumigation costs are now covered, and will not play a role in future economic analysis. SLIGHT ERRORS ARE DUE TO ROUNDING OF NUMBERS.

EXECUTIVE SUMMARY

Fire Blight:

- Over four years and in seven separate apple and pear fire blight control material trials, a dried yeast product, *Auriobassidium pulullans*, called "Blossom Protect" in Europe, controlled fire blight as well or better than the standard and test antibiotics. Issues that remain to be resolved include potential for causing russet. No russet increase was seen in these trials.
- The antibiotic kasugamycin, usually protected blossoms as well as streptomycin (AgriStrep, Fireman), and both were slightly superior to oxytetracycline (Mycoshield, FireLine.**)** The addition of oxytetracycline to kasugamycin did not improve performance.
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- This past two season's most effective treatments in both apple and pear trials were applications of acibenzolar–s-methyl (ASM, Actigard) at 50% primary bloom, followed by an antibiotic at time of inoculation. Application of this product to the soil under the test tree reduced blight infection, but not much.
- The "CougarBlight" fire blight infection risk model was upgraded in 2010 by conversion of the temperature risk values to relate directly to the hourly growth rate of E. amylovora on apple stigmas. Research by Dr. Larry Pusey, USDA-ARS Wenatchee provided the basic data used for this upgrade. The model was adapted to the WSU DAS for 2011.

Replant/fumigation:

- Fruit production started in the 3rd season of growth in the apple replant/fumigation trial. After two seasons of marked differences in vegetative development vs. the untreated replicates, the various fumigation treatments produced profoundly more fruit than the untreated portions of the orchard (see table 5.) A preliminary economic analysis indicated that economic returns, adjusted to account for fumigation, picking and packing costs, were increased by $2,600 to $4,000 per acre (table 6.) This was a 530% return over three years on the cost of the fumigation. As would be expected with higher yields; number of fruit per tree and total fruit weight per tree was improved by fumigation, as was average fruit size. Percentage of fruit per box size was documented to aid economic analysis.

Future directions:

Fire Blight: To further investigate synergistic effects following sequential applications of various compatible classes of blight control materials.

Fumigation: We will continue to document the long term production and economic impact of fumigation in a very modern apple orchard. At the conclusion in 2014, or sooner if necessary, this data will be published in both professional and popular form.

FINAL PROJECT REPORT **YEAR**: 3 of 3

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

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

Total Project Funding: Three Year Total: $ 344,866

Other funding Sources – Total cash and in-kind support from private and growers

- Private Company Funding (see below) - $209,000
- Private Company in-kind support - $150,000
- In-kind support from growers - $9,000,000 (value at $6,000/acre for 1,500 acres)

Funding

- Shin-Etsu: $80,000 to help fund evaluation of new dispenser technology
- Private Company: $60,000 for assessment of release rates, video recording and flight tunnel of behavior, and attraction of dispensers to codling moth
- Private Company: $39,000 for assessment of a novel attract-and-kill formulation
- Private Company: $30,000 for assessment of a novel mating disruption formulation

In-kind support

- Pheromone companies in-kind donation of dispensers for all trials plus donation of traps and lures to help with monitoring expenses. In-kind support over three years of this project totaled over $150,000.
- Growers provide in-kind support by allowing us to use commercial orchards for pheromone research. Over the duration of this project research was conducted on over 1,500 acres of grower-owned orchard. Based on in-kind value allowed for match in federal grants the value of this support would be over $9,000,000.

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

WTFRC Collaborative Expenses: None

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

Item	2009	2010	2011	Total
Salaries [1]				
Technical – M. Doerr (3 month)	5,278	5,489	15,833	26,600
Res. Analyst III (Hebert prog.)	8,714	9,063	9,426	27,203
GRA – 9 mo appt @ 0.50FTE	22,014	0	0	22,014
Benefits				
Technical – M. Doerr (3 months)	1,689	1,757	5,051	8,497
Res. Analyst III (Hebert prog.)	4,270	4,441	4,619	13,330
GRA – 9 mo appt @ 0.50FTE	1,881	0	0	1,881
Wages (temporary labor)[2]	7,000	8,000	14,400	29,400
Benefits (15%)	1,260	1,440	2,160	4,860
Supplies	5,000	5,000	5,500	15,500
Travel [3]	2,085	2,085	2,085	6,255
Plot Fees	0	0	0	0
Total	59,191	37,275	59,074	155,540

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

Item	2009	2010	2011	Total
Salaries	26,187	26,981	27,782	80,950
Benefits	12,847	13,506	14,107	40,460
Wages	3,000	3,000	3,000	9,000
Benefits	172	172	172	516
Supplies	1,000	1,000	1,000	3,000
Travel	1,000	1,000	1,000	3,000
Total	44,206	45,659	47,061	136,926

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

Item	2009	2010	2011	Total
Salaries	0	0	0	0
Benefits	0	0	0	0
Wages	14,000	14,000	14,000	42,000
Benefits (10% of labor)	1,400	1,400	1,400	4,200
Supplies	1,000	1,000	1,000	3,000
Travel	1,000	1,000	1,000	3,000
Total	17,500	17,500	17,500	52,500

Project objectives:

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

Significant findings 2009-2011

Optimization of hand-applied dispensers - Impacts

- This project leveraged each dollar of Commission funding into one dollar of support from private companies (cash plus in-kind support). The collaborative relationships we established with private companies provided access to new technologies, allowed us to expand the scope of our research, and influenced new product development for the benefit of the tree fruit industry.
- This project played a major role in the development of a new, more efficient, pheromone dispenser technology, Isomate CM Flex, which is now in commercial use. This technology has a more uniform pheromone release profile over time and provides flexibility in dispenser density of 200-400 per acre. Without this project's research showing the potential of maintaining CM control with lower release rates from dispensers it is very doubtful this new technology would have been developed.
- This project worked with a private company to help develop and evaluate a new automated delivery system for placing a pheromone dispenser in trees. This technology, Tangler®, reduces application time by 75%, has a high degree of retention in the canopy, and has performed equal to standard hand-applied technologies in suppressing moth capture in monitoring traps in WA and MI.
- The use of sterile CM moths obtained from the Okanogan-Kootenay Sterile Insect Release (SIR) Program allowed this project to challenge different pheromone treatments in commercial orchards. Using the SIR technology we were able to test different pheromone treatments in large replicated plots while keeping moth density the same across treatments.

Optimization of hand-applied dispensers - Research Results

- We showed that pheromone release rates and shape of dispensers influenced CM behavior, that is, attraction to and activity around pheromone sources. These behavioral changes where evaluated in field cages and small plot trials, which informed additional studies in large field plots.
- This project demonstrated that the impact of a meso-type dispenser, Isomate CM Ring, on CM was strongly dependent on dispenser density per acre. Additional studies with the Ring technology showed that rates of 20 to 40 dispensers per acre were as effective in suppressing CM behavior (attraction to monitoring traps) as a standard hand applied dispenser treatment, Isomate CM Flex, at 400 dispensers per acre.
- We showed that the addition of pear ester to the CideTrak CM dispenser did not provide additional impact on CM behavior relative to a CideTrak CM dispenser without pear ester. However, there was evidence that the impact of a meso-type CideTrak CM dispenser had a greater impact on CM behavior when pear ester was present.
- The use of video recording of CM moth behavior in the field showed that the number and duration of visits to pheromone sources varied with pheromone release rate. These results provided evidence that not only the amount of pheromone released from a dispenser was important, but that the shape of the dispenser was also important.
- A new attractant that originated from microbial chemistry was demonstrated in the field to be attractive to female and male CM. The new attractant was four times more attractive than acetic acid lures, and has then potential to be comparable to the acetic acid + pear ester attractant.

Attract and Kill (A&K) - Research Results

- Either Warrior or Assail in different formulations were shown to be good candidates for toxicants in A&K formulations. Exposure of CM moths to sublethal concentrations of a toxicant dramatically impacted the moth's ability to orient to females and mate. These results increase the potential for developing A&K technologies with low concentrations of toxicants.
- Different kinds of devices used in A&K technology have been evaluated. The shape and size of openings in an A&K device was shown to be important as well as the strength of attractant used.
- Progress has been made in developing an alternative to pear ester that could be used in A&K technologies, thus impacting both male and female CM. New technologies are important because the company holding the pear ester license does has not shown interest in using it in an A&K technology.
- Based on models and field cage studies, A&K technologies are four times more robust than mating disruption at controlling CM. Therefore, optimization of A&K technologies is worth the investment of energies and resources.
- Mini-traps looked promising in initial studies, but when evaluated in small or large field trials the designs used provided variable results. A simple tubular A&K device with a toxicant reduced male CM activity by 98% using only 50 units/A.
- There is good indication that an A&K technology would be effective to suppress populations of leafrollers.
- Video monitoring of moth behavior while extremely time consuming provides critical insights into moth behaviors associated with A&K technologies. Videos showed relative attraction to an A&K device over time and more importantly revealed barriers to source contact, which is critical to the success of this technology.

Methods

Methods used in this project were outlined in new project proposal (2009). Methods used in studies are included to some extent in the results and discussion section as a means of helping the reader understand how results were obtained.

Results and Discussion 2009-2011

Optimization of hand-applied dispensers. The first objective of this project was to improve hand-applied mating disruption systems for codling moth. A team effort focused on understanding the impact of pheromone release rates from different devices on codling moth behavior. This objective was expanded with additional resources from private companies to assess the value of meso-type and aerosol pheromone dispensing systems. Behaviors were assessed with pheromone-baited traps, in small and large field trials, field-cage studies, and with video recordings.

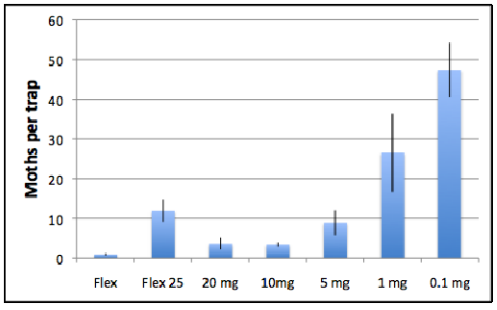


Fig. 1

Based on previous research we predicted that moth capture in traps would decrease as the pheromone release rate of a dispenser increased. With the exception of the Isomate Flex 25 tube-type dispenser, which captured more moths relative to lures loaded with 5, 10, or 20 mg of codlemone, there was a decline in moth captures as pheromone release rate increased. The release rate of the Flex 25 dispenser was expected to be the same as the 20 mg dispenser but it proved to be highly attractive when placed in a trap, though not as attractive as the 0.1 or 1.0 mg lures (Fig. 1).

Interpretation. While data from this experiment generally confirms previous studies, the relative attraction of the Flex 25 dispenser was unexpected. This result informed our interpretation of other studies and impacted the design of new dispensers now in commercial use.

Our studies continued with large field cages (enclosing 12 large apple trees) and small plot field trials. These studies were aimed at sorting out the relative impact of different pheromone treatments using dispenser densities approximating 200/A. Large cages provided the advantage of replicating treatments while keeping the density of CM constant. In small plot field trials the same treatments tested in field cages were evaluated against naturally occurring populations of CM.

In field cages there was little difference in CM disruption between low load dispensers (0.1 mg lures) and a full rate tube-type dispenser, Isomate CM Flex 100, when only males were released. However, when males and females were released in the cages there was greater disruption of CM by dispensers releasing more pheromone. In small plots there was a direct relationship between pheromone release rate and CM disruption, but the level of disruption caused by dispensers release very low amounts of pheromone was not much different from the Isomate CM Flex 100 (≈ Isomate C plus) dispenser.

Interpretation. Dispensers releasing very small amounts of pheromone can result in a significant reduction of a male moth's ability to locate a female. As pheromone release rate from a source increases there is only a slight increase in effect on male success in finding a female mimic. These results strongly suggested that to optimize a dispenser's impact its pheromone release rate should be tuned to maximize suppression of a male's ability to make multiple searches in the same night.

Video recording of moth behavior around various pheromone sources showed differences based on pheromone release rate and time of year. In May-June the number of visits was highest to dispensers with the lowest and highest release rates, while the duration of visits was highest with the highest release rate device. The percent of moths making source contact was small (10%) and only occurred with the 0.1 and 10 mg dispensers. In July the number of visits roughly doubled but the pattern was similar to the May-June observations, with a higher number of visits to the lowest and highest release rate dispensers. The average duration of visits was again highest to dispensers with the lowest and highest release rates (Fig. 2). Source contact was low (10-20%) and occurred only to the two lowest and two highest release rate dispensers.

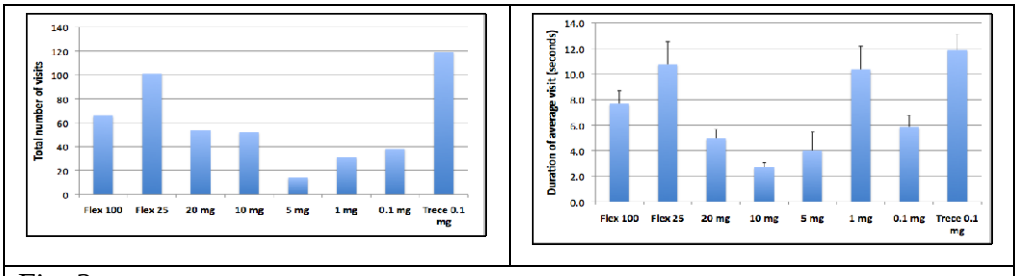


Fig. 2

Interpretation. Video recording helped us understand the relative activity of moths around different pheromone sources in the field. The number of visits was expected to mimic the capture of moths in pheromone traps; however, there were more visits and of longer duration to the high release rate dispensers than we expected. This result showed that the higher release rate dispensers were attractive and that moths often approached within at least 12 inches. This result is encouraging because, based on other evidence, we believe that moths that approach close to high pheromone release rate dispensers have a reduced likelihood of being able to search for females the same night.

We collaborated to challenge several pheromone dispensing systems over three years in large plot field trials in WA and MI. These large plot trials were informed by field cage and small plot field studies or were used to evaluated new pheromone technologies and represent our efforts to test the best treatments and products under realistic conditions. A selection of results from these studies is reported here providing insights into the most important findings from our research.

CheckMate CM-O Puffer, Isomate CM Ring (Ring) meso-type dispenser, and Isomate CM Flex (Flex) represent a range of pheromone delivery technologies that are important to the tree fruit industry. In 2010, Puffers were evaluated at 1 unit/A, Flex at 320 dispensers/A, and Rings at 1, 2, 4, 8, 16, and 32 dispensers/A (disp/A). Sterile moths obtained from the Okanogan Kootenay Sterile Insect Release Program (SIRP) were used to challenge treatments. Treatments were evaluated by comparing the relative capture of sterile moths in monitoring traps (female mimics) in each treatment.

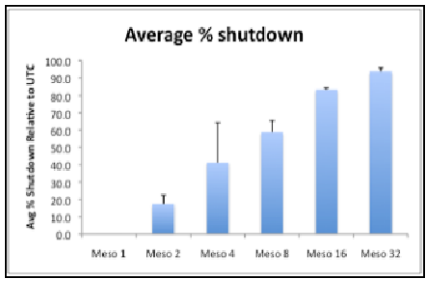


Fig. 3

There was no difference in sterile moth capture between the 1 Ring/A dispenser treatment and the no pheromone control. Trap shutdown relative to the no pheromone control increased as the density of Ring dispensers increased up to 32 disp/A, where trap shut down averaged 93.7 ± 2.0% (Fig. 3).

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

In MI Rings and Flex dispensers were compared in six orchards over two years. Treatments were Flex at 40 and 400 disp/A and Rings at 4 and 40 disp/A. The Flex at 400/A provided the greatest reduction in trap captures. There was little difference between other treatments, including the Rings at only 4 disp/A.

In 2011, the Puffer and Ring technologies were again tested. A Puffer treatment (1 unit/A) was paired with a Flex treatment (400 disp/A) in 40 acre plots at three locations. There was no difference between the capture of sterile moths in these treatments. The Isomate Ring technology was evaluated at three dispenser densities, 20, 30 and 40 disp/A, and compared to an Isomate Flex treatment at 400 disp/A. Treatments were replicated four times. There was no difference in capture of sterile moths between the four treatments (Fig. 4).

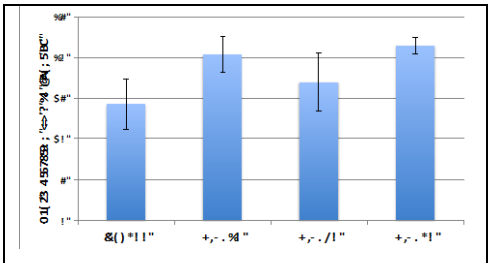


Fig. 4

In WA and MI we evaluated a reduced release rate technology, Isomate Flex in 2010. We compared Isomate C Plus (standard treatment), Isomate Flex (80), Flex 50 and Flex 25, all at 400 disp/A. The release rate from the Flex dispensers was proportional to the load. In WA there was little difference between the Isomate C Plus, Flex (80) and Flex 50 treatments though there was a slight reduction in trap shutdown with the Flex 25 treatment. In MI a no mating disruption (NoMD) control was included in studies. All three Isomate treatments, Flex (80), Flex 50 and Flex 25, reduced moth captures compared to the NoMD control in the first and second generation and the Flex 25 and Flex 50 treatments performed similarly in suppressing moth captures compared to the Flex (80) dispenser.

In 2011, MI challenged four Isomate Flex treatments with pheromone load rates from 10% (Flex 10) to 80% (Flex 80) relative to an Isomate C Plus dispenser. All Flex treatments were applied at 300-400 disp/A and replicated on eight farms. There was no difference in wild (or sterile) moths

captured in monitoring traps (Fig. 5) and no fruit injury was detected in any of the pheromone treated plots.

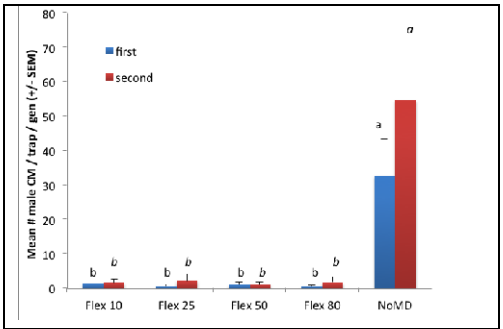


Fig. 5

The Tangler® is a newly-developed mating disruption technology developed by Ridge Quest, a Michigan-based company, and designed to automate the application process. The Tangler technology was evaluated at five sites in MI (2010). The Tangler formulation provided disruption equivalent to Isomate CM Flex. The automated deployment using the Tangler modules was nearly 4x faster than hand application of Isomate dispensers.

In 2011, the Tangler technology was evaluated in MI and WA. The launcher system (Fig. 6) was improved in 2011 and pheromone released from the module approached a zero order release rate over the summer and was similar to the release rate from the Isomate Flex dispenser. In field trials the Tangler provide suppression of moths in traps equal to an Isomate Flex treatment when both dispensers were applied at 400/A (Fig. 7). The Tangler modules were applied four to five times faster than the Isomate Flex dispensers and pheromone modules were retained in the canopy throughout the summer. Results similar to MI were observed in WA using the Tangler technologies.

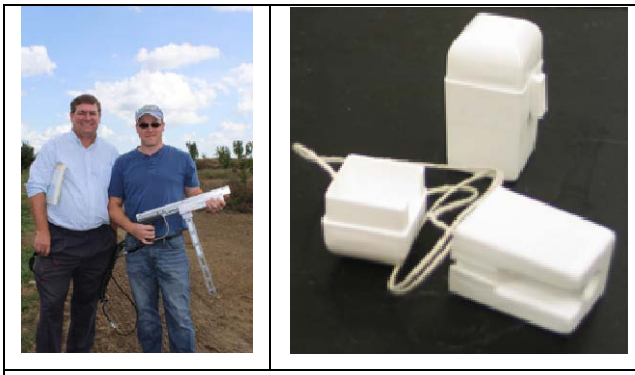


Fig. 6. Tangler launcher system left and pheromone module right.

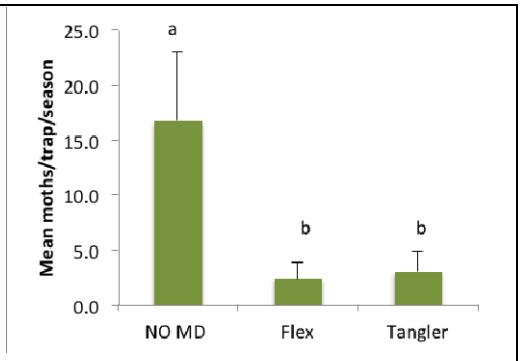


Fig. 7. Average native male captures in pheromone traps in plots treated with Flex, Tangler, and noMD.

Another pheromone company, Trécé Inc., approached us to evaluate a pheromone dispenser containing pear ester. This company had a commercially registered dispenser, Cidetrak CM. We evaluated the Cidetrak CM and a prototype dispenser Cidetrak CM/DA (with pear ester) in WA and MI in large replicated field trials. In both locations we observed no added value of including the pear ester in the standard Cidetrak dispenser. However, in MI a Cidetrak meso-dispenser (see image at right) was evaluated at 32 disp/A, with and without pear ester. In these comparisons the Cidetrak Meso CM/DA (with pear ester) increased disruption of CM from 85.9% (Cidetrak Meso CM) to 94.5%.

Interpretation. Because this project was funded by the industry we were able to access pheromone technology to evaluate and influence the development of new technology. We demonstrated that disruption of CM was possible with pheromone dispensers releasing one-tenth and one-quarter of pheromone being released by most commercial dispensers. These findings resulted in the

development of a new dispenser, Isomate Flex, which is now available to growers. We demonstrated that meso-type dispensers at rates of 20-40 dsip/A provided good suppression of CM in large replicated plots. We were also able to show some difference in a meso-type dispenser when it contained pear ester. We demonstrated that aerosol pheromone dispensing technology, Puffers, provided good suppression of CM in large commercial plots equal to a standard hand applied pheromone technology. We were also able to demonstrate that a new technology, Tangler®, to apply pheromone dispensers had great promise by reducing application time by four to five times and that it disrupted CM in large plots equal to a hand applied dispenser when used at equivalent rates per acre. These results provide evidence that a wide variety of pheromone dispensing systems can be used to control codling moth and that pheromone release rates can be dramatically reduced and still achieve acceptable levels of suppression.

Collaborations with private companies allowed us to expand our research and to influence new product development. Using the volatile capture system (VCS) developed by Dr. Hebert we showed that a new tube-type dispenser, Isomate Flex technology, had a near zero order release profile and rates proportional to loading through 150 days – (Fig. 8).

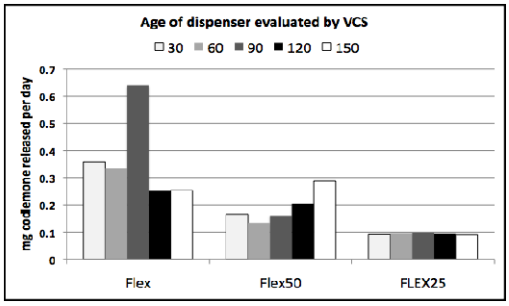


Fig. 8.

We also evaluated other hand-applied pheromone dispensers using the VCS and discovered pheromone release rate patterns that were not considered optimal. These results were shared with companies developing pheromone dispensers allowing them the opportunity to modify their products.

Interpretation. By working with commercial pheromone companies this project helped to improve dispensers in ways that should reduce costs to growers or at least maintain the current costs. Project findings played a role in the development of a more efficient dispenser that is now commercially available to growers, the Isomate CM Flex dispenser. Private companies provided financial support to scientists working on this project, which supplemented the core funding provided by the industry.

Attract and Kill (A&K) Research. The second objective of this project was to characterize behavior of adult CM and leafroller in order to optimize development of A&K technologies. A team effort focused on assessing moth behaviors in different environments; laboratory studies, large field cages, small replicated field trials, and two different A&K technologies; traps and toxic surfaces.

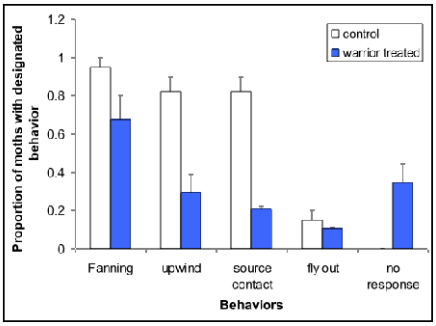


Fig. 9.

At MSU several different devices were evaluated in the wind tunnel to determine relative ability to impact codling moth. The preferred toxicant was Warrior (lambda-cyhalothrin), which caused high CM moth mortality occurred within 30 seconds. When CM male moths were exposed to a sublethal concentration of Warrior for 4 h prior to the wind tunnel bioassay they were not successful in orienting in to a pheromone source in the wind tunnel (Fig. 9). Warrior was mixed with Vaseline and aged in the field for up to 126 days in a prototype A&K device. When male CM contacted 126 day-old Warrior residues, more than 90% died.

The USDA-ARS tested A&K devices made of clear vinyl cylinder of different diameters or a section of white PVC pipe. Assail mixed in a silicone grease was used as a toxicant. The 1¼ inch

diameter cylinder showed the best ratio of moths contacting the A&K device. There was no repellency of Assail mixed with grease on moth orientation to, or contact with, the A&K device. The most effective concentration of Assail was 4% (w/w) causing 100% mortality in 24 hours.

Interpretation. There are two insecticides that quickly kill codling moth adults by contact. Exposure of moths to sublethal residues had a dramatic impact on their ability to orient to a pheromone plume. Different shapes of A&K devices, including the size of an opening, indicated that these were important factors in optimizing moth contact with a treated surface.

USDA experiments with kairomone attractants showed that and eight-component blend capture two times more moths than the AA+3-methyl-1-butanol lure (Fig. 10). Collaborations with a private company evaluating kairomones showed promise of a CM attractant capturing three to six times more moths than a pear ester lure.

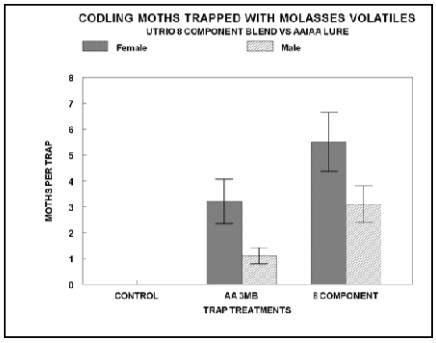


Fig. 10.

Interpretation. There are new kairomones or combinations that hold promise for capturing CM, including females, which would be very important in A&K research. Because the pear ester technology is tied up in a company with no interest in developing A&K technology it is important to continue investigations into alternative chemicals that would attract both males and females.

In MI several treatments were evaluated using the caged-tree design. In a direct comparison of male removal (delta trap with liner) and mating disruption (pheromone dispensers), male removal was shown to be four times more powerful than a full rate of pheromone dispensers in preventing mate location. Several kinds of A&K devices showed promise, although the large delta trap with a liner proved superior (Fig. 11).

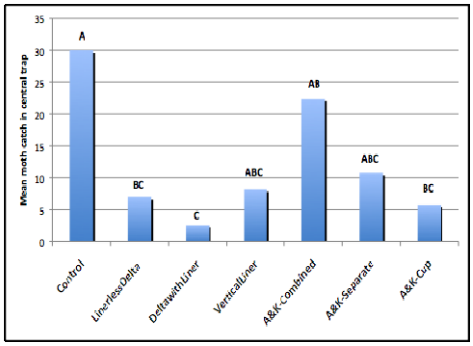


Fig. 11.

Interpretation. Field-cage trial results demonstrated that A&K was a more robust tactic than pheromone mating disruption when moth densities were controlled. The reason for the greater impact of A&K is because it limits the number of visits per night to a pheromone source (female).

In MI seven treatments, two experimental commercial formulations, two prototype A&K devices, a pheromone lure only, a large lined delta trap and untreated control were compared for male removal efficiency in replicated small plots (0.1 acre). Treatments were applied at a rate of 100 A&K devices/A. There was a significant reduction in male captures in monitoring traps but only the large delta trap with liner showed a significant reduction compared to a lure only treatment.

In MI direct comparisons of the efficiency of different trap types in capturing CM moths was part of an effort to develop a "trap out" system that would be economically feasible since field cage studies had shown that A&K is potentially four times more robust than mating disruption (see discussion above). Four different trap types were compared to the standard large delta trap in two different field studies. None of the traps captured as many moths as the large delta but two, the lantern and box traps, showed some promise. When these different traps were taken to the field and evaluated in a replicated small plot study with release of sterile moths the delta trap removed more

moths but the box and lantern traps captured 60-70% of the delta trap with much smaller trapping surfaces.

The effects of varying the density of MSU micro-traps on inhibition of CM catch in a central monitoring trap was evaluated in a small plot study in MI. Treatments included a no pheromone check, Isomate Flex at 200 disp/A, micro-traps at 50/A, 100/A, 200/A, and 400/A. All micro-traps were baited with L2 lures. A single monitoring trap baited with an L2 lure was placed centrally in each plot to assess treatment effects. All pheromone treatments reduced the capture of CM in the central monitoring trap. The three highest micro-trap application rates significantly reduced CM capture in the monitoring trap compared to the lowest micro-trap rate. CM capture rates were statistically equal between the mating disruption standard at 200 disp/acre and the micro-trap rates of 100/acre and 400/acre (Fig. 12).

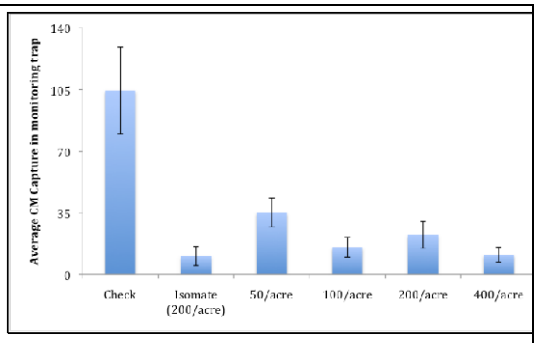


Fig. 12.

In MI a large plot attract-and-remove trial was conducted to compare a standard mating disruption program to a trap-out control scenario for reduction of CM male activity. Treatments included a no pheromone control, Isomate Flex, non-sticky micro-traps, and sticky micro-traps. Treatments were applied to 0.5-acre plots at an application rate of 200/A. All micro-traps were baited with L2 lures for first flight and 0.1mg lures for second flight. All pheromone treatments were effective at reducing the capture of CM in central monitoring traps (Fig. 13). During first flight, the attract-and-remove approach was more effective at reducing CM capture in the monitoring traps than either of the other treatments. In the second flight the attract-and-remove approach was statistically superior to the commercial mating disruption standard, even with a use of lower load lures, 0.1 mg.

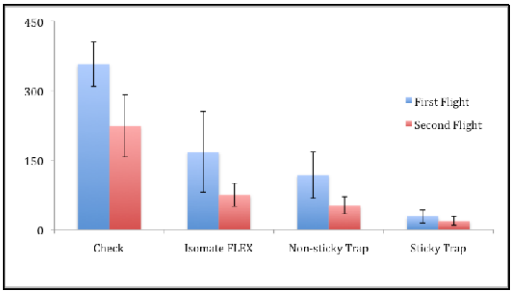


Fig. 13.

In WA an attract-and-remove study was conducted as a replicated small plot trial using lantern mini-traps at 200/A, 200 0.1 mg lures/A, an Isomate Flex treatment (400 disp./A), and a no pheromone control. In the second generation pheromone trap captures were lower in the Flex treatment in pheromone monitoring traps but not in acetic acid/pear ester (AA/PE) baited traps. There was no difference in the sex ratio of CM captured in AA/PE traps, which was expected if the lantern mini-traps were removing males from the population at a significant rate. At the end of the first generation there was about half the fruit injury in the Flex treatment compared to other treatments. At harvest fruit injury was high in all treatments with no differences between treatments.

Because mating disruption for leafrollers is not as robust as it is for CM, A&K or attract-and-remove (trap out) is a possible alternative approach. A small plot attract-and-remove trial was conducted in MI to compare a standard mating disruption treatment to a trap out program for OBLR. Treatments included Isomate OBLR/PLR, Pherocon IIB traps baited with standard OBLR lures (attract-and-remove treatment), and a no pheromone control. Treatments were applied to 0.5-acre plots at 200-point sources per acre. Both pheromone treatments were effective at reducing the capture of OBLR leafroller in monitoring traps. During both flights, the attract-and-remove approach was

more effective at reducing OBLR capture in the central monitoring traps than the commercial mating disruption standard (Fig. 14). The attract-and-remove approach for OBLR appears to have greater potential compared to a mating disruption approach. Developing a cheap device to remove leafroller males from orchards could reduce the need for chemical control treatments or, when combined with soft chemical controls like Bt, enhance biological control in IPM programs.

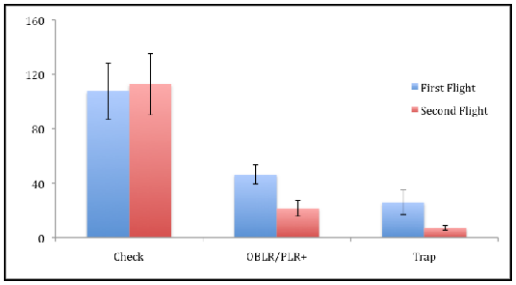


Fig. 14.

A similar attract-and-remove approach for OBLR was tried in WA in replicated small plots. Treatments include an untreated control, Pherocon IIB traps baited with OBLR lures, and OBLR lures only. The OBLR treatments were applied at 200 trap or lures/A. The treatments went out at the end of the overwinter generation flight. OBLR capture in pheromone monitoring traps was suppressed in the trap and lure only treatments by about 85% relative to the untreated control. In AA/PE monitoring traps, there was no difference in moth captures between any of the treatments and the sex ratio was similar in each. Trap out does not seem to be a robust enough approach for managing OBLR, at least in these small plots.

A private company approached us to evaluate an A&K technology they were developing. Treatments were established in WA and MI that were compatible but not duplicative. In MI the A&K technology, with and without the toxicant, was compared to an Isomate Flex treatment and untreated control. The A&K treatments suppressed moth capture in traps relative to the control and similar to the Isomate Flex treatment. However, there was no difference between the A&K technology treatment with and without the toxicant, suggesting that there was a mild mating disruption effect but not an A&K effect. In WA different rates of the A&K technology was tested in replicated small plots challenged with release of sterile moths. There was a slight suppression of CM capture in pheromone monitoring traps relative to the untreated control, but with no real differences between the different rates of the A&K technology. In AA/PE baited monitoring traps there was also no differences between CM captures, males or females, between the untreated control and A&K technology treatments. Data from these studies has been shared with the company, which is evaluating its next steps with the technology.

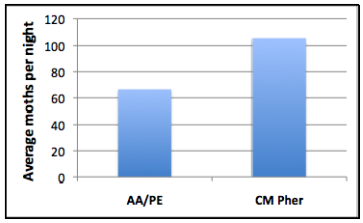
Attract and kill stations were deployed in apple orchards at a rate of 50/acre to determine their impact on catches of CM moths in monitoring traps baited with pheromone lures or a feeding attractant lures. The station design was a short length of white tubing, a lure at the bottom center of the inside of the tube, and a killing agent coating the interior of the tube. Each attract-and-kill experiment was conducted over 8 days, with comparisons of treated versus untreated plots, in both spring and summer flights attract-and-remove. The percent reductions in moths captured in pheromone monitoring traps were initially low and not statistically significant, but steadily improved to reach 98% reductions of males and 80% reductions of females in traps baited with codling moth/pear ester lures, and 82% and 85% reductions of males and females in traps baited with acetic AA/PE lures.

Interpretation. All of the field trials point to a good potential for use of attract-and-remove or attract-and-kill as a strategy for CM and possibly for OBLR management. There is developing technology that could make this approach economical. The micro-trap is much smaller than the delta trap, yet still catches 25-60% as many moths. This is encouraging considering it was designed to be deployed at densities of 100/A.

Video monitoring of behavior around A&K devices is an effective tool in identifying limitations of designs and the relative effect of different attractants. For example, a kairomone in the USDA-ARS A&K device was only half as attractive as the device baited with a pheromone lure (Fig. 15-left),

however, the duration of visits was equal between the two attractants (Fig. 15-right). Contact with the A&K device was low, 3%, and was the same for both lure-types.

Video recordings to a private company's A&K device showed that there was no impact of attractant (pheromone) load rate on the number of visits but the duration of visits increased slightly with load rate and was similar to a 0.1 mg pheromone lure. With this A&K device there was a consistent but low level of contact by moths, 10-25%.



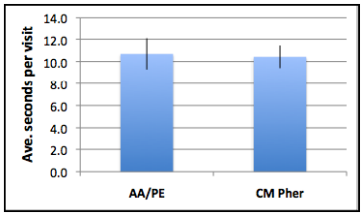


Fig. 15.

When another companies A&K technology was evaluated using video cameras, results showed that the technology was attractive, though much less so than a 0.1 mg lure. The attraction of the A&K technology remained for over three months and actual duration of visits increased as the A&K technology aged. However, the contact of CM moths with the A&K technology, which is critical for efficacy, was very low relative to the 0.1 mg lure. These data support observations of the small plot field trial with this technology where the effect seemed attributable more to a mild mating disruption effect than to an A&K effect.

Interpretation. Video monitoring was shown to be a powerful tool to demonstrate the relative attraction to potential A&K technologies and the actual level of contact with devices. The lack of moth contact with experimental devices suggests that lure strength is important in enhancing contact. Our results also showed that the shape of a device is important in enhancing or limiting contact. These results helped point to ways to improve upon A&K devices and attractants employed in them.

Executive Summary

This project developed collaborative relationships with private industry and leveraged each dollar of Commission funding into one dollar of support from private companies (cash plus in-kind support). The collaborative relationships provided access to new technologies, allowed us to expand the scope of our research, and influenced new product development for the benefit of the tree fruit industry. An impact of this project's research was the development of a new, more efficient, pheromone dispenser technology, Isomate CM Flex, which is now in commercial use. This project significantly influenced the development of the Tangler® technology which reduces application time of pheromone dispensers by 75% while providing CM control equal to hand-applied dispensers. The use of sterile CM moths obtained from the Okanogan-Kootenay Sterile Insect Release (SIR) Program allowed this project to challenge different pheromone treatments in commercial orchards. Using the SIR technology we were able to test different pheromone treatments in large replicated plots while keeping moth density the same across treatments.

We showed that pheromone release rates and shape of dispensers influenced CM behavior. These behavioral changes where evaluated in field cages and small plot trials, which informed additional studies in large field plots. A new meso-type dispenser, Isomate CM Ring, showed that rates of 20 to 40 dispensers per acre were as effective in suppressing CM behavior as a standard hand applied dispenser treatment, Isomate CM Flex, at 400 dispensers per acre. We also showed that the addition of pear ester to the CideTrak CM hand-applied dispenser did not provide additional impact on CM behavior. However, there was evidence that the impact of a meso-type CideTrak CM dispenser with pear ester was had a greater impact on CM behavior than when pear ester was absent. Video recording of CM moth behavior in the field proved to be a valuable tool providing evidence that not only the amount of pheromone released from a dispenser was important, but that the shape of the dispenser was also important. A new attractant that originated from microbial chemistry was demonstrated in the field to be attractive to female and male CM. The new attractant was four times more attractive than acetic acid lures, and has then potential to be comparable to the acetic acid + pear ester attractant.

Based on models and field cage studies, A&K technologies were shown to be four times more robust than mating disruption at controlling CM. Therefore, optimization of A&K technologies is worth the investment of energies and resources. This project demonstrated that either Warrior or Assail in different formulations were good candidates for toxicants in attract-and-kill (A&K) formulations. Exposure of CM moth adults to sublethal concentrations of a toxicant dramatically impacted the moth's ability to orient to females and mate. These results increase the potential for developing A&K technologies with low concentrations of toxicants. We showed that the strength of attractant and the shape and size of openings in an A&K device are important in optimizing efficacy. Mini-traps looked promising in initial studies, but when evaluated in small or large field trials the designs used provided variable results. However, a tubular A&K device with a toxicant reduced male CM activity by 98% using only 50 units/A. There is good indication that an A&K technology would be effective to suppress populations of leafrollers. Video monitoring of moth behavior while extremely time consuming provides critical insights into moth behaviors associated with A&K technologies. Videos showed relative attraction to an A&K device over time and, more importantly, revealed barriers to source contact, which is critical to the success of this technology. Progress has been made in developing an alternative to pear ester that could be used in A&K technologies, thus impacting both male and female CM. New technologies are important because the company holding the pear ester license is not interested in using it in an A&K technology.

FINAL PROJECT REPORT

Project Title: Identification of critical physiological targets in codling moth

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

Total Project Funding: $130,989

Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	$14,000	$15,000	$16,000
Benefits	$ 1,019	$ 1,024	$ 1,204
Wages	$ 4,800	$ 4,992	
Benefits	$ 466	$ 484	
Equipment			
Supplies	$22,000	$22,000	$12,000
Travel	$ 500	$ 500	
Plot Fees			
Miscellaneous	$ 5,000	$ 5,000	$ 5,000
Total	$47,785	$49,000	$34,204

ORIGINAL OBJECTIVES

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

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

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

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

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

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

Year 1:

- A gene transcript encoding the codling moth receptors for three neuropeptide/peptide hormones (PBAN, Neuropeptide F and Insulin receptor) were cloned.
- Transcriptomes from codling moth eggs, neonate larvae, and adult male and female chemosensory organs were prepared.
- Full length transcripts of six heat shock proteins (HSP) were cloned and expression profiles for three HSPs in various codling moth stages were determined.

Year 2:

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

Year 3:

- Completed characterization of codling moth transcriptomes and identified several classes of proteins that are critical for regulation of the codling moth's sense of smell. Gene transcripts encoding sensory neuron membrane proteins (SNMP) and odorant degrading enzymes (ODE) were identified.
- Characterized gene transcripts encoding three codling moth general odorant binding proteins and determined their expression profiles in chemosensory tissues of pupal and adult moths.

RESULTS & DISCUSSION

The chemosensory system is responsible for the detection of chemical compounds present in the insect's environment. The senses of smell (olfaction) and taste (gustation) play crucial roles in insect survival, often regulating feeding and reproductive behaviors. For the codling moth, the chemosensory system is critical for finding food sources, mates, and oviposition sites. Many codling moth control programs already include semiochemicals targeting the chemosensory system. Codlemone, a sex pheromone, is currently used in the orchard for mating disruption. A further understanding of the molecular pathways that are used by pheromones will allow for the potential discovery of new, more effective compounds that can be used in codling moth control programs.

Identification of major proteins involved in the detection of pheromones

To gain a better understanding of the codling moth sense of smell, the major research focus of this project has been to identify and characterize the main proteins involved in insect olfation. An overview of the components of the moth olfactory system is presented in Figure 1. Important proteins involved in the detection of an odorant, such as codlemone, include odorant binding proteins (OBP), odorant degrading enzymes (ODE), sensory neuron membrane proteins (SNMP), and odorant receptors (OR). As an odorant enters the antennal sensilla, it is bound by an OBP in the sensillar lymph. The odorant/OBP complex makes its way through the sensillar lymph until it reaches the olfactory neuron membrane, where interactions with its OR and SNMP occur. Odorant interaction with its OR causes the nerve to fire, sending a signal to the brain which can cause a behavioral response, such as the tracking response used by codling moth males when it is exposed to codlemone. To control nerve firing, ODEs degrade the odorants stopping the signals sent from the OR. Several studies indicate that each of the protein classes involved in the sense of smell are needed for the proper functioning of the moth's olfactory system.

We have analyzed transcriptomes generated from codling moth chemosensory tissues to identify gene transcripts encoding proteins that may be critical to the detection of codlemone and plant derived odorants. Last year we reported the identification of gene transcripts encoding 18 codling moth OBPs, of which 8 are predicted by homology to potentially bind pheromones and plant derived kairomones. In further analysis of the codling moth transcriptome data, we have now identified two transcripts encoding SNMPs and transcripts encoding several ODEs. It is interesting to

note that ODEs belong to the same family as insecticide degrading enzymes, and for codling moth we identified 10 glutathione *S*-transferases, 12 carboxylesterases, and 20 mixed function oxidases (Table 1). We are in the process of cloning the gene transcripts encoding codling moth OBPs, ODEs and SNMPs for future functional studies to determine their viability as targets for insect control.

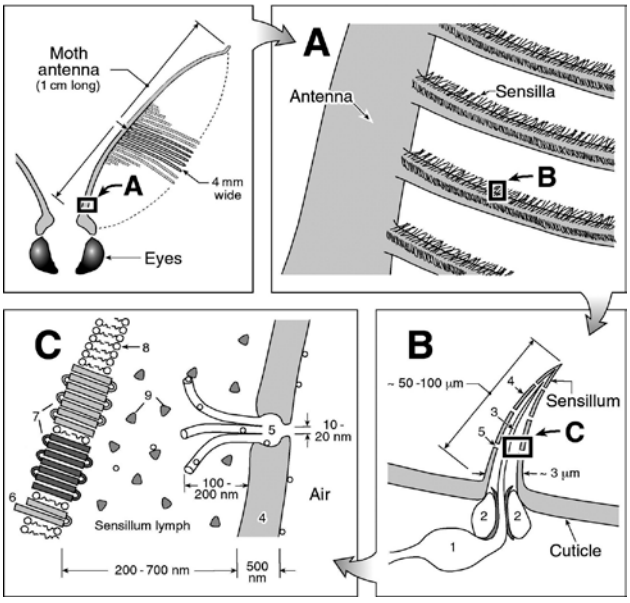

Figure 1. The route a pheromone takes through the sensillum lymph on its way to the olfactory nerve. A moth antenna (A) is highly branched, and the branches are covered with sensory hairs (sensilla). The sensilla are hollow cuticular structures that are innervated by a few olfactory neurons (1 in panel B). The cuticle of the hair (4) is traversed by pores (5). The dendrite of an olfactory neuron (3) protrudes into the space of the hair and is bathed by an electrolyte-rich sensillar lymph (panel C). The sensillar lymph contains odorant binding proteins (9 - triangles in panel C) and ions (Na+, K+, Cl–, phosphate). The dendritic (olfactory neuron) membrane (8 in panel C) houses the odorant receptors (7 in panel C) and the sensory neuron membrane proteins (6 in panel C).

Table 1. Potential odorant degrading enzymes annotated from codling moth transcriptome.

Tissue Source	Annotated Hits by Homology	GST	Esterase	Cyt P450
Male Antennae	**542**	**4**	**4**	**5**
Female Antennae	**475**	**4**	**3**	**3**
Male Legs and Mouthparts	**431**	**3**	**0**	**5**
Female Legs and Mouthparts	**350**	**1**	**4**	**6**
Eggs (Embryos)	**660**	**2**	**0**	**3**
Neonate Larvae	**617**	**4**	**1**	**4**
All Tissues	**2267**	**10**	**12**	**20**

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

Characterization of gene transcripts encoding codling moth general odorant binding proteins

General odorant binding proteins (GOBPs) are members of a subfamily within the clade of OBPs. GOBPs are mainly expressed in antennae and have been shown to bind pheromones and plant volatiles, suggesting possible roles in mate and host finding. Because GOBPs bind both pheromones and plant compounds, we wanted to see where the gene transcripts are expressed for clues as to the types of compounds they may bind. First we cloned the full-length gene transcripts encoding the three GOBPs identified by transcriptome analysis. The transcript for antennal CpomGOBP1 is 698 nucleotides (nt), encoding for 163 amino acids, that for CpomGOBP2 is 1289 nt, encoding for 160 amino acids, while the transcript for CpomGOBP3 is 702 nt, encoding for 169 amino acids (data not shown).

To determine where the CpomGOBP transcripts are expressed in males and females, an expression profile was performed using RT-PCR to detect RNA transcripts in a variety of codling moth tissues (Fig. 2). CpomGOBP1 expression was limited to pupal heads, and adult antennae and mouth parts, with PCR products more strongly visualized in adult male and female antennae (Fig. 2, top panel). CpomGOBP2 transcripts were more broadly expressed, with PCR products detected in late pupae heads, and antennae, heads, mouthparts of adults from both sexes, and female abdomen tips (Fig. 2, second panel). Expression of CpomGOBP3 was similar to that of CpomGOBP1 with PCR products mainly detected in antennae and mouthparts, and also faintly detected in female heads (Fig. 2, third panel). CpomActin, which should be expressed in all tissues, was used as a control (Fig. 2, bottom panel).

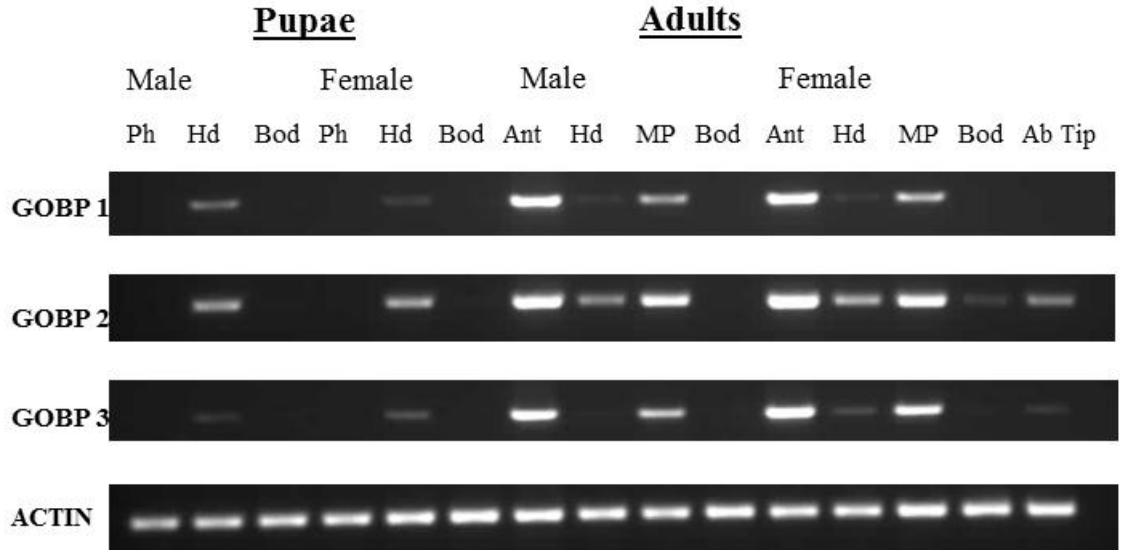

Figure 2. CpomGOBP1, CpomGOBP2, and CpomGOBP3 expression profile in cDNA prepared from total RNA extracted from pharate pupae (Ph), male and female pupae heads (Hd) and bodies (Bod), adult male and female antennae (Ant), heads (Hd), mouthparts (MP) and bodies (Bod), and adult female abdomen tips (Ab Tip). Top Panel: Detection of CpomGOBP1 using transcript specific primers in RT-PCR reactions. Second Panel: Detection of CpomGOBP2 using specific primers in RT-PCR reactions. Third Panel: Detection of CpomGOBP3 using specific primers in RT-PCR reactions. Bottom Panel: Detection of CpomActin using specific primers in RT-PCR reactions. For CpomGOBP1, CpomGOBP2, CpomGOBP3 and CpomActin, PCR products were visualized on 1.5% agarose gels by ethidium bromide staining and UV illumination. Bands produced were of the size predicted for the transcript specific primer pair.

Last year, we reported detecting abnormalities in messenger RNAs (mRNA - gene transcripts that encode proteins), that code for pheromone receptors, and we have detected similar abnormalities in mRNAs that code for GOBPs. Figure 3 shows a diagram representing the structural features of mRNA molecules. Of major interest is the 3'untranslated region (3'UTR) and the Poly-A tail. What we have found is that several gene transcripts that code for pheromone receptors and the three GOBPs alter the length of their 3'UTRs by alternating the places where the poly-A tail is added. By altering the length of the 3'UTRs of these gene transcripts, the insect can regulate how much protein gets made and localize where the protein is inserted into the membrane. This type of mechanism would be important in codling moth detection of sex pheromone and would allow for males to increase protein amounts as they detect increasing sex pheromone concentrations as they approach females. Having transcripts with a variety of 3'UTR lengths can potentially regulate the number of receptors on a nerve membrane, or increase the number of OBPs in response to pheromone detection. This rare mechanism has been previously cited as a means for translational control of gene transcripts in mammals, but has yet to be reported for insects. Collaborative research projects to explore this mechanism for detection of pheromones have been initiated with Richard Newcomb at Hort Research in New Zealand.

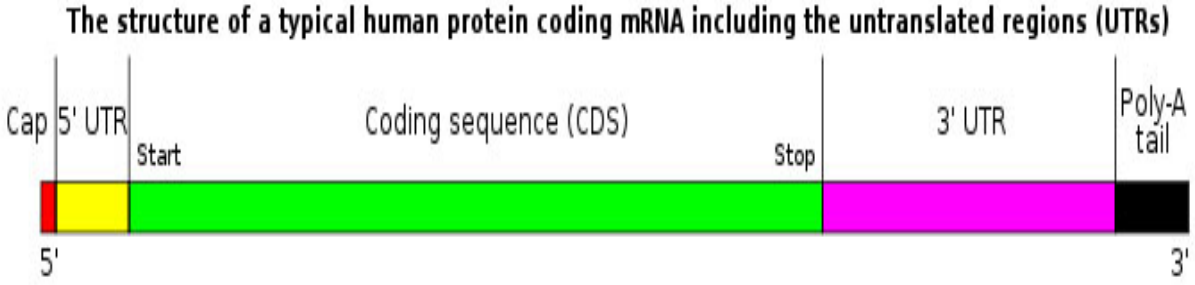

Figure 3. The structure of a typical mRNA molecule (also referred to as a gene transcript). At the extreme 5' end of the mRNA is a Cap structure which helps protect the molecule from degradation. The Cap is followed by a 5' untranslated region (5'UTR) which is a variable stretch of nucleotides that contains regulatory elements. Following the 5'UTR is the Coding sequence (CDS) which has a specific nucleotide sequence that encodes for a specific amino acid (protein) sequence. After the CDS is the 3'untranslated region (3'UTR) which is a stretch of nucleotides that contain regulatory elements and sequences that signal the addition of a Poly-A tail.

EXECUTIVE SUMMARY

The chemosensory system, which includes the senses of taste and smell, is responsible for the codling moth's ability to detect chemical cues in its environment. The codling moth, as well as other pest insects, use the senses of smell and taste to detect food sources and to locate mates or oviposition sites. The goal of this project was to determine protein components expressed in chemosensory organs and other stages of codling moth by analyzing transcriptomes prepared from key tissues housing the machinery that the insect used to detect food and odorant sources. Transcriptome analysis, coupled with cloning and characterization of key olfactory proteins, has provided fundamental biological information on codling moth physiology that can be used to identify new targets for enhanced pest control by disruption of the insect's sense of smell.

To gain a better understanding of the codling moth sense of smell, proteins encoding the major constituents of the molecular machinery used to detect odors in antennae were identified. Gene transcripts encoding pheromone and other odorant receptors (membrane proteins which are involved in nerve signaling in response to a pheromone or other odors), odorant binding proteins (small soluble proteins that are thought to shuttle pheromones and other odors to the membrane receptors), odorant degrading enzymes (proteins that degrade pheromones and other odorants) and sensory neuron membrane proteins (membrane proteins which are involved in the interaction of pheromones and pheromone receptors) were identified, cloned and characterized. A major finding was that gene transcripts encoding pheromone receptors and general odorant binding proteins use a mechanism in which they produce variant 3'untranslated regions of their mRNA. This is potentially important in explaining how a moth can detect concentration gradients of sex pheromones and other attractive odorant sources.

The results of the research performed in this project has led to new ideas on how moth's detect pheromones and other odorants, and has led to the initiation of several new collaborations with research groups in California, New Zealand and France. Research on the interaction of pheromones with their membrane receptors is already providing insights on the design of parapheromones that interact more strongly with pheromone receptors, and are more attractive to moths. These same design strategies can be used to synthesize novel codlemone derivatives which may provide better codling moth control in the orchard. Research involving altering odorant degrading enzyme function is also underway. Several enzyme inhibitors are available and can be used to determine the role of odorant degrading enzymes in the molecular detection of pheromones. It is hypothesized that alteration of odorant degrading enzymes will cause an habituation-like affect and perhaps be a useful supplement in mating disruption strategies. Lastly, several researchers are now interested in studying the regulation of the production and localization of pheromone receptor proteins by alteration of the 3'untranslated region of the mRNAs that encode them. If we can understand how a moth produces proteins in response to detecting pheromone concentration gradients it will help us understand the basis of how mating disruption is working and provide clues on how to enhance its effectiveness.

FINAL PROJECT REPORT

Project Title: Evaluating use of sterile codling moth in apple IPM programs

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Cooperators: Gary Judd and Howard Thistlewood, Canada Agriculture, Summerland BC; Mike Doerr, WSU TFREC, Wenatchee.

Total Project Request: **Year 1:** $41,106

Other funding sources

Agency Name:	California Walnut Board
Amt. requested/awarded:	$19,000
Notes:	Funding to support CA project only

Budget 1 History

Item	**2011**
Salaries (M. Doerr – one month)	5,278
Benefits (M. Doerr)	1,684
Salaries (Research Assoc. - five months)	12,900
Benefits (Research Assoc.)	6,024
Wages	4,800
Benefits	720
Equipment	0
Supplies	1,000
Travel	1,000
Miscellaneous	0
Total	33,406

Budget 2 History: Okanogan Kootenay SRI

Item	**2011**
Salaries	0
Benefits	0
Wages	0
Benefits	0
Equipment	0
Supplies (SIRP moths - $4/dish)	7,200
Travel	500
Miscellaneous	0
Total	7,700

Objectives:

1. Establish a mechanism for the SIRP to move moths across the US/Canada border.
2. Determine the best method(s) for releasing SIRP moths to achieve desired results.
3. Evaluate the capability of SIRP moths to control codling moth in selected orchard situations – bin piles, orchard borders, hot spots in orchards, and late season orchard treatments.

SIGNIFICANT FINDINGS:

- Due to regulations on moving insects across international boundaries we were not able to obtain permission for the Okanogan Kootenay SIRP facility in Osoyoos to bring sterile codling moths across the US/Canada border. We relied in the existing USDA-APHIS permit to bring moths across the border on a weekly schedule. While this process worked well for the current project if there was any interest from the industry in accessing sterile CM for commercial purposes a different method of transport would need to be worked out.
- We did not achieve a high sterile to wild moth ratio in traps at most of our SIR release sites, only four of eleven and two of these were sites where no mating disruption was used.
- Most of the sites selected were good tests of border problems in orchards dealing with immigrating codling moth.
- Fruit injury data at harvest was highly variable and more injury occurred on average in blocks where SIR moths were released. While these results could have been influenced by how we set up our study sites it is not very encouraging result.
- There was no difference in moth activity, number recaptured, at different distances from release sites. This would allow for SIR moths to be more rapidly distributed in the orchard should this technique be developed into a commercial venture.

Methods

Insects: Sterile Codling moths were produced at the Okanogan Kootenay SIRP facility in Osoyoos, British Columbia. The first moths were received in Washington state on June 1, 2011 and released at SIR trial sites on June 1 and 2, 2011. The last release date was August 31, 2011. The codling moths were packaged and shipped in Petri dishes (Fig. 1 - left) inside of Styrofoam coolers with ice packs to keep them from damaging themselves during transport. Each Petri dish was filled by weight and contained approximately 800 moths. The codling moth caterpillars were reared

Figure 1. Sterile codling moth were obtained from the Canadian SIR facility in petri dishes of 800 moths (left) and were released by dumping the cooled moths into a release cup that protected the moths and allowed them to exit through holes (right).

using a diet with red dye, which provided a fast way to tell wild moths from sterile moths captured in traps during these studies.

Traps: Both treatment (SIR release) and control (no release) orchard blocks were monitored using white delta type traps baited with codling moth pheromone lures. At the beginning of the season we used 0.1 mg codlemone lures, but due to low trap catches in mating disruption treated orchards we changed to Trécé L2 lures, commonly referred to as a 1x lure. Trap catches improved after this lure change.

Sterile moth release devices: Release devises where constructed from 16 ounce clear plastic deli cups, by cutting four 1 inch diameter holes in the sides and attaching a wire for hanging them from trees (Fig. 1 - right). These release devices gave the moths a somewhat protected area to become acclimatized to the release site and fly out when they were ready. Moths are released using a blower from the back of a 4-wheeler in British Columbia. This method is efficient for the delivery of large numbers of moths, but was method for the relatively small number of moths released in this study. Using the release devices also provides a degree of consistency across the different treatment areas which otherwise might have had very different moth survival rates due to differences in ground cover, irrigation practices, and orchard structure. One entire Petri dish of moths, containing approximately 800 moths (400 males), was released into each release device every week.

Study sites: It was originally intended that there be four different categories of sites for this study: 1) bin piles, 2) orchard borders adjacent to external CM problems, 3) internal orchard hot spots, and 4) late season orchard treatments. When orchardists were contacted to locate good candidate sites for this study, the most commonly described problem areas were borders adjacent to problem areas, usually neighboring orchards or in one case a bin pile. Sites were chosen in eight orchards in central Washington. Three of these orchards contained two different problem areas treated as separate sites for this study. Orchards were located near Brewster, Chelan, Manson, Selah, Wapato, Buena and Royal City. The Brewster site was the last one to be set up and releases began on June 22, 2011. The second Manson site (Buck) was set up two weeks later than the other sites and releases started at this location on June 15, 2011. All of the orchards included in this study employed mating disruption for the management of codling moths, with one exception: the Wapato Orchard. One of the study sites was so small that a separate treatment and control block could not be established with any expectation that sterile moths would not totally infiltrate the control block. This site, the second Manson area site, therefore, had no control for comparison.

Site descriptions: The *Brewster* location had historically been a problem area for codling moth due to the strange long narrow shape of the orchard. It is situated between a railroad and the Columbia River and is between one and four rows wide and nearly half a mile long. This has made it difficult to manage and made insecticide treatments less cost effective. This orchard is essentially one long border with nearby backyard orchards, which in general have notoriously high codling moth populations. The year prior to this study very high codling moth damage noted, especially in the northern half of the orchard, which was at least partly due to a lack of management for the second half of the season.

The first *Manson* location (Fry) is a relatively small plot of older red and golden delicious trees which is bordered very closely on three sides by apple orchard and also interspersed with homes, making it difficult to manage for codling moth. Historically this orchard has had trouble along the northwestern border which is where the SIR moth releases were focused.

The second *Manson* location (Buck) is the northernmost edge of a large orchard. It is also the highest edge of the orchard, which is on a slope. Historically this edge has had high codling moth damage,

possibly due to moth immigration from neighboring yards or feral apple trees. Inspection of this orchard found it to be a mixture of varieties and sizes of trees with gaps between trees and evident horticultural problems. The trap catch in this orchard prior to the first release showed that there was a large number of codling moths present.

The *Chelan* location was the only site we found representing the bin pile category. The orchard was near to and uphill from the Chelan Fruit warehouse bin pile and has reportedly suffered effects from codling moth due to it close proximity to the bin pile. Bin piles are notorious for causing difficult codling moth management situations for nearby apple growers because codling moth larvae are not killed during storage in controlled atmosphere storage (CA), and they emerge from diapause after being removed from storage. These moths are continuously emerging from bins that are processed and moved to the bin pile area. Moths are also not typically in sync with the natural life cycle of the codling moth making it difficult or impossible to correctly time insecticide treatments for management. Unfortunately there were no trees or sites for placing traps between the bin pile and the orchard, but release cups were attached to metal fence posts and placed along the edge of the bin pile. The edges of the orchard nearest to the bin pile were monitored with traps and sterile moths were released there as well (Fig. 2A).

The *Selah* location contained two separate sites where codling moth had been of concern. One site was bordered by residences and backyard apple trees (Fig. 2B - Selah West) and the other was a section of the orchard with large old golden delicious variety trees (Fig 2B - Selah East). Both of these sites had histories of high codling moth damage and trap catches.

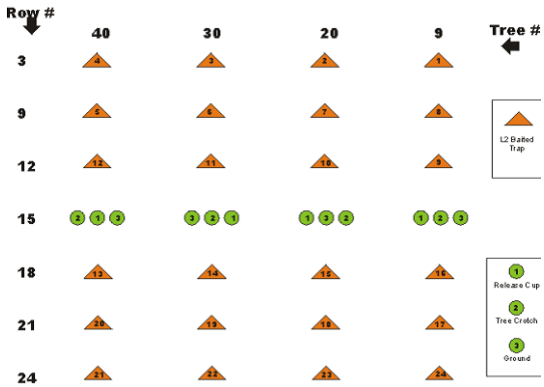
The *Wapato* location had some unique problems. The orchard was a mix of tree crops including pears, cherries and peaches and different varieties of apples planted in several different planting layouts. The orchard was on a hill with significant elevation differences between the different apple plots, making it difficult to evaluate degree days and timing of pesticide treatments in the different areas. The orchardist reported two specific problem areas. One was a small block of triple row planted Gala apples at the top of the hill (Fig. 2D - Wapato Gala) and the other was a slightly larger area of large Golden Delicious trees (Fig. 2D - Wapato Gold). This was the only orchard in this study that did not use mating disruption, which resulted in higher moths catches compared to the other locations.

The *Buena* location was larger and more uniform than most of the other locations. This orchard contained two separate border areas that had experienced effects of poorly managed neighboring orchards. Bordering one of the sites, *Buena NE* (Fig. 2C) was a pear orchard in which there was clear evidence of codling moth damage throughout the season. The other site, *Buena SW* was adjacent to another apple orchard which showed signs of codling moth damage throughout the season and also had historically received pressure from orchards across the road (Fig. 2C). The manager of this orchard reported having to hand remove damaged apples along these border areas before harvesting the previous year because there was so much injury from codling moth.

The *Royal City* location had a reported border problem along one edge shared with another apple orchard. The bordering orchard was small and did not extend the entire length of the study orchard providing only a small area for a release treatment and a control block.

Damage surveys: Codling moth damage was evaluated in each location two times over the course of the study; after the first codling moth generation and again prior to harvest. Fruit damage was evaluated in each SIR release treatment and control by randomly selecting 40 trees distributed throughout each block and inspecting forty half-fruit on each tree. In cases where there were fewer than forty half-fruit visible on a selected tree the evaluation was continued on neighboring trees until a total of forty half-fruit were counted. The number of codling moth injuries found was recorded for

each tree. The trees in the orchard at the *Chelan* location had very few apples, and the SIR release and control blocks were very small necessitating a smaller sample size of twenty half-fruit per tree.

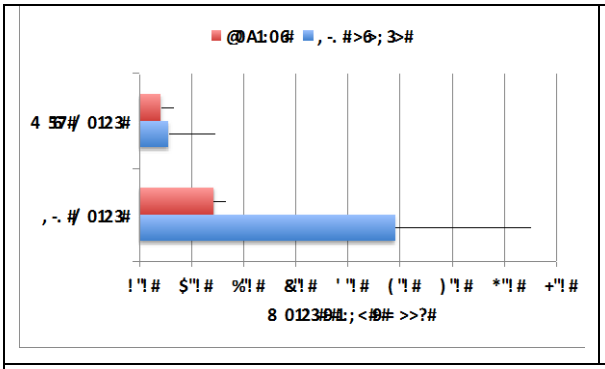


Release method comparison: Moths were colored with florescent powder and released using three different release methods at point sources in an organically managed orchard east of Wenatchee. Pheromone baited traps were placed 60, 120 and 180 feet north and south of these release points (see image to right). Three different release methods were used: the release device described above, pouring the moths onto the ground around the base of a tree, and quickly distributing the moths into the tree itself, mostly in large crotches of the trees and on horizontal branches. Some of the moths that were place in the trees did end up on the ground, but this simulated the actual results that could be expected using this release method in practice. Moths were released once per week for eight weeks, from July 8, 2011 to September 2, 2011. Traps were checked twice per week at four and seven days after release. Trap bottoms with moths were brought back to the lab and checked with a UV lamp to determine florescent powder color. One wild codling moth was captured during this trial.

Results & Discussion

Efficiency evaluation: Most of the time and money invested in distributing the sterile codling moths for this study was spent in driving to the moth pickup site and between all of the release sites. This may or may not be an expense included in the implementation of an SIR release for coding moth control depending on the location of releases and method of transportation chosen in the future. Because the moths should be released soon after receipt, efforts to coordinate speedy distribution of moths to release sites and make timely application would be a concern and will probably be the most expensive and complicated part of adopting this management technique. For this study the crew from Wenatchee spent a total of 9 hours driving to the SIR moth drop off site and between release sites each week covering approximately 430 miles. These figures do not include the driving time or miles driven by the person who picked up the moths up in Canada each week and deliver them to the drop off site in Brewster. Relative to the driving time, the time spent actually releasing the moths in the orchards was very small.

As discussed above the moths were release into release cups and the lid was replaced, so this took



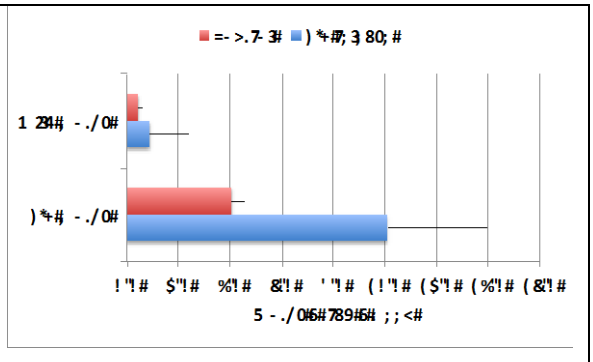


Figure 3. Average moths per trap per week captured in 0.1 mg lures (June) and L2 lures (July-Aug.) in sites with releases of SIR moths and in control (no release) sites (N=11).

more time than applying them onto the ground would have, but either way most of the time was spent walking between the release point. Subtracting the time spent checking the monitoring traps in the treated areas, releasing sterile moths into the release devices at a rate of one device per acre took approximately 15 to 20 minutes for each plot with between five and seven release devices in each. Extrapolating these values gives a figure of between 3 and 4 minutes per acre. If the applicator were driving a four-wheeler and/or distributing the moths on the ground or directly onto the trees instead of putting them in release devices this time could be shorter.

The components of the release devices themselves are inexpensive but they are time consuming to assemble using the current method. This process could be streamlined and made much more efficient for mass production if necessary.

Trap catches: Sterile moths were consistently captured in traps baited with either the 0.1 mg or L2 lure in the control blocks (Fig. 3). Due to the small size of the orchard plots used for this study this result was understandable and expected. When averaged over the 11 test sites the number of sterile moths captured was lower in control compared to the SIR release blocks (Fig. 3). The average ratio of sterile moths to wild moths in the SIR release blocks was 8.7 and 11.3 when using the 0.1 mg lure

result was understandable and expected. When averaged over the 11 test sites the number of sterile moths captured was lower in control compared to the SIR release blocks (Fig. 3). The average ratio of sterile moths to wild moths in the SIR release blocks was 8.7 and 11.3 when using the 0.1 mg lure and the L2 lure, respectively. In the control blocks the average ratio was 3.4 and 8.8 when using the 0.1 mg lure and the L2 lure, respectively. The target ratio for using SIR to control codling moth is 40:1, however, this ratio is usually determined without the influence of mating disruption which we know reduces the efficiency of monitoring traps. In the SIR release blocks four of the 11 had ratios of sterile to wild moths greater than 40:1 (50:1-127:1), with two of these being the Wapato sites where no pheromone mating disruption was used. An additional SIR release block had a ratio of 36:1 (Chelan bin pile site) and another a ratio of 18:1 (Manson Fry). In the control blocks only two of 11 had a sterile to wild moth ratio greater than 40:1, 137:1 and 104:1, one of which was a Wapato site and the other Wapato site had a ratio of 28:1. These data suggest that there should have been some impact of the SIR releases on codling moth.

At some sites the capture of SIR moths was very low relative to the wild moths. At these sites it is highly likely that grower spray programs could have negatively impacted the survival of sterile

Table 1. Percent fruit injury in different treatments at each study location after the first CM generation and prior to harvest.

Location	Treatment	1st gen	Harvest
Chelan	SIR release	0.00%	0.00%
Chelan	Control	0.00%	0.00%
Manson Fry	SIR release	*0.17%*	*0.17%*
Manson Fry	Control	*0.00%*	*0.00%*
Manson Buck		3.33%	9.00%
Brewster	SIR release	*0.83%*	*0.33%*
Brewster	Control	*0.00%*	*0.17%*
Selah W	SIR release	0.00%	*0.67%*
Selah W	Control	0.00%	*0.17%*
Selah E	SIR release	0.00%	0.00%
Selah E	Control	0.00%	0.00%
Wapato Gala	SIR release		**1.17%**
Wapato Gala	Control	**1.83%**	**1.83%**
Wapato Golden	SIR release	**0.00%**	**1.00%**
Wapato Golden	Control	**2.50%**	**1.67%**
Buena SW	SIR release	0.17%	**0.33%**
Buena SW	Control	0.17%	**0.50%**
Buena NE	SIR release	0.00%	*4.83%*
Buena NE	Control	1.50%	*2.50%*
Royal Slope	SIR release	0.00%	*0.50%*
Royal Slope	Control	0.00%	*0.17%*

moths. We are still working on getting spray records from the cooperating locations to assess their possible impact on SIR released moths. At the Manson Buck location the average capture of wild moths was high, 5.9 moths/trap/week, which was essentially the same as captures of sterile moths. At the Buena location the ratio of sterile to wild moths was very low, 1.5:1 and 2.8:1 in the two SIR release sites and there was little difference in ratios on the control sites.

The primary goal of this project was to see if release of SIR moths could help problems sites within apple orchards. The bottom line for this determination is to see if fruit injury was reduced in locations where SIR moths were released.

Table 1 summarizes the fruit injury results from each orchard site. After the first generation it was found that there was more injury in two study sites, Manson Fry and Brewster, in the SIR release blocks than the control blocks. The Brewster site was set up late and was very small and injury was in the northern part of the orchard where it had been the worst the previous season. The fruit injury was lower at harvest in the SIR release block while injury in the control block had increased.

At the Mason Fry location fruit injury in the SIR release area was higher than the control treatment after the first generation but did not increase by harvest.

At the Buena SE site the SIR release blocks had lower levels of injury than the control blocks but at the Buena NE site the SIR release block at harvest had higher fruit injury than the control block.

At the Royal Slope location the SIR release block had a higher level of fruit injury than the control blocks at harvest.

At the Wapato location the SIR release block has less injury than the control blocks, though injury overall was high at this location, likely because no mating disruption was used.

Fruit injury data would not support the value of releasing SIR moths using the methods in this study. We did select sites with high codling moth pressure, which was the intent of the project. In some of the locations it was difficult to get a good control to compare with the SIR release area and this more than anything could have skewed the fruit injury results. Often we set up the SIR release area where most of the external pressure would be felt while the control block was typically more removed from the area of highest codling moth pressure.

Release method comparison: When SIR moths were marked with florescent powder and released using different methods there was a definite and consistent pattern. The further monitoring traps were from the release locations the fewer moths were recaptured and fewer moths were recaptured upwind of the release sites (Fig. 4 all data pooled).

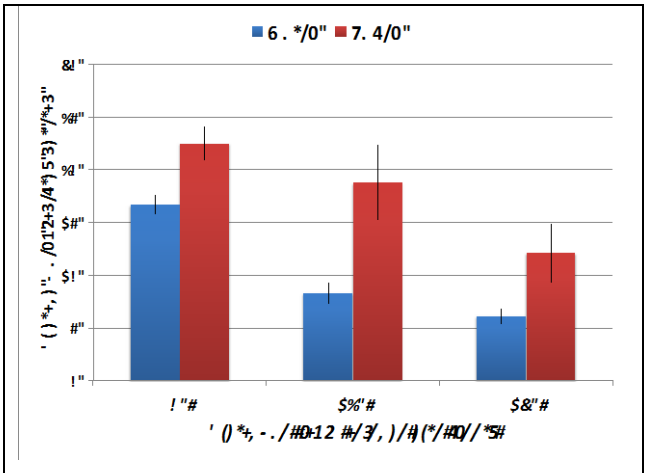


Figure 4. Average sterile moth capture in traps placed at different distances from the release site.

The average number of moths recaptured from the three different release methods was similar at each distance from the release locations (Fig. 5). These data suggest that releasing SIR moths by dumping them on the ground or putting them in the crotch of a tree would not hinder their activity. These releases were done in summer so the temperatures were warm and dew on the ground cover would not have been an issue. There were overhead irrigation in the block that was used in this study but if there was any effect from this practice it impacted all moth activity from all release methods the same.

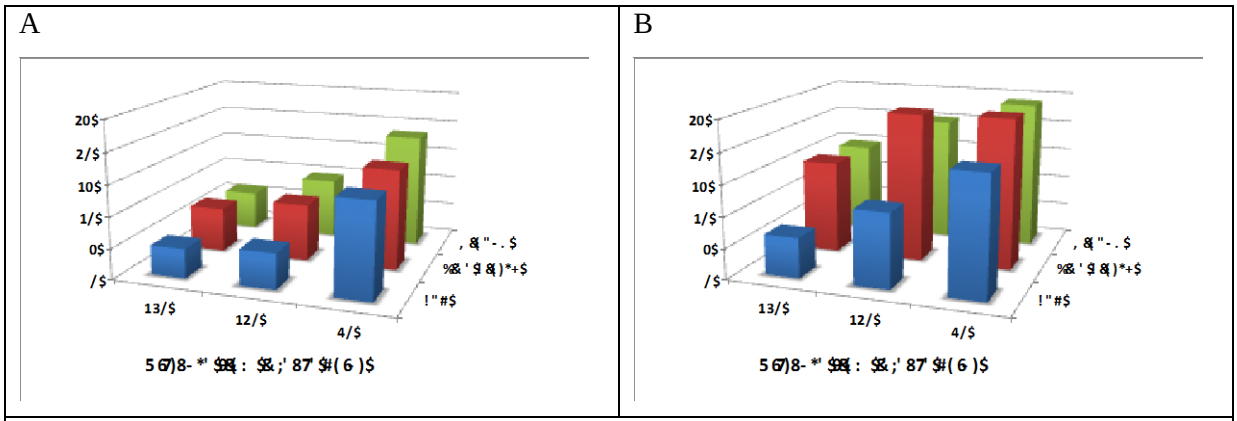


Figure 5. Average sterile moth capture in traps placed at different distances from the release site. A - moth capture to the north of the release sites and B - moth capture to the south of the release sites. Wind direction tended to be north to south.

Executive Summary

Moving insects across international boundaries for the Okanogan Kootenay SIRP facility in Osoyoos was found to be fraught with difficulty. The personnel at the Okanogan Kootenay SIRP facility were excellent to work with and they produced a very good product. We, therefore, relied on the existing USDA-APHIS permit to bring moths across the border on a weekly schedule. If there were interest from the industry in accessing sterile CM for commercial purposes a different method of transport would need to be worked out. We did not achieve a high sterile to wild moth ratio, 40:1 being the target, in traps at most of our SIR release sites, only four of eleven achieved this ratio and two of these were sites where no mating disruption was used. Most of the sites selected were good tests of border problems of orchards dealing with immigrating codling moth. Fruit injury data at harvest was highly variable and more injury occurred on average in blocks where SIR moths were released than the "control" blocks at the same location. While these results were likely influenced by how we set up our study sites it is not a very encouraging result. There was no difference in moth activity, number of moths recaptured, at different distances when we released moths using different methods, release cup, dumped on ground and placed in the crotch of a tree. These results would allow SIR moths to be more rapidly distributed in the orchard should this technique be developed into a commercial venture.

FINAL PROJECT REPORT

Project Title: Chlorochroa ligata pheromone and development of management strategies

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

Other funding sources

Agency Name:	**California Pistachio Commission**
Amt. requested/awarded:	$19,353
Notes:	Work to be done in CA

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

Agency Name:	**SCRI**
Amt. requested/awarded:	funded at $
Notes:	Collaborative national project

Total Project Request: **Year 1 & 2 plus BMSB**: 67,226

Budget History

Budget 1 – Jay Brunner

Item	2010	2011	2011-BMSB	2011-Total
Salaries (Doerr; 0.0166 FTE)	0	5,247	0	5,247
Benefits (Doerr; 0.0166 FTE)	0	1,590	0	1,590
Wages	7,000	6,400	4,000	10,400
Benefits (14.8%)	1,036	1,036	600	1,636
Equipment	0	0	0	0
Supplies (traps and lures)	5,000	0	5,000	5,000
Travel	500	1,000	500	1,500
Miscellaneous	0	0	0	0
Total	13,536	16,373	10,100	25,373

Budget 2 – Jocelyn Millar

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

Budget 3 – Peter Shearer

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

Budget 4 – Todd Murray

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

OBJECTIVES

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

SIGNIFICANT FINDINGS:

- Synthesis of *C. ligata* pheromone was challenging due to two steps in the five-step process that represented bottlenecks in production of high quality pheromone. These synthesis problems will make it a challenge to interest companies in making a *C. ligata* pheromone lure or the lures will be very expensive. The problem is exacerbated by the fact that it is necessary to use large doses of pheromone (50-100 mg) in each lure.

- There was little difference in release rates of *C. ligata* pheromone from polyethylene lures of different thickness under laboratory or field conditions.

- Over two years traps baited with *C. ligata* pheromone lures made of polyethylene of different thickness captured low numbers. The thinnest polyethylene lure, 1.5 mil, captured more *C. ligata* than a commercially produced fiber lure in 2011. Low capture levels were due to low densities of *C. ligata* in the areas trapped coupled with a low release (evaporation) rate of the pheromone from lures tested. Placing multiple *C. ligata* pheromone lures (3 or 10) in the same trap increased captures by five to six times compared to a trap with only one lure.

- *E. conspersus* pheromone release rates from polyethylene lures increased as the thickness decreased. In 2011 the thinnest lure's release rate was about four times that of a commercial lure. In the spring traps baited with the thinnest polyethylene *E. conspersus* lures (= highest release rate) captured more adults compared to thicker polyethylene lures and a commercial lure. However, in the summer traps baited with a commercial lure and the thickest polyethylene+foil lure captured more *E. conspersus* adults than traps baited with thinner polyethylene lures.

- Traps baited with the *E. conspersus* or *C. ligata* pheromones captured primarily conspecifics, that is, their own species. When lures of both species (combo lure) were placed into the same trap there was no interference in the capture of either species.

- Trap captures of *E. conspersus* and *C. ligata* tracked seasonal activity, indicating that each species has only one generation per season (2010 and 2011).

- Laboratory screening of insecticide residues showed that Thiodan, Lannate and two pyrethroids (Danitol and Warrior) were most effective in killing *E. conspersus* adults. Treating the panels of pyramid traps with insecticide did not prove to be an effective way of intoxicating stink bugs adults, probably because they tended to crawl up the edges of panels.

- Pyramid trap efficiency is limited because stink bug adults can escape. For example, 70% of the stink bug adults placed inside jugs escaped within four days. Placing a kill strip inside the jug or treating the inside of the jug with an insecticide dramatically reduced the number of escapes.

- Large numbers of *E. conspersus* adults, 527, were trapped from native habitat near a apple orchard notorious for high fruit injury from stink bugs. The area closest to the high density trapping area had the lowest fruit injury, 0.4% compared to the orchard average of 4.4%.
- When trap captures of summer *E. conspersus* adults near orchard boarders was compared with fruit injury in the border row there was a good relationship ($R^2 = 0.88$), giving hope that traps could be a useful tool in indicating risk of crop damage.
- BMSB was detected at four sites in southwestern WA, representing a slight increase in its distribution in WA. BMSB was also detected in Hood River, WA. No BMSB were detected in eastern WA or Milton-Freewater, OR.

METHODS

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

Optimize release rate of stink bug pheromone. Pheromones of *C. ligata, E. conspersus and T. pallidovirens* were put in sealed polyethylene pouches with a cotton wick (Fig. 1), which were then placed in a fume hood in the laboratory and weighed at regular intervals to determine the evaporation (= release rate). After a range of release rates was identified candidate lures were placed in the field to determine which attract the most of each species. Traps baited with different lures were checked every 2-4 days. Bugs trapped during each sample period were identified, counted and sexed. Lure trials were replicated at four to five locations. Unbaited traps served as controls.

Fig. 1

Traps. In 2010 new pyramid traps were constructed from expanded PVC panels and painted yellow. Traps were 4 feet tall with an 18" base. A one-gallon clear plastic jar was fixed to the top to collect bugs (Fig. 2 - left). In 2011 smaller traps, black in color, were used in the trap out (attract and kill) study and were used to monitor the red-shoulder stink bug, *Thyanta pallidovirens*, and the BMSB (Fig. 2 - right).

Fig. 2

Life history of *C. ligata*. Trapping began in May to capture overwintering bugs and continued into September. By monitoring over this period we were able to characterize the seasonal life history of *C. ligata* and compare it to *E. conspersus*. Trapping focused on the border areas of orchards. Traps were checked weekly, bugs removed, counted, sexed, and life stages recorded.

Dual attraction of *C. ligata* and *E. conspersus*. A new trap was placed at each of four locations where we were already sampling for *C. ligata* and *E. conspersus* that was baited with a lure for each

species (= combo trap). The number and sex of each stink bug was recorded from August 23 through September 13.

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

In 2011 we placed 20 traps in an uncultivated area of an orchard, which historically had high pressure from *E. conspersus* to determine if removal of bugs from this area could reduce fruit injury in the nearby orchard. Traps were checked two times per week and the number and sex of bugs recorded. Fruit injury samples were taken in the nearby orchard, Tillman site, prior to harvest in October by examining 20 fruit per tree in four rows starting with the edge tree and each tree at 25 meter intervals within each row.

BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug. Additional funding was provided to sample for the BMSB. Most of the trapping was done with small black pyramid traps (Fig. 2) with emphasis on the southern part of WA and northern OR. Traps with pheromone lures (*Plautia stali (E,E,Z)*- 2,4,6-decatrienoate)) were placed out in August. In addition, Master Gardeners were trained by WSU Extension personnel to trap and search for BMSB in western WA.

RESULTS AND DISCUSSION:

Synthesis of stinkbug (*C. ligata and E. conspersus)* pheromone. The Millar laboratory began synthesizing *C. ligata* pheromone in late winter of 2009-10. The pheromone synthesis process must have as few steps as possible to minimize costs. Each step must produce clean product with minimal byproducts. Starting materials and intermediates should be as cheap as possible. The synthesis process was challenging because two of the five steps had low yields. Twenty grams of pheromone were delivered on 1 April, 2010 and we began lure release rates studies and trapping studies with polyethylene lures (Obj. 2). The Millar laboratory synthesized another 16 grams of *C. ligata* pheromone by 1 July, 2010, which was sufficient to complete planned studies lure testing and phenology studies. The Millar laboratory provided another 50 grams of *C. ligata* pheromone for 2011 research activities, but it is unlikely that the synthesis process can be tweaked to increase yield. We worked with a commercial company, Scentry Biologicals, interested in producing *C. ligata* pheromone but they ran into the same barriers to efficient synthesis as experienced by the Millar laboratory. They did, however, provide some pheromone and a fiber lure for testing in 2011. Synthesis obstacles will make production of the *C. ligata* pheromone expensive and, therefore, it is unlikely available as a commercial product given the relative importance of this species.

In 2010 we obtained commercial *E. conspersus* lures and removed pheromone to make our own polyethylene lures (Fig. 1) used in release rate studies. In 2011 the Millar laboratory converted (pear ester) to the *E. conspersus* pheromone so we could conduct field studies on optimization of lures for *E. conspersus* capture and for trap out (attract and kill) studies.

Millar's laboratory also provided pheromone of another stink bug species, *Thyanta pallidovirens*, the red-shoulder stink bug, which was used in monitoring at some sites in 2010 and 2011.

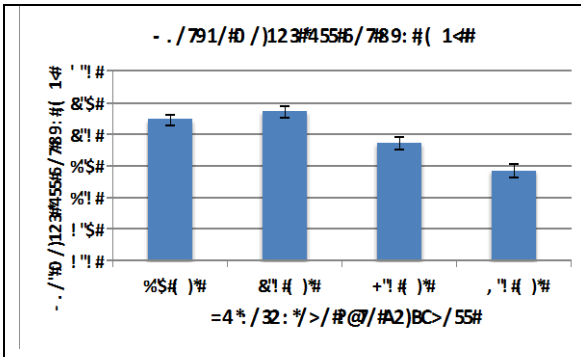

Fig. 3. Average total weight loss (n=4) of *C. ligata* pheromone from polyethylene lures of different thickness.

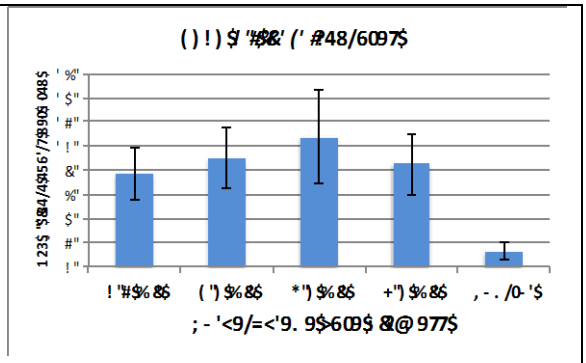

Figure 4. Average cumulative capture of *C. ligata* in traps baited with polyethylene lures of different thickness.

Optimize release rate of stink bug (*C. ligata and E. conspersus)* pheromone. Under laboratory (fume hood) conditions the release rate of *C. ligata* pheromone from polyethylene lures of variable thickness showed very little difference (Fig. 3). Evaporation of *C. ligata* pheromone from a cotton wick was only about 25% higher than the thinnest, 1.5 mil, polyethylene lure. When *C. ligata* pheromone lures were aged in the field for 49 days and weighed every 7 days pheromone release rate (net weight loss) was low, similar to the laboratory study, with no real differences observed between lures of different polyethylene thickness.

Average (total) capture of *C. ligata* in traps baited with polyethylene (pheromone) lures was low with no differences in between lures of different thicknesses over a 47-day period (Fig. 4). In 2011 we compared a 1.5 mil polyethylene lure with a *C. ligata* fiber lure provided by Scentry Biologicals. Captures of *C. ligata* were low but more adults were captured (LSD p-value = 0.03) in traps baited with the polyethylene lure in the spring, due primarily to a capture of more males while in the summer the same lure captured more adults (LSD p-value = 0.02) due to capturing more females.

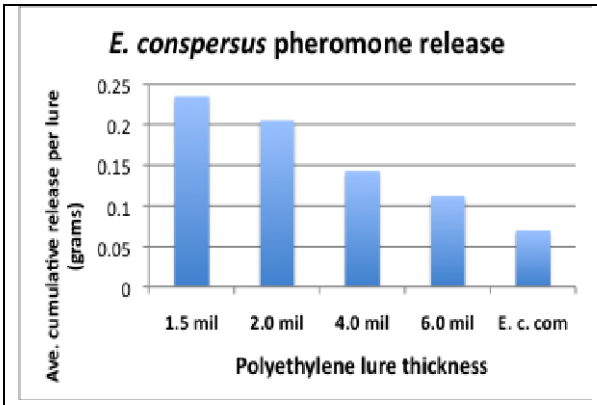

Fig. 5. Average weight loss of *E. conspersus* pheromone from polyethylene lures of differing thickness, 2010.

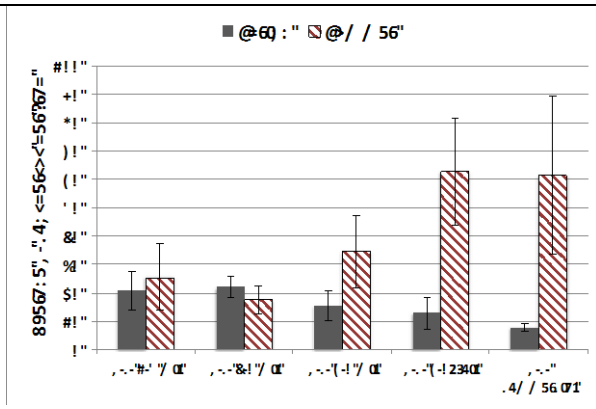

Fig. 6. Average capture of *E. conspersus* adults in pyramid traps baited with polyethylene lures of differing thickness loaded with aggregation pheromone during the spring and summer of 2011.

In an attempt to enhance the attraction of *C. ligata* pheromone we baited traps with a single lure, three lures, and ten lures. The trap baited with three and ten lures captured between five and six times more *C. ligata* adults than the trap baited with the single lure. There is some hope that the attraction of *C. ligata* could be enhanced by using multiple lures in the same trap, but the expense of producing the pheromone could limit this approach.

E. conspersus pheromone is a smaller molecule relative to the *C. ligata* pheromone and therefore evaporates faster from a substrate or lure. Polyethylene lures of varying thickness provided a very good release profile (Fig. 5). The thinnest *E. conspersus* lure, 1.5 mil, released 95% of the total pheromone in 10 days while the thickest lure, 6.0 mil, released 42% and a commercial lure released 27% of the total pheromone.

In 2011 we evaluated *E. conspersus* polyethylene lures of different thickness along with a commercial lure from AlphaScents during the spring and summer. In the spring, more *E. conspersus* adults were captured in the thinnest lures, 1.5 and 4.0 mil (Fig. 6). Most of the *E. conspersus* adults captured were females, 70%. In the summer the commercial lure and the thickest polyethylene lure, 6.0 mil+foil, which captured the most *E. conspersus* adults (Fig. 6), but the female bias was diminished to only 55% during this period. It is unclear as to why there would be a difference in the capture of *E. conspersus* adults in traps baited with lures of different release rates in the spring versus summer. The most important time for monitoring *E. conspersus* adults is in the summer so lures releasing pheromone at rates similar to the thickest polyethylene lure with foil and the commercial lure would be the best choice.

We conducted some preliminary release rate studies with the *T. pallidovirens* pheromone using polyethylene lures. The release rate of this pheromone was even faster than that of *E. conspersus* with the thinnest lure releasing 81% of the pheromone in only five days in the laboratory. We monitored *T. pallidovirens* using its pheromone in pyramid traps in several southern WA sites in 2011 but very few bugs were captured.

Stink bug pheromones, at least for the species we have worked with, are highly specific, that is, they primarily attract members of their own species with very little cross over attraction.

Life history of *C. ligata* and *E. conspersus*. By combining data from all trapping studies in 2010 and 2011 we were able to characterize the seasonal life history of *E. conspersus* and *C. ligata*. Both species have only one generation per year, confirming what has been reported in the literature and earlier studies in WA. New *C. ligata* adults began appearing in early July in 2010 with captures peaking in late July and early August (Fig. 7). New *E. conspersus* adults did not appear until late July with a definite peak in mid-August. In 2011 the cool year delayed the appearance of new *C. ligata* adults and peak activity was in late August and early September. Similar delays in the summer appearance of new *E. conspersus* adults and peak activity were observed in 2011. (Fig. 7) These data could have been influenced by orchard border spraying, which began in mid- to late-July in most orchards.

Dual attraction of *C. ligata* and *E. conspersus*. There appeared to be no impact on placing lures of *C. ligata* and *E. conspersus* in the same trap (combo trap). Over a 21 day period in August-September the total number of *E. conspersus* captured in four combo traps was 96, while the number caught in a trap baited with the commercial lure was 88. The number of *C. ligata* captured during this period was low but slightly higher in the combo trap, 12, compared to the trap baited with the 1.5 mil polyethylene *C. ligata* lure, 6 bugs. Certainly there was no negative impact of putting the pheromones of both species in the same trap.

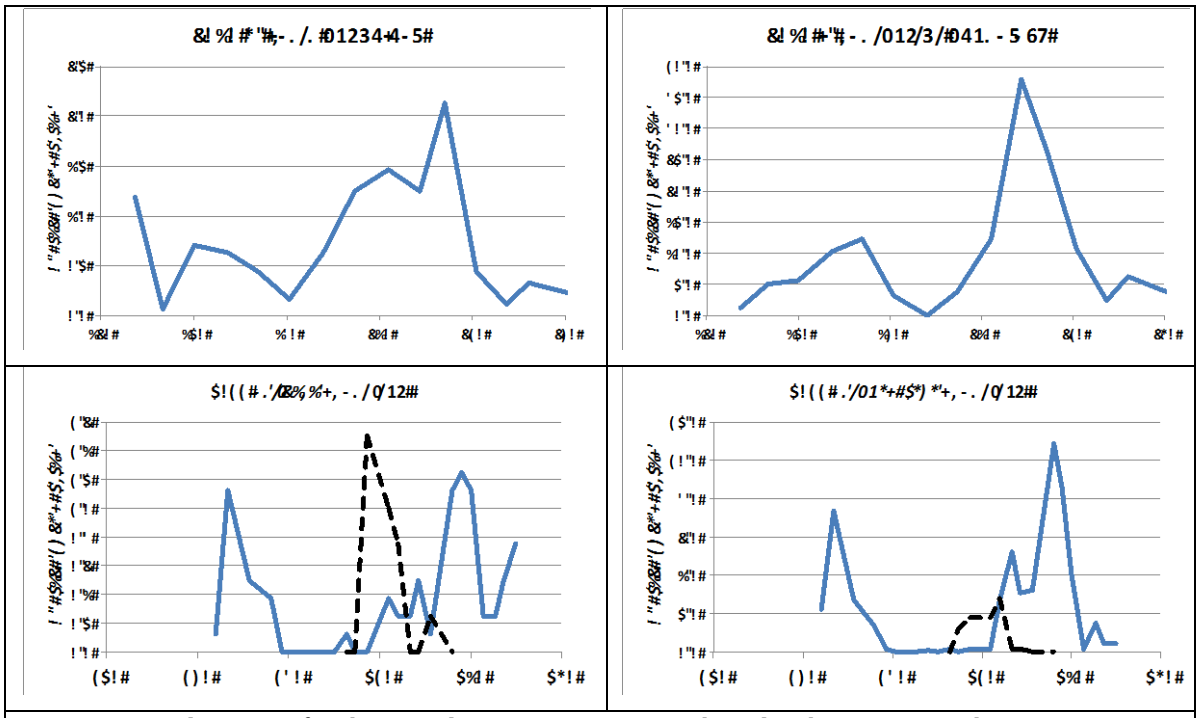

Fig. 7. Seasonal capture of *C. ligata* and *E. conspersus* in traps baited with aggregation pheromone. Dashed line data from Milton-Freewater, OR.

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

Fig. 8

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

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

We treated trap panels with insecticides, Thiodan, Warrior or a Thiodan-

Warrior mix, and put the traps in a collecting tray and released 15 *E. conspersus* adults on the tray (Fig. 8). The release was repeated 5 times for each treatment (75 total bugs). Twenty-four hours after release the location of bugs and mortality was recorded. Many bugs left the trays and were not found. In the control 41% were accounted for after 24h. Few bugs were found dead in the trap, indicating that they were not intoxicated while climbing panels on the way to the trap. This may in part be due to their behavior of climbing primarily on the edges of the panels when moving towards the trap. A few bugs were found dead in the trays suggesting that they became intoxicated when climbing on the panels.

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

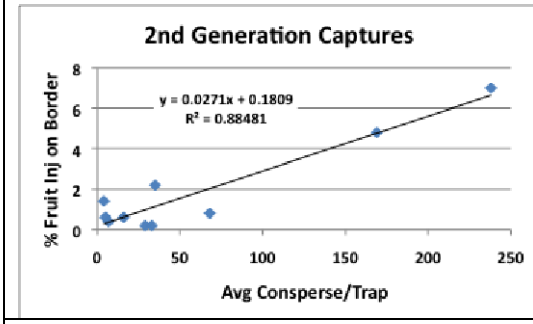

Fig. 9

We monitored stink bugs at ten locations in WA during 2010, five over the entire season and five only in the second generation. Since previous research had shown that the highest level of fruit injury from stink bug feeding occurs on orchard borders we sampled ten trees in on the border closest to where traps were placed. We then compared the total capture only of *E. conspersus* with the fruit injury. There was a good relationship between total captures for the entire season, and for capture only in the summer, and fruit injury (Fig. 9). While these data should be considered preliminary they show promise of using trap captures of *E. conspersus* as an indicator of the risk of an orchard for fruit injury.

Twenty small black pyramid traps (Fig. 2) were placed in an area of approximately 2 acres of native habitat near an apple orchard with a notorious problem with stink bug damage, Pittman orchard near Manson (Fig. 10 A, C). The 20 traps in the native area captured 527 *E. conspersus* adults, an average of 26.3 per trap. Traps associated with a lure test located at the southern end of the orchard captured another 410 *E. conspersus* adults. Monitoring traps placed near the orchard border captured an average of 33.0 *E. conspersus* adults per trap. These data suggest that the high density of traps had little impact on movement of *E. conspersus* adults into the orchard.

Just prior to harvest three of the eight rows were sampled, plus a partial row which was closest to the native area, for stink bug injury to fruit. The average percent fruit injury across the entire block was 4.4%. The average percent fruit injury on the northern border, edge and trees in 25 meters in from the edge, was 14.2% (Fig 10D). There was an area of relatively low fruit injury that was closest to the high density trapping area, 0.4%, which suggests an impact of removing a large number of *E. conspersus* adults. While it is unwise to place too much weight on results from a single location these results may point to a hope of using border trapping at high density as a way to mitigate the impact of *E. conspersus* adults moving into orchards in late summer.

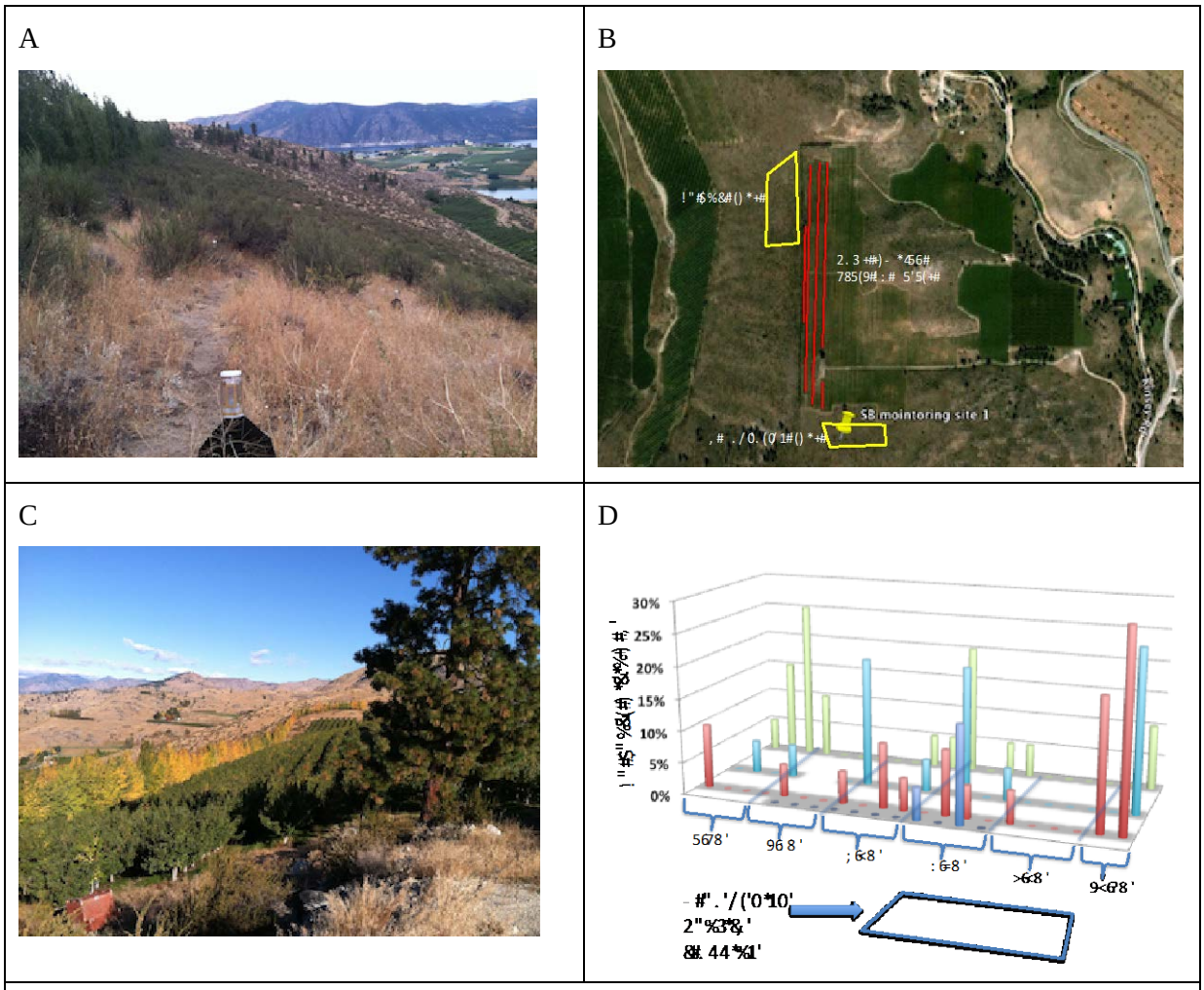

Fig. 10. A - Native area where 20 *E. conspersus* pheromone baited traps were placed (June - Sept.). B - Aerial view of the Pittman orchard site. Red lines show rows of apple sampled for fruit injury. C - View of the Pittman orchard looking southeast to northwest. The site is surrounded by native habitat and is long an narrow. D - Percent fruit injury in the Pittman orchard by tree and by section of orchard.

BMSB: Evaluate/develop monitoring tools and determine distribution of the brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stal). This is an exotic stink bug species first discovered in the US in the mid-1990s in Pennsylvania (Allentown). In 2004 it was reported from OR, and is now well established in the Portland, OR area. It has since spread north to Vancouver, south to Corvallis, east to Sandy, and has recently been found in Arlington, OR, just across the river from Roosevelt, WA. In 2010 the eastern US reported high levels of damage to many soft fruit and apple orchards and researchers in the mid-Atlantic region report high captures in traps.

In 2010, BMSB was confirmed in Vancouver WA. In 2011, Master Gardener volunteers were recruited to host the modified panel traps through the I-5 corridor in three counties (Clark, Cowlitz and Lewis) and into the Columbia River Gorge (Skamania County). Twenty-seven volunteers (13 in Clark, 6 in Cowlitz, 5 in Lewis, and 3 in Skamania) were recruited and given trap instructions and maintenance requirements. Since no pesticides were used in the survey, volunteers were instructed to check traps each morning. Identification factsheets were also provided to help volunteers distinguish

possible BMSB specimens from other true bugs. Along with maintaining the traps, volunteers were directed to make observations of susceptible hosts for the presence BMSB in the immediate area.

Trapping occurred from mid-September to late October. By November 10, 66% of the traps have been returned with potential specimens of BMSB and collections from the immediate area. No BMSB were caught in traps, however, BMSB adults were hand-collected at 4 trapping sites documenting its presence. At the low densities that BMSB is currently present in southwest WA, traps baited with the *Plautia stali* pheromone do not effectively attract adults. While densities of BMSB in WA are low, the volunteer survey confirmed an expanded distribution to northern Clark County (La Center WA) and as far east as Prindle WA in Skamania County.

No BMSB were collected in traps or from visual observations in eastern WA (Brunner and Walsh) or northeastern OR (Milton-Freewater). A single BMSB was detected in Hood River, OR and numerous BMSB were trapped and collected in the Portland, OR area and south in the Willamette Valley of OR.

Executive summary

This project met the objectives outlined in the original proposal. While we demonstrated that the aggregation for *Chlorochroa ligata* was attractive selective it was very difficult to synthesize which will likely limit its commercial availability due to high cost. The *C. ligata* pheromone is a large molecule and, therefore, has a slow release rate from different lures tested. Efforts to optimize capture of *C. ligata* by using lures with different pheromone release rates was not successful, as all lures captured roughly the same number of bugs. Using multiple lures in a trap did increase capture of *C. ligata* but the cost of using multiple lures seems impractical because of the expected cost of lures. We were able to show that the optimized pheromone release rate for *Euschistus conspersus* was different in the spring, overwintered adults, compared to the summer adults. The good news is that the best commercially available *E. conspersus* lure has an optimized attraction in late summer to detect adults moving into orchard from native habitats. We also showed that putting lures of two stink bug species, *E. conspersus* and *C. ligata*, in the same trap did not inhibit capture of either species. Monitoring *E. conspersus* adults in late summer and fall appeared to be a good indicator of risk of the orchard to fruit injury, that is the more *E. conspersus* adults captured the more injury was detected to fruit on the orchard border. Laboratory screening showed that the carbamates Lannate (methomyl) and Carzol (formetanate hydrochloride), the chlorinated hydrocarbon, Thiodan (endosulfan) and the synthetic pyrethroids Danitol (fenpropathrin) and Warrior (lambda-cyhalothrin) caused highest mortality of *E. conspersus* adults. Treating the panels of pyramid traps with toxicants did not prove to be a good method for attract and kill as bugs tended to crawl up the edges of the panels and were exposed to very little toxicant. Bugs entering traps were able to escape, but putting a Vaportape II kill strip in the trap or coating the inside of the trap with a toxicant reduced or eliminated bugs escaping traps. A mass trapping study provided some encouragement that this approach could provide some protection from orchards at high risk from immigrating stink bug adults in late summer and fall.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: CP-10-104A

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

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Cooperators: Zirkle Fruit Company, O'Brien Orchards,

Budget: **Year 1**: $79,117 **Year 2**: $79,866 **Year 3**: $**78,764**

TOTAL PROJECT REQUEST $237,316

Other funding sources
Agency Name: WTFRC - Technology Subcommittee
Amt. requested: $19,000 + $15,000 (2012 + 2013)
Notes: A proposal to use foam application technology for spraying lacewing eggs on foliage

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

Item	2010	2011	**2012**
Salaries[1]	$29,230	$29,230	$29,230
Benefits[1]	$ 8,770	$ 8,770	$ 8,770
Wages			
Benefits			
Equipment			
Supplies[2]	$ 4,000	$ 4,000	$ 3,750
Travel			
Plot Fees			
Miscellaneous			
Total	$42,000	$42,000	**$41,750**

Footnotes: [1]Partial support for GS-6 technicians (benefits at 30%)
[2]Purchase of insectary-reared green lacewings, wetting agents.

Budget 2
Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker
Telephone: 509-335-7667 **Email address:** mdesros@wsu.edu

Item	2010	2011	**2012**
Salaries[1]	$22,014	$22,895	$23,811
Benefits	$ 1,851	$ 1,925	$ 2,002
Wages[2]	$ 8,580	$ 8,923	$ 9,280
Benefits	$ 172	$ 178	$ 185
Equipment			
Supplies	$ 1,500	$ 1,500	
Travel[3]	$ 3,000	$ 2,000	$ 1,500
Plot Fees			
Miscellaneous			
Total	$37,117	$37,649	**$37,014**

Footnotes: [1]Project Assistant (Graduate student, academic year);
[2]Summer wages (graduate student); [3]Travel to field sites

OBJECTIVES

1. Interview organic orchardists and managers to discover problems associated with releases and supply, and revise research details accordingly.

   This objective was largely completed in 2010-2011 but we will conduct additional interviews with orchardists through year 3 of project. In 2012 we will seek hard data on the timing used for predator releases by growers and other approaches to supplement lacewing or mite releases such as manual removal of aphid colonies and sucker removal.

2. Develop our capacity to differentiate between insectary-reared and naturally occurring predators using morphological traits.

   This objective is important for better understanding of our predatory mite complex. We will continue the survey of mites species on central Washington apples began in 2011; this will be a major thrust in 2012 together with additional predator mite release experiments.

3. Make releases of lacewings and lady beetles, or predatory mites and monitor populations of both pests and predators at release sites and non-release sites.

   Lacewing and mite release were conducted in grower orchards in 2011 and will be expanded in 2012. Lacewing egg releases will employ improved egg application technology developed in 2011. Predator mite releases will be reduced in scale and will only be conducted in orchards that show very low predator mite densities with high pest mite populations. Immunomarking of released predator mites will be used to better estimate success of mite releases.

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

   Because we have stopped ladybeetle studies we will not test attractants. Lacewing egg releases will be made in early spring and mid spring. These timings approximately correspond to earliest curled leaves, 2 to 4 weeks later depending on weather. The earliest releases will be approximately 1 month earlier than those made in 2011. In 2012, we will also emphasize the comparison of liquid carriers for eggs to enhance retention on trees.

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

   Comparison of feeding capacities of Chrysoperla rufilabris (insectary) versus Chrysopa nigricornis (endemic) species on rosy apple aphid and pear psylla are largely completed. Comparative feeding studies at low temperatures will be finished in 2012. The feeding and reproductive capacity of endemic Amblydromella caudiglans and Galendromus occidentalis will be compared to insectary produced Galendromus occidentalis.

SIGNIFICANT FINDINGS

- ✓ Insectary produced *C. rufilabris* eggs will hatch at temperatures corresponding to late March (2010)
- ✓ ***C. rufilabris* neonates get trapped in woolly apple aphid honeydew (2010)**
- ✓ Hibernating ladybeetle vary greatly in quality and availability (2010)
- ✓ ***C. rufilabris* show similar feeding rates with native *C. nigricornis* (2010)**
- ✓ **Pesticides interfered with predatory mite reproduction (2010)**
- ✓ ***C. rufilabris* readily feeds on Rosy apple aphid even at late March temperatures**

- ✓ ***C. rufilabris*** **feeds readily on pear psylla**
- ✓ ***C. rufilabris*** **eggs survive pump pressures up to 25 psi**
- ✓ **50-80% of hand-sprayed *C. rufilabris* eggs are lost at contact with leaves**
- ✓ **Gum spray solutions can be used as suspensions for *C. rufilabris* eggs leading to a doubling in adhesion to tree surface after contact and initial dry down**
- ✓ **No *C. rufilabris* larvae were found following large releases of eggs in apple and cherry orchards**
- ✓ **No evidence of establishment of *G. occidentalis* was seen in a release study in orchard**
- ✓ **The dominant predator mite found in 5 orchards was** *Amblydromella caudiglans* – **a big surprise**

METHODS

Objective 2 -Differentiating species.

Comparison of feeding capacities of *Chrysoperla rufilabris* provided by Beneficial Insectaries were examined at 8 and 15**°C** . Individual neonate larvae were confined with known number of third instar rosy apple aphid on an aphid leaf for 24 h; the number consumed was determined at the end of the exposure (10 replicate predators per temperature). Similarly, *C. rufilaris* was confined with eggs and neonate pear psylla on leaves for 24 h at 21**°C** and numbers of psylla consumed enumerated.

100-leaf samples were collected from 27 locations throughout eastern Washington, including apple, cherry, and wild blackberry leaves. All phytoseiid predatory mites (and prey species when present) were transferred to 70% ethanol. Date collected, location with GPS coordinates, prey species available, plant species, orchard name, block number, crop cultivar, and orchard type (conventional or organic) was recorded . These samples are currently being slide mounted and identified. Each phytoseiid mite will be mounted on a slide in modified Berlese's solution and identified to species. Additional samples include cardboard bands placed in orchard trees in late summer at three orchards. These provide overwintering sites for predator mites and often have many eggs of European red mite. Additional sampling in 2012 will use the same methods.

Objective 3- Releases in grower orchards

Mites were released in a mature block of 'Golden Delicious' apples near Pasco, WA. Trees were planted at 7 x 15 ft row spacing. Replicates consisted of 100 trees in 5 row x 20 tree sections, with treatments randomly assigned within each of the six replicates. Western predatory mites (*Galendromus occidentalis*) from the BioBest were deployed onto the release trees on 8 July. The treatments consisted of predatory mite levels corresponding to 0, 15000, and 50000 mites per acre. Mites were released by placing bean leaves containing predatory mites, with predator numbers estimated by subsampling. Predator and prey mite populations were assessed from mite brushing of 100 leaves picked from a 3-row by 13-tree area in the center of each replicate plot by removing 2 or 3 leaves per tree. All stages of *Tetranychus urticae*, *Panonychus ulmi, G. occidentalis, Aculus schlechtendali*, and *Zetzellia mali* were counted. Leaf samples were taken and counted once weekly for six weeks. The first sample was a pre-release sample to establish native predatory mite numbers and to determine a baseline population count of herbivorous mites.

Lacewing eggs were released in an organic cherry and apple orchards. With large cherry trees, plots consisted of 7 adjacent trees in a row and 3 plots were created in each row. Treatment levels were 0,

7000, and 14000 *C. rufilabris* eggs and the three treatments were replicated in 6 rows. A similar design was used in small trellised apples but plots consisted of 29 trees. Again, the 3 treatment plots were placed in a single row with random assignment of 0, 7000, 14000 eggs/plot and 6 replicates. Pre-samples were 1) visual counts of rolled leaf colonies on each tree in the cherries and over whole plots in the apples, and 2) in cherries, 35 aphid colonies were removed from each plot, typically at 5 colonies per tree. In apple plots, 54 colonies were removed per plot. Colonies were placed in Berlese funnels to drive aphids and predators into a salt/soap bath to facilitate counts of predators. The same procedure to detect released predators was used 8 and 10 days after releases in cherries and apples.

A bioassay to test the survival, fecundity and prey consumption of the commercially-reared *G. occidentalis* was conducted using residual pesticides on orchard leaves from a commercial orchard where the predators were released and an untreated research orchard at WSU-TFREC. Non test-arthropods were removed from leaves with a paint brush. Leaf disks 2 cm in diameter were cut from the leaves and placed on water-saturated cotton arenas with the lower surface facing up. *T. urticae* on bean leaves from BioBest Insectary were brushed onto a glass plate. Some of the contents of the glass plate (all stages, mainly eggs and larvae) were smeared onto each leaf disk to provide sufficient food for the predatory mites. This transfer technique provided pesticide-free food for the test predators. One female *G. occidentalis* (either from BioBest or from the WSU-TFREC laboratory colony) was placed on each leaf disk. A treatment consisted of all possible combinations of leaf source and mite source, creating a total of four treatments. Each treatment was replicated six times with five subjects per replicate. The bioassay was evaluated non-destructively at 48 h. *G. occidentalis* females were recorded as live, dead or runoff and the number of *G. occidentalis* eggs was counted. *G. occidentalis* egg positions were marked with a felt-tip pen. After the 48 h evaluation, *G. occidentalis* females were removed from the disks. On the fifth day after the 48 h evaluation, the number and status of *G. occidentalis* life stages were counted (hatched and unhatched eggs, live and dead larvae).

Objective 4- Conduct field experiments

A large series of studies were conducted to determine the value of various solutions to enhance lacewing adherence to apple leaves and to aid in suspension of lacewing eggs in solution to support even dispersal of eggs in sprays. Assays consisted of spraying various test solutions onto seedling apples or apple trees in the field using a hand-held 2 liter pump sprayer which was pressurized manually. Solutions of various gums, sugars, and starches were first screened in the laboratory without spraying. In this case eggs were placed in solutions for 30 minutes and moved to filter paper and dried. Subsequent egg hatch was measured by moving eggs onto a sticky label which allowed hatch but preventing neonate larvae from eating one another. These tests eliminated toxic adjuvants. Subsequently, test solutions were sprayed on to apple plants and either leaves were removed to petri dishes to estimate hatch rates or marks were placed next to eggs with white typewriter correction fluid and egg retention and hatch were estimated by repeated visits to test plants.

RESULTS & DISCUSSION

Objective 2 -Differentiating species

Chrysopa rufilabris fed readily on Rosy apple aphid in reduced temperatures corresponding to conditions which would be experienced under typical field releases (Figure 1). Neonates readily consumed pear psylla, of

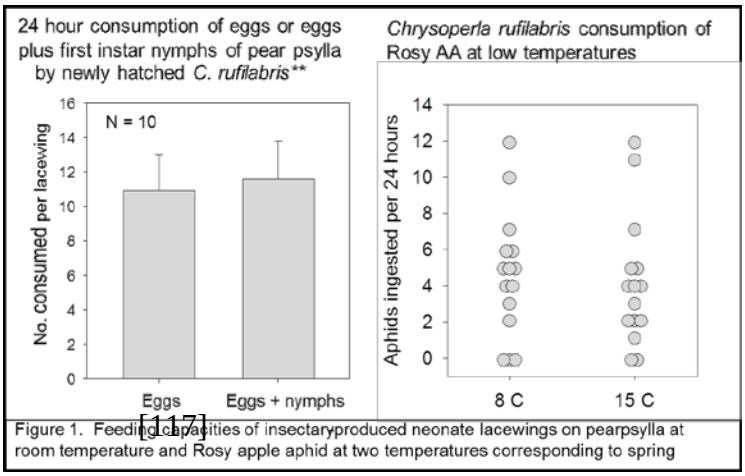

Figure 1. Feeding capacities of insectaryproduced neonate lacewings on pearpsylla at room temperature and Rosy apple aphid at two temperatures corresponding to spring

interest to Beneficial Insectary as a potential expansion of the market (Figure 1). Comparative studies using endemic *Chrysopa nigricornis* and *Chrysoperla plorabunda* are planned for 2012 once we obtain field collected material in spring.

Five phytoseiid predator mite species were found in apple orchards: *Amblydromella caudiglans, Galendromus occidentalis, Galendromus flumenis, Typhlodromus citri* and *Typhlodromus pyri.* Surprisingly, the most broadly distributed and most abundant was *Amblydromella caudiglans* a more generalist predator that *G. occidentalis* and known to have a relatively lower reproductive rate than *G. occidentalis.* Because all of these species look very similar and usually require slide mounting for authoritative identification, the evaluation of releases of *G. occidentalis* takes another step up in difficulty. This represents a potent argument for using external dusting and possibly internal marking (pollen food laced with protein marker) of predators prior to release studies. More importantly are plans to compare the capacities of *A. caudiglans* versus *G. occidentalis* to suppress spider mite populations.

Objective 3- Releases in grower orchards.

Results: Counts of spider and rust mite were low at the time of predator release in early July. *P. ulmi* was the dominant phytophagous mite species and it increased during the test (Fig. 2). There were no statistical differences between treatment means for any mite species or group on any date. These findings provide no evidence that the released predators became established or had any effect on pest mites.

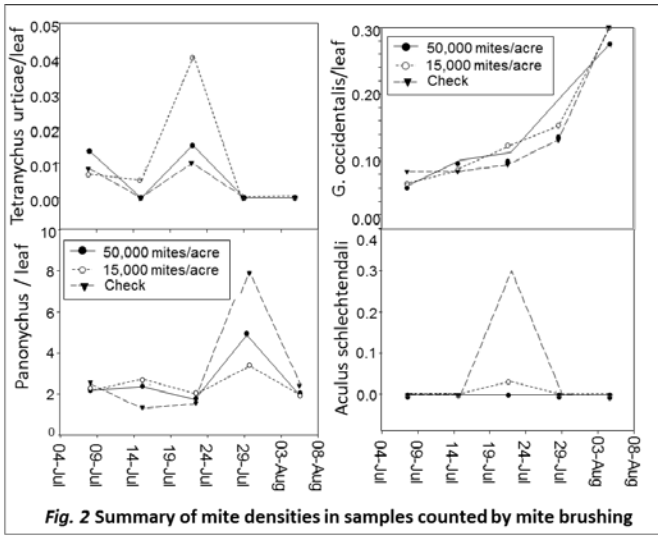

Fig. 2 **Summary of mite densities in samples counted by mite brushing**

In a 2010 release, residual pesticides were suspected of having sublethal toxic effects on the predatory mites and leaves from a sprayed orchard appeared toxic. In contrast, our 2011 bioassay does not show evidence of any form of sublethal toxicity from commercial orchard leaves on the predatory mites from either Sterling Insectary or the laboratory colony (data not shown). Unfortunately, results from the 2011 bioassay suffered from problems associated with the feeding method used causing low oviposition and high mortality of controls, possibly due to a fungus that developed a few days after application. These problems will be corrected in future assays. No significant treatment effects were observed (data not shown).

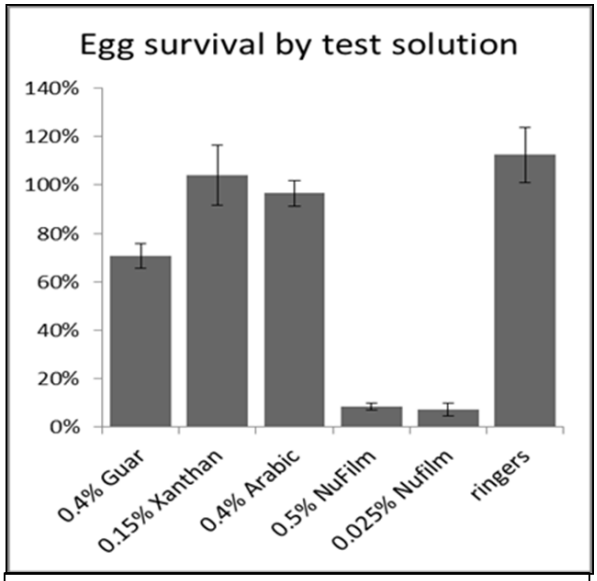

Figure 3 C. rufilabris egg survival in 6 solutions as a percent survival in water controls

Releases of lacewing eggs in apple(June 9) and cherry (June 6) orchards resulted in no recoveries of *Chrysoperla rufilabris* larvae, pupae or eggs at 8 or 10 day post release follow up samples. Several endemic predator

species, *Chrysoperla plorabundus* larvae, unidentified syrphid and ladybeetle larvae were all found in low numbers.

Objective 4- Conduct field experiments Of several test solutions for stickers only three gums allowed for high egg survival that was comparable to water. Starches and sugar solutions reduced survival and were dropped early in the studies. Figure 3 shows a comparison of gum solutions with the organic sticker NuFilm and is represented as percent of survival in water controls (typically water controls vary between 80 to 95% survival depending on egg age; eggs ready to hatch perform better). The results show that xanthan and gum Arabic provide good survival while NuFilm is quite toxic. In release studies conducted in 2011 Guar gum was used because it is organically approved for application in the field.

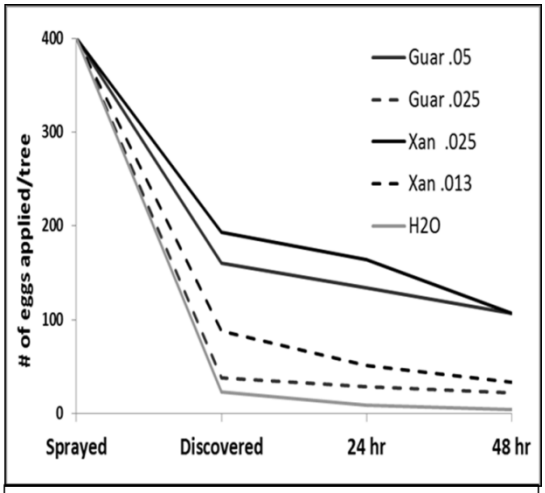

Figure 4. Number of *C. rufilabris* eggs retained 1 hr (discovered) and 24 and 48 post-spray

The value of these gums in enhancing adherence of lacewing eggs was demonstrated in several field studies. Figure 4 shows the results of an experiment where eggs were located visually on apple trees one hour following sprays of 400 eggs/tree (4 trees/treatment). Leaves containing eggs were marked close to the egg. Two people repeatedly searched each tree to maximize discovery of eggs. The results show the significant loss of eggs that occur at spray contact with the tree. Lacewing eggs in water and low concentrations of the gums are discovered in much lower numbers suggesting the critical retention period is at spray and shortly thereafter. Not shown, but of importance, is the hatch rate in the two concentrated gum treatments, which both exceeded 85%.

Plans for 2012 are reviewed under the recap of our objectives above. Release studies of mites will be conducted in smaller plots with an emphasis on clear delineation of the species present. Given the dominance of *Amblydromella caudiglans* at some sites and the virtual absence of *Galendromus occidentalis* at several sites, it may be feasible to assess establishment of released mites based on morphology. The immunomarking marking tests begun in 2011 will be continued in 2012 to adapt and refine the techniques for these small arthropods, often less than 1 mm in length. We will expand the marking approach to test internal marking produced by letting the predators feed on pollen mixed with the marker protein such as soy flour. The marking technique will be used to differentiate released mites from native mites in release studies.

Comparative feeding capacities of the two endemic lacewings will be compared to *C. rufilabris* in simple assays as presented above. *Chrysopa nigricornis* should be available from bands we have collected from orchards and a colleague (Peter Shearer currently has a colony *C. plorabunda* allowing us to finish this effort).

New media for releases of lacewing eggs will be a major focus of the lacewing work. Preliminary studies testing some foaming agents have begun (and may be supported by separate funding). Additional testing of applications using an ATV-mounted handgun using gums combined with foam-inducing air induction nozzles will continue through the winter and spring in the greenhouse to prepare for field trials in 2012.

CONTINUING PROJECT REPORT **YEAR**: Year 3 (no-cost extension was granted)
WTFRC Project Number: CP-08-806

Project Title: Honey Bee Colony Health

PI:	Walter S. Sheppard	**Co-PI(2):**	Richard S. Zack
Organization:	Wash. St. University	**Organization:**	Wash. St. University
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Address 2:	PO Box 646382	**Address 2:**	PO Box 646382
City:	Pullman	**City:**	Pullman
State/Province/Zip	WA 99164-6382	**State/Province/Zip:**	WA 99164-6382

Cooperators: Eric Olson, WA Commercial Beekeeper

Total Project Request: Year 1: $10,000

Other funding sources
Agency Name: Personal Donations from Beekeepers including Eric Olson (WA) and Tom Hamilton (ID)
Amount awarded: Each beekeeper donated $10,000 ($20,000 total)
Notes: additional funding from these sources has totaled over $30,000 since 2009

Agency Name: WSDA, Washington State Bee Registration Program
Amount awarded: $20,000
Notes: Funding was recommended at an emergency meeting of the Apiary Advisory Board on 3 April 2008. Funding was received

Agency Name: WSU, Agricultural Research Center (ARC)
Amount awarded: $55,000 per annum for two years
Notes: Funding that has allowed the Apiculture Program to hire a technical assistant for a period of two years to assist Dr. Sheppard in coordinating the Colony Health Program

Budget 1:

Organization: WSU	**Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564	**Email:** carriej@wsu.edu;

Item	**2008**		
Salaries			
Benefits			
Wages	$9,124		
Benefits	$876		
Total	**$10,000**		

Funds obtained from the WTFRC were used to supplement the salaries of individuals hired to staff a Colony Health Lab that examines parasitic mites and diseases of honey bees in an attempt to understand bee kills in Washington and the Pacific Northwest.

Note: Through an oversight of the Principal Investigator (WSS) the original funds from the WTFRC were not identified in accounting documents and remained unspent until 2011. Funds were expended in 2011 and there remains $3635.75 to be expended in 2012.

Recap Objectives

The Apiculture program requested funds from the WSTFRC to assist in setting up a diagnostic laboratory to perform examinations of mite and pathogen loads in Washington State beekeeping operations. This laboratory was set up using a compilation of funds from numerous sources and has continued to function to serve Washington State Beekeepers in this role since 2008. Numerous beekeeping organizations and individual beekeepers have donated money to keep the laboratory functional and we also received some Honey Bee USDA-CAP funding in 2010 and 2011.

We conducted a targeted survey in 2008 to:

1. Determine seasonal numbers of tracheal and Varroa mites
2. Determine the presence of *Nosema ceranae* in Washington bee colonies
3. Determine spore counts of *Nosema* in relation to seasonal changes

Significant Findings

1. *Nosema cerana* was found to be omnipresent in the Pacific Northwest. With the exception of 2 samples out of several thousand evaluated, only *Nosema ceranae* was detected. The previous *Nosema* disease causing organism well-known to beekeepers (*Nosema apis*) was largely absent.
2. The seasonality of *Nosema ceranae* in the Pacific Northwest was verified and published. Beekeepers could adjust treatment regimens to account for natural seasonal variation.
3. Additional research derived from the diagnostic laboratory indicated that sub-lethal pesticide exposure had pronounced effect son the susceptibility of individual honey bees to *Nosema*.

Methods

Beekeepers in Washington State can now submit their colony samples to the Honey Bee Diagnostic Laboratory for examination. Collection methods and details of shipment are available on the WSU Entomology Website.

We have increased the productivity of the laboratory and in almost all cases, diagnostic results are available to the beekeeper within 2 weeks. One trained person can analyze about 10-12 colony samples for tracheal mite infestation rates /day.

The determination of *Nosema ceranae* species identity is accomplished through a PCR-based molecular protocol that examines genetic variation in small subunit ribosomal DNA.

Results and Discussion

This project resulted in a clear understanding of the incidence and distribution of the major honey bee pests within honey bee populations in WA. *Nosema ceranae* was found to be widespread and omnipresent in PNW honey bees. Additional research in our laboratory also identified sub-lethal pesticide exposure and *Nosema* disease interactions. As a result we were able to inform beekeepers

that regular replacement of wax brood comb could substantially assist in removing a major source of internal hive contamination.

Overall, *Nosema ceranae* is a pathogen that beekeepers now have to live with in their operations in Washington State. The diagnostic laboratory is a valuable tool for management decision-making and this laboratory receives over a thousand samples a year from Washington State beekeepers, both commercial and smaller operators. The information returned to the beekeepers allows them to make management decisions based on actual infestation or prevalence data, rather than using a scheduled treatment system. Current annual winter losses of honey bees in the PNW are around 30% per year.

Reliable treatment and control for *N. ceranae* remains elusive and one of the most promising approach is our WSU breeding effort to develop honey bees that are more tolerant/resistant to *Nosema* infection in the PNW. Ongoing WSU research on honey bee breeding and genetic measures to deal with pathogens have led to importation of honey bee semen from Old World sources to increase genetic diversity for breeding. Since 2008, we have been able to import honey bee semen directly for breeding and have released genetic material to the western US queen production industry.

Overall, the diagnostic laboratory has significantly assisted the beekeeping industry as measured by its use and continued support form beekeepers themselves. The funds provided by the WSTFRC in support of the set up of this laboratory helped ensure that commercially available colonies of bees were available to meet the pollination needs of the Washington agricultural community.

CONTINUING PROJECT REPORT **YEAR**: Year 1 of 3
WTFRC Project Number: CP-11-103

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

PI:	Jay F. Brunner	**Co-PI**:	Cameron Peace
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Co-PI: Kate Evans
Organization: WSU Tree Fruit Research and Extension Center
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Address: 1100 N. Western Ave.
City/State/Zip: Wenatchee/WA/98801

Cooperators: Mike Doerr, WSU TFREC, WA.

Total Project Request: **Year 1:** $37,904 **Year 2: $53,399** **Year 3:** $42,648

Other funding sources: NONE

WTFRC Collaborative expenses: NONE

Budget 1
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston; Kevin Larson
Telephone: 509-335-4564; 663-8181 X221 **Email:** carriej@wsu.edu; kevin_larson@wsu.edu

Item	2011	2012	2013
Salaries (grad student) [1]	22,901	23,817	24,770
Benefits [1]	1,895	1,970	2,050
Wages [2]	10,094	15,840	10,918
Benefits [3]	514	2,772	1,910
Equipment	0	0	0
Supplies [4]	1,500	6,500	500
Plot fees [5]	0	2,000	2,000
Travel [6]	1,000	500	500
Miscellaneous	0	0	0
Total	37,904	**53,399**	42,648

Footnotes:
[1] Joseph Schwarz, PhD student.
[2] Temporary summer labor – three people at $11/h @ 40h/wk for 12 weeks.
[3] 17.3%.
[4] Rearing supplies, leafroller colony rearing supplies, liners budding costs.
[5] Maintenance fees for 2 acres of orchard at TFREC.
[6] Within State Travel.

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.

Methods:

***Identify and characterize resistance*.** The bioassay method used for years to evaluate the impact of pesticides against the obliquebanded leafroller (OBLR), the leaf-disk method, did not prove robust enough to characterize mortality, development, and reproductive parameters when comparing different apple cultivars. The primary issue was deterioration of leaf tissues that impacted OBLR larval survival. Therefore, a significant amount of effort went into developing a bioassay method for leafrollers. The bioassay method that provided leaf quality over time involved using a whole leaf placed in a large petri dish 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 – See Fig. 1. Newly hatched OBLR larvae were placed into the petri dish arena and development was checked every 7 days. At each evaluation the larval stage (instar) was recorded along with mortality. After pupation occurred the pupal weight was recorded and development was followed daily until adult emergence. Adult weight was recorded and

Figure 1. Whole leaf bioassay method developed for OBLR.

adults were placed inside an oviposition cage and the number of egg masses deposited was recorded. Egg masses were placed individually in a small plastic petri dish until hatch and the number of larvae per egg mass was recorded.

Bioassays were also conducted for CM by exposing fruit available on the varieties below to newly hatched larvae in plastic containers. Because the development of CM larvae could not be observed the number of mature larva that emerged from the apple and time to emergence was recorded, as was the subsequent time to adult emergence. Adult CM were placed into an oviposition chamber and the number of eggs laid was recorded along with the number of eggs that hatched.

The apple varieties (accession number) evaluated in 2011 for possible OBLR and CM resistance were: Antonovka 1.5 (107196), PRI 1346-2 (589785), Redfree (594111), Florina (588747), Cox's Orange Pippin (5888853), Northern Spy (588872), Liberty (588943), Russian Seedling (589312), Jonafree (589962), Cortland (588848), Yellow Transparent (588859), Viking (589434), Lady (589053), ***Jonathan*** (890185), Virginiagold (588778), Trent (589490), ***Delicious*** (589841), Poeltsamaa Winter Apple (383515), Haralson (589469) and ***Granny Smith*** (588880).

Localize genes for resistance - see original proposal for methods.

Develop predictive markers for resistance - see original proposal for methods.

Results and Discussion:

Identify and characterize resistance. Data on the development time and mortality of OBLR larvae reared on an artificial (pinto bean) diet was collected in order to have an independent standard to compare with the development of OBLR on an apple leaf. Under controlled temperature conditions, OBLR larvae were primarily in the 2nd instar after seven days, in the 4th instar after 14 days, in the 5th and 6th instars after 21 days and in the pupal stage after 28 days (Fig. 2).

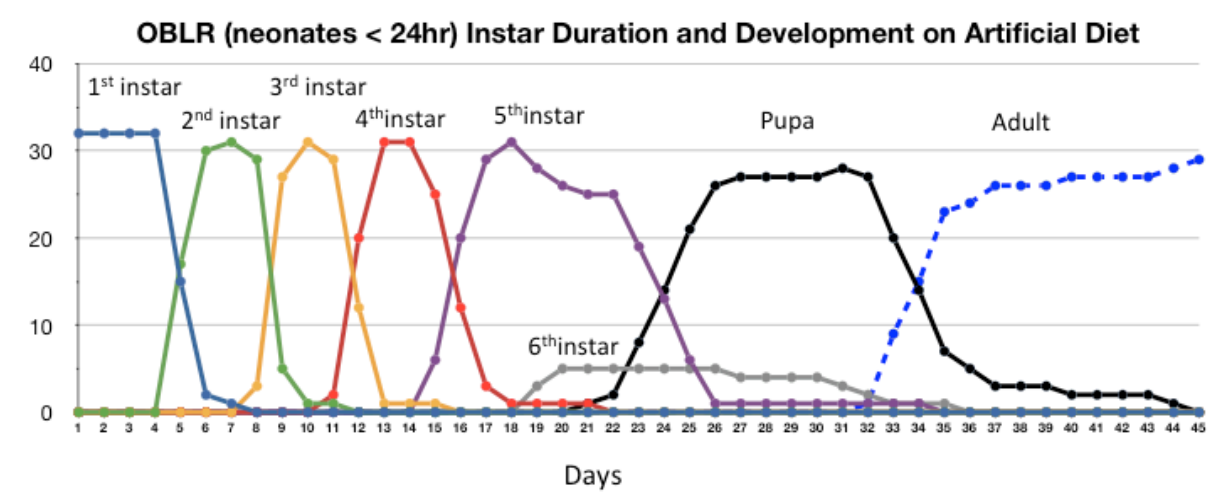

Figure 2. Number of OBLR larvae in a life stage when reared on artificial diet.

These development data provided a time line on which to evaluate OBLR development on leaves. When leaves were checked every seven days most larvae were in a specific instar and not in the process of molting. Transferring an insect larva during a molt can increase mortality.

For each apple variety 30 to 32 newly hatched OBLR larvae were exposed to leaves using the bioassay method described above. One measure of larval development is the average days required to complete development, that is, to reach the pupal stage. Fig. 3 shows the average days to pupation for male and female OBLR sorted from longest duration to shortest. There are no data for Northern Spy or Lady for females because no larvae survived to the pupal stage. Larvae development on certain varieties took considerably longer than on others. The pinto bean diet treatment is included for

reference. Standard apple varieties Delicious and Granny Smith are also highlighted for reference. Larvae feeding on seven varieties, Viking, Yellow Transparent, Liberty, Virginiagold, Antonovka and Poeltsamaa, had the longest development time suggesting some inhibition of normal development. Larval development time on Granny Smith and Delicious was similar to the pinto bean diet and several other varieties (Fig. 3).

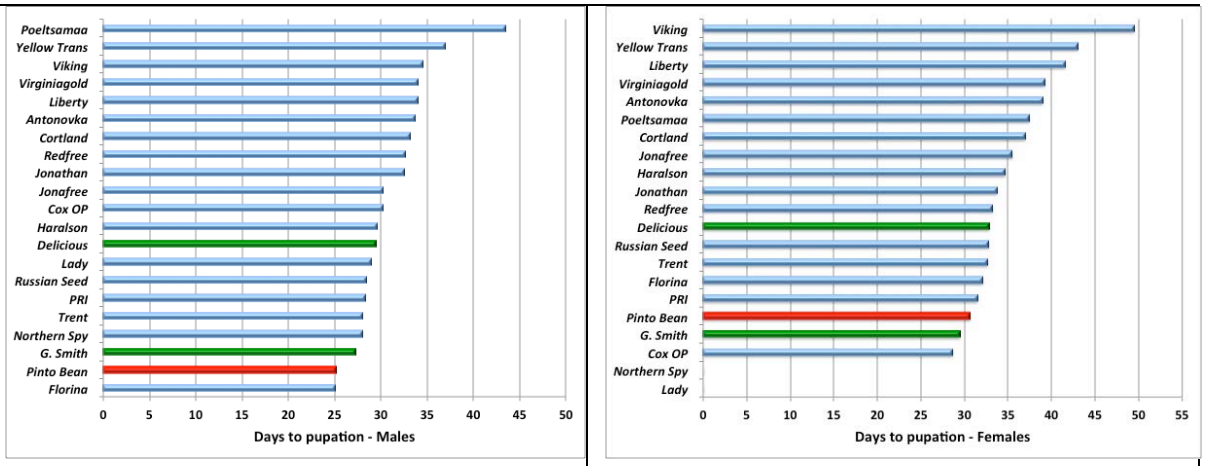

Figure 3. Average days to pupation for male and female OBLR reared on different apple varieties.

Another measure of the impact of different apple varieties on OBLR larvae is the size, or weight, of pupae. Male OBLR pupae are smaller and develop faster (Fig. 3) than female OBLR (Fig. 4). Larvae reared on the pinto bean diet weighed more than larvae reared on any of the apple varieties. No data are shown for females of Northern Spy or Lady as none survived to the adult stage. There was less variation in the weight of male OBLR pupae reared on different apple varieties than for female pupae. Some pupae reared on apple varieties were small and some of these, Antonovka, Liberty and Viking, were the same apple varieties where OBLR larvae took longest to develop to the pupal stage, Viking, Yellow Transparent, Liberty, Virginiagold, Antonovka and Poeltsamaa (Fig. 3).

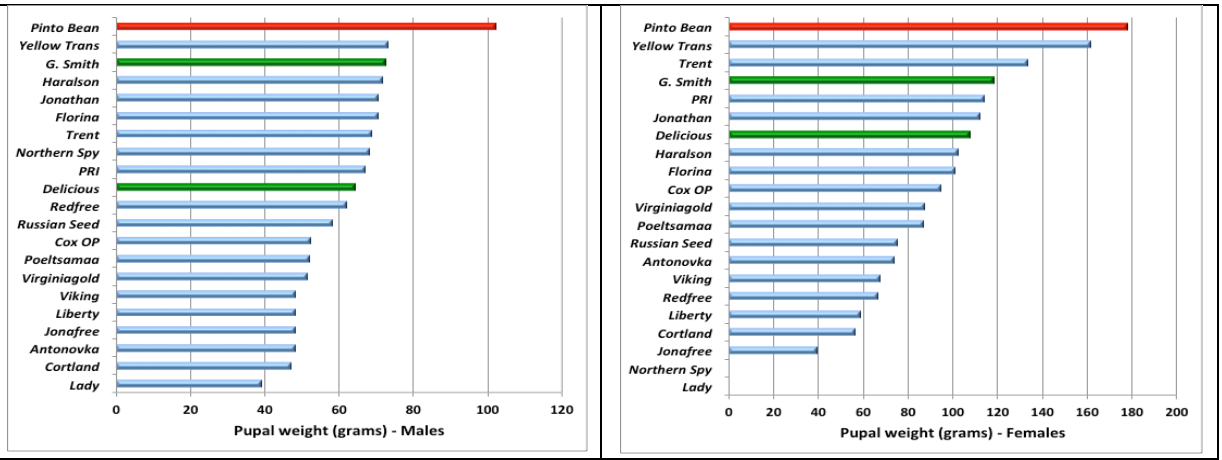

Figure 4. Average pupal weight for male and female OBLR reared on different apple varieties.

Survival on different apple varieties is one of the most important parameters to determine resistance of an apple variety to OBLR. Figure 5 shows the average percent survival of larvae feeding on different apple varieties and the pinto bean diet. There was wide range of survival from

none for OBLR larvae (females) feeding on Lady and Northern Spy to very high percent survival for larvae feeding on Cox Orange Pippen (males but not females), Florina, Haralson, Granny Smith, and Delicious with some differences between male and female larvae (Fig. 5). There was very low survival of OBLR larvae feeding on Lady, Northern Spy, Viking, Yellow Transparent, and Antonovka, some of the same apple varieties, which showed longer development times and smaller pupal weights (Figs 3 and 4).

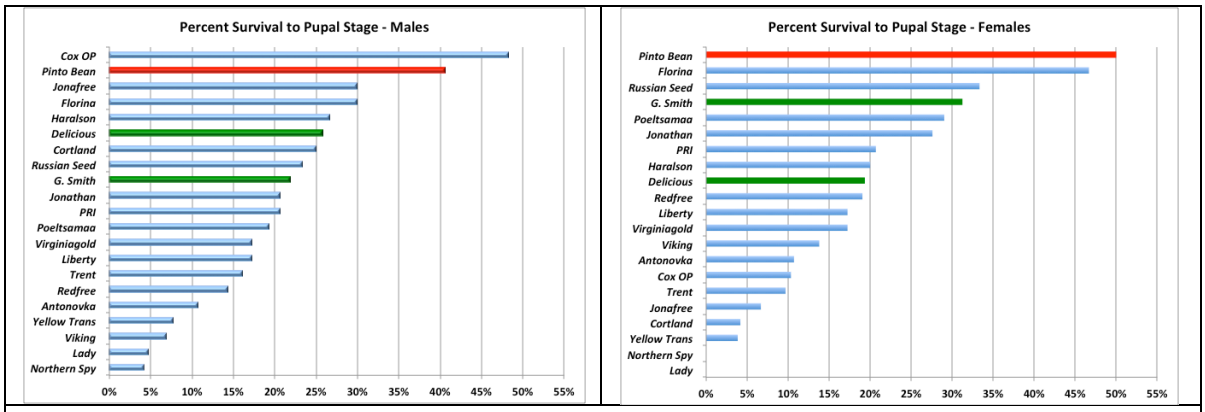

Figure 5. Average percent survival for male and female OBLR reared on different apple varieties.

We can gain additional information on when larval mortality occurs by examining survivorship curves. Figure 6 shows three types of survivorship curves for a select group of apple varieties, which represent patterns of survivorship curves for other apple varieties. 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. 6). 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.

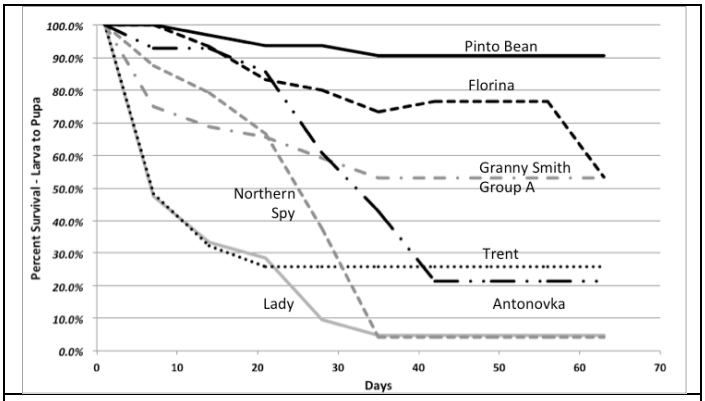

Figure 6. Average percent survival of OBLR larvae reared on different apple varieties. Group A Varieties – Delicious, Haralson, Poeltsamaa WA, PRI 1346-2, Russian Seedling, Jonathan.

The reproductive assessment of OBLR adults showed that when reared on some varieties fecundity was significantly reduced. Some of the apple varieties that negatively impacted OBLR larval survival and development time (Figs. 3 and 5) showed negative impacts on fecundity. Data on reproductive effects while interesting are difficult to obtain and limited by the need to obtain larger numbers of adults in order to have more confidence in the data, which in turn requires starting with many more OBLR larvae in the leaf bioassays. We may select some apple varieties to assess reproductive effects but will focus on larval bioassays in order to screen larger numbers of apple varieties in 2012.

At the time of this report data on CM bioassays were still being collected and results will be reported on at the research review.

Next Steps – Plans for 2012. The leafroller bioassay developed in 2011 provided a good screening method for examining different expressions of resistance in apple varieties. This method is necessary to screen apple varieties but is highly labor intensive and we are therefore requesting additional funding for labor in year two. The bioassay being used for CM appears to provide the kinds of data required to evaluate resistance in different apple varieties, though we may modify this bioassay after we have all the data in from 2011.

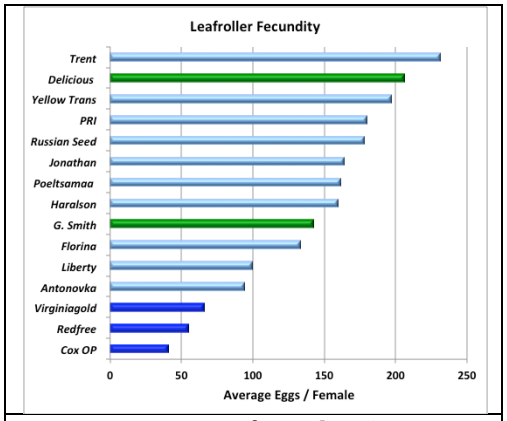

Figure 7. Average fecundity (eggs per female) of OBLR larvae reared on different apple varieties.

We will be evaluating three to four selections in the WSU apple breeding program along with Gala and Fuji for resistance to leafrollers and CM. In addition, we will evaluate a large number, 80-100, of other apple varieties based on input from the apple breeding team so we will be better able to evaluate genetic linkages informed by information generated through the RosBREED project. In order to screen this many varieties we will shorten the bioassay for leafroller to 21 days, which will provide data on development time and larval mortality. We will not be able to follow OBLR development through to the adult stage and collect reproductive data on these bioassays as the time and labor required to do so is prohibitive. We will also repeat bioassays on some varieties tested in 2011 to confirmed observed resistance.

Localize genes for resistance. Year 3 of the project.

Develop predictive markers for resistance. Year 3 of the project.

CONTINUING PROJECT REPORT **YEAR: 2 of 2**
WTFRC Project Number: CP-11-100A and CP-11-100B

Project Title: Identification of neuropeptides and receptors in codling moth and SWD

PI: Stephen F. Garczynski
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Co-PI (2): Amit Dhingra
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Total Project Request: **Year 1:** $40,049 **Year 2: $20,000**

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

Item	Year 1	Year 2
Salaries	$9,666.80	
Benefits	$ 739.51	
Wages		
Benefits		
Equipment		
Supplies	$9,593.69	$10,000
Travel		
Plot Fees		
Miscellaneous		
Total	$20,000	**$10,000**

Footnotes:

Budget 2
Organization Name: WSU **Contract Administrator:** Mary Lou Bricker
Telephone: 509 335 7667 **Email address:** mdesros@wsu.edu

Item	Year 1	Year 2
Salaries	$3,100	$3,100
Benefits	$ 949	$ 949
Wages		
Benefits		
Equipment		
Supplies	$4,000	$6,000
Travel		
Plot Fees		
Miscellaneous[1]	$12,000	
Total	$20,049	**$10,000**

Footnotes: [1]Money allocated for transcriptome sequencing

OBJECTIVES:

Because many insecticides target proteins produced by the brain and nervous system, the main goal of our project is to provide fundamental biological information on critical physiological functions of these organs through the generation and analysis of transcriptomes (a compilation of genes that are being actively expressed) of heads from various larval and adult stages of codling moth. Our approach will be to identify proteins produced in the codling moth brain and nervous system by determining the nucleotide sequences of gene transcripts that encode them, and then to categorize these proteins by biological function. Completion of this project will provide a "catalog" of potential protein targets that can be used by codling moth researchers to enhance existing or develop new strategies and tools for control of this major pest of apple.

1) Extract messenger RNA (mRNA) from heads of codling moth larvae, pupae and adults. Convert RNA transcripts to complementary DNA (cDNA). (Garczynski)

Because protein encoding genes are expressed as mRNA, and brain and nerve tissue is contained in heads, our first objective is to extract mRNA and prepare cDNA from heads dissected from various stages of codling moth. To obtain a most complete “catalog” of expressed genes, all stages of codling moth (larvae through adults) will be used.

2) Determine sequences of cDNAs representing brain mRNA transcripts using 454 sequencing technology. (Dhingra)

Massive parallel sequencing of the transcriptome is now an established methodology using the 454 technology. The Dhingra lab has recently published the apple genome which serves as a host for the codling moth and transcriptomes from strawberry, which has a complex chromosome number. The 454 sequencing platform is ideally suited for generating large quantities of nucleotide sequence data, making it a preferred choice for transcriptome investigations.

3) Analyze sequences of assembled brain cDNAs to identify those encoding neuropeptides, peptide hormones, and other potential protein targets for codling moth control. (Dhingra, Garczynski)

To identify expressed genes encoding potential protein targets for codling moth control, a bioinformatic approach will be used. Computer programs will be used to determine the identity of the proteins encoded by the various head expressed genes, and once identified, biological functions will be assigned. Completion of this objective will allow us to identify neuropeptides and receptors, as well as other potential protein targets that might be exploited in codling moth pest control programs.

4) Clone and characterize cDNAs from Spotted winged Drosophila that encode neuropeptides and receptors involved in regulation of feeding and reproduction. (Garczynski)

Spotted winged Drosophila (SWD) is an emerging pest of tree fruit in the Pacific Northwest. The purpose of this objective is to clone and characterize gene transcripts encoding neuropeptides and receptor proteins that regulate feeding and reproduction in *Drosophila suzukii* larvae and adults. Successful completion of this objective will provide basic information that can be used by other researchers in attempts to control this insect pest. In addition, clones of gene transcripts that encode neuropeptide receptors will be expressed in a mammalian cell line which can be used in future work to discover compounds that potentially disrupt SWD feeding and reproduction. Specific targets include: neuropeptide F and short neuropeptide F receptors (involved in regulation of feeding and growth), and the sex peptide receptor (a modulator of female reproduction/male receptivity).

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS):

Year 1

- Gene transcripts encoding the spotted winged Drosophila sex peptide and its putative receptor have been cloned.
- Gene transcripts encoding spotted winged Drosophila neuropeptide F, short neuropeptide F, and their putative receptors have been cloned.

- A codling moth colony started from field collected insects was generated to provide insects more closely resembling those in the orchard. This colony was infused regularly with newly collected insects until sufficient numbers of insects were collected for RNA extraction and cDNA synthesis.
- Heads of thousands of codling moth larvae, pupae and adults were dissected and used to isolate mRNA for transcriptome sequencing and analysis.

METHODS

1) Transcriptome generation and analysis.
    A) mRNA was isolated from heads dissected from larvae, pupae and adults.
    B) mRNA was converted to cDNA, and the cDNA will be sent to Amit Dhingra for sequencing using 454 technology.
    C) Nucleotide sequences of cDNAs will be assembled and then annotated using bioinformatic software packages.
    D) Annotated sequences will be analyzed, and potential targets, focusing on neuropeptides and peptide hormones, will be identified.

2) Cloning and expression of gene transcripts encoding neuropeptides and their receptors from SWD.
    A) Total RNA and mRNA was isolated from SWD larva and adults.
    B) RNA was converted to cDNA and used as template for cloning neuropeptides and neuropeptide receptors of interest.
    C) Oligonucleotide primers were designed for each of the targets of interest using bioinformatics approaches.
    D) Gene transcripts were amplified using PCR, and PCR products were cloned and their nucleotide sequences were determined.

3) Characterization of SWD neuropeptides and their receptors.
    A) Neuropeptides of interest will be chemically synthesized for use in cell based assays.
    B) Neuropeptide receptors will be expressed in cell lines for use in assays.
    C) Binding assays will be performed to verify neuropeptide/neuropeptide receptor pairs.

RESULTS & DISCUSSION

Because protein components of the insect brain and nerves are targets of insecticides, the main goal of this project is to develop a catalog of gene transcripts encoding proteins expressed in the codling moth brain. This gene transcript catalog can be used to help us understand codling moth response to insecticides, and help explore potential resistance mechanisms. This catalog will also be useful in the identification of potential new targets that can be exploited for the development of future forms of codling moth control.

Generation of codling moth for dissection

The first step in generating codling moth transcriptomes was to collect heads and brains from various insect stages. Initially we planned to use codling moth collected from a maintained colony, however the goal of this project is to identify gene transcripts relevant to insects that have to survive in the orchard setting. Therefore, we collected insects from various infested apple trees and reared them through to adults. These adults were mated and the next generation of larvae were reared on artificial diet. Each subsequent generation was mated with orchard collected codling moth until we had a colony that was not inbred or far removed from natural environmental conditions. While this took an enormous effort, we feel that the transcriptome profile will more closely reflect that of wild populations of codling moth.

Once our codling moth colony was generating a constant source of insects, the heads of the various larval and adult stages were collected for RNA extraction. Tables 1 and 2 summarize the materials collected for RNA extraction, and subsequent transcriptome generation. A major effort was needed to collect enough neonate larval heads for efficient RNA extraction. Rearing the wild codling moth took a full-time technician six months to generate enough neonate larvae for dissection, and the dissections required the efforts of a full time technician and six part-time technicians to complete. It was not until late November that all materials were collected. We are now in the process of preparing the RNA templates that will be sequenced in the Dhingra laboratory this upcoming year.

Table 1. Adult heads collected for transcriptome analysis.

	Mated adults		***Unmated adults***	
Hrs post emergence	**Males**	**Females**	**Males**	**Females**
0-24	0	0	28	28
24-48	17	17	22	20
48-72	20	20	21	19
72-96	20	20	23	20
96-120	21	21	35	28
Total	**78**	**78**	**129**	**115**

Table 2. Larvae and pupa heads collected for transcriptome analysis

Immature Stage	***Number of heads dissected***
Neonates	2000
2nd instar	200
3rd instar	100
4th instar	50
5th instar	30
Pupa	100

Identification and cloning gene transcripts encoding SWD neuropeptides and receptors

Spotted winged Drosophila is a new, potentially devastating pest of various fruits grown in the Pacific Northwest. As part of this project, it was proposed that we clone and characterize cDNAs from that encode neuropeptides and receptors involved in regulation of feeding and reproduction. For this, we chose to focus on sex peptide and its receptor which are involved in female receptivity and oviposition; neuropeptide F and short neuropeptide F and their receptors, which in insects are involved in the regulation of feeding and larval growth.

A bioinformatics approach was used to identify conserved regions of gene transcripts encoding the neuropeptides and their receptors of interest from insects whose mRNA sequences have been previously determined. We used alignments of protein and mRNA sequences determined for 12 other species of Drosophila to identify conserved regions that could be exploited for oligonucleotide primer design to amplify transcripts encoding related proteins in SWD. These oligonucleotide primers were used in PCR reactions to amplify and clone gene transcripts encoding the SWD neuropeptides and receptors of interest. We have been successful in cloning transcripts encoding the SWD sex peptide and receptor, neuropeptide F and its receptor, and the short neuropeptide F receptor. We will continue this upcoming year to clone the transcript encoding SWD short neuropeptide F.

A collaboration has been initiated with researchers at the USDA-ARS in Maricopa, AZ to further characterize SWD sex peptide and receptor. These collaborators are interested in developing methods to control insect mating by disruption of the sex peptide signaling pathway. While their research focuses on *Lygus* bugs, they would like to work with the clones I have generated for the SWD peptide-receptor system. It is our hope that further characterization of the sex peptide signaling system will yield new forms of insect control, exploiting this physiological regulator of female mating receptivity.

CONTINUING PROJECT REPORT **YEAR**: Year 2

Project Title: Monitoring leafrollers and codling moth with one non-pheromone lure

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Total Project Request: Year 1: $26,000 **Year 2: $27,000**

Other funding sources

Agency Name: Trécé Inc., Adair, OK
Amt. requested: $12,000

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

Item	2011	2012
Salaries		
Benefits		
Wages	9,655	9,850
Benefits	705	760
Equipment		
Supplies	1,500	1,600
Travel	1,100	1,250
Plot Fees		
Miscellaneous		
Total	$12,960	**$13,960**

Budget 2
Organization Name: WSU-TFREC **Contract Administrator:** ML Bricker; Kevin Larson
Telephone: 509-335-7667; 663-8181 X221 **Email address:** mdesros@wsu.edu; kevin_larson@wsu.edu

Item	2011	2012
Salaries		
Benefits		
Wages	9,600	9,600
Benefits	1,440	1,440
Equipment		
Supplies	1,000	1,000
Travel	1,000	1,000
Plot Fees		
Miscellaneous		
Total	$13,040	**$13,040**

OBJECTIVES

1. Refine the correlation of moth catches of Pandemis and oblique banded leafroller in traps baited with the combo lure used with an acetic acid with local larval leafroller populations within orchards.

2. Evaluate the relative attractiveness of other host plant volatiles in combination with acetic acid for both leafrollers and codling moth.

SIGNIFICANT FINDINGS

- ✓ The addition of an AA lure to the sex pheromone-pear ester combo lure-baited traps significantly increased codling moth catches in one of four studies conducted in Washington, Oregon, and Utah.

- ✓ A commercial acetic acid lure, Pherocon AA, was developed for codling moth as a result of this research.

- ✓ The optimal daily release rate of acetic acid from lures required to be effective for both codling moth and leafrollers was found to be between 10 – 50 mg that is higher than the rate of the Pherocon AA lure, < 5 mg/d.

- ✓ AA lures can be formulated that are effective for both Pandemis and Obliquebanded leafrollers.

- ✓ Five other host plant volatiles in addition to pear ester combined with codling moth's sex pheromone and used with an acetic acid lure performed similarly in traps for Pandemis leafrollers, and both beta ocimene and nonatriene were as attractive as pear ester for codling moth.

- ✓ The use of the AA lure with the sex pheromone-pear ester combo lure provided useful predictive capabilities to detect either the presence of either leafroller species within the orchard or the threat of nearby leafroller populations invading the orchard. Within orchards isolated from potential leafroller infestations the traps generally did not catch any leafroller adults.

METHODS

Studies planned for 2012 will continue to evaluate these lures in apple and pear orchards situated near Brewster, Wenatchee, Yakima, and Medford. Orchards will be classified according to three categories: within-field leafroller infestation, adjacent to potential leafroller infested sites, or distant from any leafroller infested sites. Orchard sites will again be selected to include both Pandemis and obliquebanded leafrollers. Sites will be monitored with traps baited with the sex pheromone-pear ester combo lure for codling moth, the combo lure plus an AA lure, and leafroller sex pheromone lures. Orchards will be sampled in the spring and mid-summer for the presence of leafroller larvae and late in the summer for injured fruits.

Replicated tests will be conducted in several codling moth and leafroller infested orchards to refine the optimal release rate of acetic acid for both codling moth and leafrollers. Experimental lures will be provided by Trécé Inc. and compared with vial lures that we construct. Residual weight loss of all lures will be conducted to estimate lure emission rates.

The potential use of alternate host plant volatiles will be evaluated in one or two orchards infested with obliquebanded leafrollers. Unfortunately, a trial comparing host plant volatiles for the oblique

banded leafroller in 2011 was erroneously established in an orchard without a within-orchard leafroller population and no leafrollers were caught. Several potential orchards were identified after harvest this year and will be evaluated for current leafroller infestations early in the season.

RESULTS & DISCUSSION

Benefit of Adding AA to CM Combo lure. Previously we reported that the addition of AA to the sex pheromone-pear ester combo lure either increased or left unchanged the total number of codling moths caught in traps. During 2011 this was more widely tested in Brewster and Quincy, WA, Medford, OR, and near Logan, UT (Table 1). We found a strong beneficial effect of adding the Pherocon AA lure to sex pheromone-pear ester combo baited-traps in six Brewster orchards treated with Isomate CM Flex dispensers. Orchards were monitored from 14 July to 9 September and the combo + AA baited traps caught 3x and 7x more males and female codling moth than the standard combo baited traps in these orchards. Similar studies conducted in four orchards near Quincy by Mike Hodge, seven pear orchards under mating disruption near Medford by Rick Hilton, and in four orchards in Utah by Dr. Diane Alston treated with either hand-applied dispensers or puffers all found that the addition of the Pherocon AA lure did not significantly increase total moth catches in traps baited with the combo lure. However, in general catches of female codling moths were numerically higher with the combo + AA lure versus just the combo lure.

Table 1. Codling moth catches with the Pherocon CM DA Combo lure with and without the addition of a Pherocon AA lure in six orchards in Brewster, four orchards in Quincy, seven orchards in Medford, and four orchards near Logan, UT, 2011.

		Mean (SE) moth catch per trap		
Site	Lure	Male	Female	Total
Brewster	Combo	16.8 (2.5)	2.5 (0.3)	19.3 (2.8)
	Combo + AA	46.2 (5.0)	17.8 (1.2)	64.0 (5.8)
ANOVA: $_{df = 1, 10}$		$F = 27.07$ $P < 0.001$	$F = 140.1$ $P < 0.0001$	$F = 47.83$ $P < 0.0001$
Quincy	Combo	7.8 (2.3)	1.6 (0.8)	9.4 (3.1)
	Combo + AA	9.4 (3.4)	2.2 (0.4)	11.4 (3.6)
ANOVA: $_{df = 1, 6}$		$F = 0.12$ $P = 0.74$	$F = 0.87$ $P = 0.38$	$F = 0.21$ $P = 0.66$
Medford	Combo	28.0 (13.5)	3.9 (1.5)	31.9 (14.2)
	Combo + AA	33.6 (15.4)	14.1 (6.3)	47.7 (21.0)
ANOVA: $_{df = 1, 12}$		$F = 0.09$ $P = 0.85$	$F = 2.05$ $P = 0.18$	$F = 0.19$ $P = 0.67$
Utah	Combo	13.1 (5.9)	3.4 (1.5)	16.5 (7.3)
	Combo + AA	9.8 (3.8)	6.3 (2.4)	16.0 (6.1)
ANOVA: $_{df = 1, 30}$		$F = 0.81$ $P = 0.38$	$F = 2.01$ $P = 0.18$	$F = 0.11$ $P = 0.75$

These data suggest that the use of the AA lure will not substantially change the performance of traps for monitoring codling moth except to increase the trap's ability to detect female moths. Growers' ability to track female codling moths has been shown to improve management.

Optimal AA loading for lure. Over the past four years we have been working with Trécé Inc. to develop an acetic acid co-lure to improve the combo lure for codling moth. This led to the Pherocon AA lure which has now been added to their catalogue. Our studies have found that the emission rate of this lure is much lower than the vial lures we originally tested, but that this lure has a similar level

of attractiveness for codling moth. Unfortunately, studies conducted in 2011 found that this AA lure is not optimal for catching leafrollers (Table 2). A higher emission rate is required and thus we replaced all of the Pherocon AA lures this summer with a vial with a 3mm hole as quickly as we could. At present it is not clear what the optimal rate would be for both codling moth and leafrollers. Trécé Inc. provided us with a new AA lure (TRE0421) with a higher emission rate than Pherocon AA for further testing. This lure looked promising in our initial test.

Table 2. Moth catches of Pandemis leafrollers in two trials with traps (N = 10) baited with the Pherocon CM DA Combo lure plus one of several AA co-lures.

	27 June – 7 July		5 Aug – 29 Sept	
AA co-lure	Lure wt loss (mg/d)	Moth catch	Lure wt loss (mg/d)	Moth catch
Vial, 3 mm hole	40	11.5	55	3.0
Vial, 1.6 mm hole	17	9.3	20	4.3
Pherocon AA	3.5	1.6	4	1.4
TRE0421	-	-	12	3.9

Further research is needed to test AA lures to bracket the optimal emission rate needed for dispensers to be attractive for both codling moth and leafrollers under typical temperatures in Washington State. In addition, we want to run this comparison with both species of leafrollers.

Comparison of Host Plant Volatiles with AA. Studies were conducted to compare six host plant volatiles as lures for codling moth and Pandemis leafroller in an orchard situated near Naches, WA (Table 3). Host plant volatiles were equally effective in catching leafroller adults when combined with the AA lure. The pear ester, beta ocimene, and nonatriene lures were similar in attractiveness for codling moth. A second study established in mid August near Pasco, WA in an orchard purported to be infested with obliquebanded leafroller was a bust as leafroller counts in the sex pheromone traps were very low (5<) and none were caught with host plant volatile lures.

Table 3. Comparison of moth catches of codling moth and Pandemis leafroller in traps (N = 10) baited with one of six host plant volatiles in combination with the sex pheromone of codling moth and the addition of a AA vial with a 3 mm hole, Sept. 2011.

	Codling moth		Pandemis	
Host plant volatile	Total	Females	Total	Females
Pear ester	0.9	0.3	9.4	3.4
Beta ocimene	0.8	0.3	12.0	4.6
Nonatriene	0.9	0.4	9.0	4.2
Farnesol	0.5	0.1	10.1	3.3
Beta farnesene	0.0	0.0	8.6	2.8
Butyl hexanoate	0.4	0.1	8.5	2.5

Additional testing of pear ester, nonatriene, and beta ocimeneis needed, especially with the obliquebanded leafroller.

Use of one trap for codling moth and leafrollers – correlation with leafroller populations
Studies were conducted near Brewster, Quincy, Wenatchee, and Yakima, WA and Medford, OR. Sites for 2011 outside of the Yakima and Brewster studies were chosen based on some expectations that orchards would be infested with leafrollers. Visual sampling for leafroller larvae and the presence of fruit injury late in the season were conducted in most orchards. No leafroller adults were caught in traps in 12 orchards. Among these no signs of leafroller larvae were found except for spring larvae were sampled in the Wenatchee2 site which was also nearby known infested blocks. Low levels of leafroller adults < 1 moth was found in two sites in which larvae or fruit injury was not detected. These were both sprayed orchards. In five orchards leafrollers were caught in traps and no larvae or injury was found in the monitored block but known infested hosts, such as mature and non-bearing cherry blocks and backyard unsprayed fruit trees, were nearby the orchard. The most interesting block in this category was Naches1 that had very high levels of leafroller adults without any injury. At harvest the grower unexpectedly found high levels of fruit injury in a 'Honeycrisp' block that was < 0.2 miles away. In addition, the Naches1 orchard was surrounded by several cherry blocks thatwere not sampled. Traps in all blocks in which leafroller fruit injury was detected caught leafroller adults.

Several factors were identified that can influence the catch of leafroller adults in traps baited with Pherocon CM DA plus AA. These include the spray practices used within the orchard and the potential immigration of leafroller adults from nearby infested hosts. Both species of leafrollers can attack a large number of non commercial hosts and populations develop on cherry and can increase in these orchards after harvest.

Table 4. Summary of moth catches and leafroller infestations in orchards monitored during 2011 with the Pherocon CM DA Combo lure plus a vial with a 3 mm hole loaded with AA.

	Mean catch PH trap		Mean catch Combo + AA trap		Infestation presence or potential
Orchard	PLR	OBLR	CM	LR	
Naches1	294	81	61	24	Nearby injury & hosts
Naches2	66	9	86	0.5	No
West Valley	23	35	30	0	No
Wiley City	42	31	21.5	0	No
Moxee1	2	18	41	0	No
Moxee2	19	123	23	0.5	Nearby hosts
USDA Farm W	53	40	30	7	3.6% injury
USDA Farm E	248	36	46	30	14.0% injury
Wapato1	74	97	59	0	No
Wapato2	88	62	57	0	No
Wapato3	79	43	8	2	N.A.
Wapato4	86	60	28	1	N.A.
Brewster1	-	30	0	0	No
Brewster2	-	11	1	0	No
Brewster3	-	28	1	0	No
Brewster4	-	61	1	0	No
Pasco	-	4	8.5	0	No
Medford1	-	298	55	6	Some fruit injury
Medford2	-	260	126	5	Some fruit injury
Medford3	-	320	1	1	No
Medford4	-	57	0	0	No
Medford5	-	335	4	4	Some fruit injury
Medford6	-	149	122	4	Some fruit injury
Medford7	-	408	26	8	June larvae

Quincy1	-	217	6	4	High fruit injury
Quincy2	-	227.5	6	1	Some fruit injury
Quincy3	-	41.5	21	1	Nearby hosts
Quincy4	-	52	12.5	3.5	Nearby hosts
Wenatchee1	0	45	-	1	Spring/summer larvae
Wenatchee2	0	94	-	0	Spring larvae
Wenatchee3	1	125	-	4	Nearby hosts
Wenatchee4	20	184	-	4	Spring/summer larvae
Wenatchee5	2	3	-	2	Spring larvae
Wenatchee6	0	15	-	7	?

These data support the use of a single trap baited with the sex pheromone of codling moth plus pear ester and acetic acid to monitor both codling moth and leafrollers. The use of one trap to monitor both pests would be a no-cost benefit to growers. Further confirmation of this approach in year 2 of this grant is needed to expand its potential use among WA growers.

2011 WTFRC APPLE PESTICIDE RESIDUE STUDY

Spray application 1 day before harvest

In response to industry interest in apple pesticide residues, the Washington Tree Fruit Research Commission (WTFRC) recently conducted a trial in 'Gala' at the WSU Sunrise Research Orchard near Rock Island, WA. Ten insecticides and six fungicides were applied using a Rears airblast sprayer according to either an "aggressive" (maximum label rates at minimum retreatment and pre-harvest intervals) or "standard" (typical industry rates and timings) protocol. Apples from each protocol were sampled at harvest with half of the fruit being subjected to a simulated commercial packing process. Fruit samples were delivered the day of harvest to Cascade Analytical (Wenatchee, WA) for processing in advance of chemical analysis by a Portland, OR lab specializing in pesticide residue screening.

TRIAL DETAILS

- 5th leaf 'Pacific' Gala / M.9 Nic.29 trained to central leader/spindle on 3' x 10' spacing
- 2 x 25 gal Rears Pak-Blast sprayer calibrated to 100 gal / acre
- All chemicals applied with 8 oz Regulaid / 100 gal water / acre
- No overhead irrigation or precipitation for duration of trial

Measured residues vs. maximum residue levels (MRLs) for STANDARD industry pesticide programs utilizing typical rates, application timings, and retreatment intervals on apples. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2011.

Chemical name	Trade name	Application rate[1]	Application timing(s)	Field run fruit	Washed fruit	US MRL[2]	Lowest export MRL[3]
		oz per acre	*days before harvest*	*ppm*	*ppm*	*ppm*	*ppm*
Diazinon	Diazinon 50W	64	35 & 21	<0.01	**0.017**	0.5	0.01 (EU)
Endosulfan I	Thionex 50W	64	35 & 21	<0.01	0.018	1	0.05 (EU)
Endosulfan II	Thionex 50W	64	35 & 21	0.016	0.024	1	0.05 (EU)
Methoxyfenozide*	Intrepid*	8*; 16	35; 21	<0.01	<0.01	1.5	1.5 (many)
Acetamiprid*	Assail 30SG*	1.7*; 3.4	35; 21	<0.01	<0.01	1	0.1 (EU)
Spinetoram*	Delegate WG*	3.5*; 7	35; 21	<0.01	<0.01	0.2	0.05 (many)
Chlorantraniliprole*	Altacor*	2.3*; 4.5	35; 21	<0.01	<0.01	1.2	0.3 (CAN)
Trifloxystrobin	Flint	2.5	28 & 14	0.015	0.012	0.5	0.5 (many)
Fenpropathrin*	Danitol	21.3	28 & 14	<0.02	<0.02	5	0.01 (EU)
Zn dimethyldithiocarbamate	Ziram 76DF	96	28 & 14	**1.3****	**0.23****	7	0.1 (EU)
Triflumizole	Procure 480SC	12	28 & 14	0.016	<0.01	0.5	0.5 (many)
Difenoconazole	Inspire Super	12	28 & 14	<0.01	<0.01	1	0.5 (many)
Cyprodinil	Inspire Super	12	28 & 14	<0.01	<0.01	1.7	0.05 (many)
Imidacloprid***	Nuprid 2SC***	16***	21	<0.01	<0.01	0.5	0.5 (many)
Carbaryl	Carbaryl 4L	64	21 & 3	**2.0**	**0.71**	12	0.05 (EU)
Thiophanate-methyl	Topsin 70WP	24	14 & 3	0.014	<0.0097	2	0.5 (EU)
Captan	Captec 4L	128	14 & 1	0.78	0.69	25	3 (EU)
Pyraclostrobin	Pristine	14	7 & 1	0.068	<0.01	1.5	0.3 (EU)
Boscalid	Pristine	14	7 & 1	0.24	0.050	3	2 (many)

[1] Materials applied with Rears Pak-Blast sprayer at 100 gal water/acre

[2] 30 Sep 2011. http://www.nwhort.org/AppleMRLs.html

[3] Major export markets for WA apples; 30 Sep 2011. http://www.nwhort.org/AppleMRLs.html

* Initial applications of Intrepid, Assail 30SG, Delegate WG, and Altacor (35 dbh) were made at 50% of intended rates

** Thiocarbamate residues cannot be directly measured; values are estimates based on analysis of the degradation product carbon disulfide

*** Nuprid 2SC was accidentally applied at 2.5x (16 oz) the labeled rate of 6.4 oz/acre

Results of this lone unreplicated trial are shared for informational purposes only and should not be construed as endorsements of any product, reflections of their efficacy against any insect or fungal pest, or a guarantee of similar results regarding residues for any user. Apple growers should consult with their university extension staff, crop advisors, and warehouses to develop responsible pest control programs.

Soapy fruit during simulated packing

FRUIT WASHING DETAILS

1. 3 minutes of gentle agitation in 90°F water with 80 ppm chlorine
2. 30 seconds on brush bed with EpiClean soap (Pace International)
3. 30 second overhead rinse with cool water
4. 2 minutes dry time on clean brush bed
5. No waxes applied

CONCLUSIONS

Neither aggressive nor standard use patterns of all but three products evaluated produced residues which exceed either US or foreign MRLs. Application of **Diazinon 50W** produced residues very near the European Union (EU) MRL, which has essentially been set at the current limit of quantification; even though diazinon residues are not reported for field run fruit, they were detected just below the reporting limit. **Ziram 76DF** residue levels also exceeded EU tolerances, but are inherently imprecise in that current instrumentation does not allow for direct quantification of thiocarbamate residues; aggressive use of Ziram also produced a residue (2.8 ppm) very near MRLs for Taiwan (2.5 ppm) and India (3 ppm) in unwashed fruit. All applications of **Carbaryl 4L** tested produced residues far in excess of the EU MRL for carbaryl, which is also set at the current limit of quantification; unwashed apples also exceeded the carbaryl MRL for Taiwan (1 ppm). Washing fruit in a simulated packing process generally reduced residue levels for most products tested; diazinon and endosulfan residues were relatively persistent in our study. For more information on MRLs, visit the Northwest Horticultural Council website, www.nwhort.org.

Measured residues vs. MRLs for AGGRESSIVE pesticide programs utilizing maximum label rates and minimum pre-harvest and retreatment intervals on apples. 'Gala'/M.9 Nic.29, Rock Island, WA. WTFRC 2011.

Chemical name	Trade name	Application rate[1]	Application timing(s)	Field run fruit	Washed fruit	US MRL[2]	Lowest export MRL[3]
		oz per acre	*days before harvest*	*ppm*	*ppm*	*ppm*	*ppm*
Diazinon	Diazinon 50W	64	35 & 21	<0.01	**0.010**	0.5	0.01 (EU)
Endosulfan I	Thionex 50W	64	35 & 21	<0.01	0.010	1	0.05 (EU)
Endosulfan II	Thionex 50W	64	35 & 21	0.015	0.016	1	0.05 (EU)
Trifloxystrobin	Flint	2.5	28 & 14	0.018	0.012	0.5	0.5 (many)
Fenpropathrin	Danitol	21.3	28 & 14	<0.02	<0.02	5	0.01 (EU)
Methoxyfenozide	Intrepid	16	28 & 14	<0.01	<0.01	1.5	1.5 (many)
Zn dimethyldithiocarbamate	Ziram 76DF	128	28 & 14	**2.8***	**0.10***	7	0.1 (EU)
Imidacloprid**	Nuprid 2SC**	24**	21	<0.01	<0.01	0.5	0.5 (many)
Triflumizole	Procure 480SC	16	21 & 14	0.028	0.010	0.5	0.5 (many)
Difenoconazole	Inspire Super	12	21 & 14	<0.01	<0.01	1	0.5 (many)
Cyprodinil	Inspire Super	12	21 & 14	<0.01	<0.01	1.7	0.05 (many)
Acetamiprid	Assail 30SG	8	21 & 7	<0.01	<0.01	1	0.1 (EU)
Carbaryl	Carbaryl 4L	96	21 & 3	**3.1**	**0.62**	12	0.05 (EU)
Spinetoram	Delegate WG	7	14 & 7	<0.01	<0.01	0.2	0.05 (many)
Chlorantraniliprole	Altacor	4.5	14 & 5	<0.01	<0.01	1.2	0.3 (CAN)
Captan	Captec 4L	128	14 & 1	1.1	0.82	25	3 (EU)
Thiophanate-methyl	Topsin 70WP	24	7 & 1	0.037	0.017	2	0.5 (EU)
Pyraclostrobin	Pristine	18.5	7 & 1	0.078	0.014	1.5	0.3 (EU)
Boscalid	Pristine	18.5	7 & 1	0.35	0.089	3	2 (many)

[1] Materials applied with Rears Pak-Blast sprayer at 100 gal water/acre

[2] 30 Sep 2011. http://www.nwhort.org/AppleMRLs.html

[3] Major export markets for WA apples; 30 Sep 2011. http://www.nwhort.org/AppleMRLs.html

* Thiocarbamate residues cannot be directly measured; values are estimates based on analysis of the degradation product carbon disulfide

**Nuprid 2SC was accidentally applied at 3.75x (24 oz) the labeled rate of 6.4 oz/acre

For more information, contact Tory Schmidt (509) 669-3903 or email tory@treefruitresearch.com

WASHINGTON TREE FRUIT RESEARCH COMMISSION

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

Project Title: Sustainable postharvest decay control

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

Cooperators: Selected packinghouses across central Washington State

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

Other funding sources:

Agency Name: Washington State Commission on Pesticide Registration
Amt. awarded: $11,247
Notes: A proposal on control of Pristine-resistant *Botrytis cinerea* was funded by WSCPR for the 2011 season.

WTFRC Collaborative expenses:

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

Budget 1
Organization: WSU-TFREC **Contract Administrator:** Carrie Johnston; Kevin Larson
Telephone: 509-335-4564; 509-663-8181 x221 **Email:** carriej@wsu.edu; kevin_larson@wsu.edu

Item	2010	2011	2012 (extension)
Salaries[1]	43,747	45,747	**0**
Benefits	17,149	18,550	**0**
Wages[2]	4,000	4,000	**0**
Benefits	592	384	**0**
Equipment	0	0	**0**
Supplies[3]	8,000	8,000	**0**
Travel[4]	2,000	2,000	**0**
Plot Fees	0	0	**0**
Miscellaneous	0	0	**0**
Total	75,488	78,681	**0**

Objectives:

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

Significant Findings:

This project deals with postharvest diseases. Some experiments conducted on the 2011 crops are still in progress and will have results in summer 2012. The final report of this project will be available for the next research review. Progress as of the end of 2011 is presented in this report.

A one-year extension of this project without additional funds has been approved by the WTFRC.

- Resistance to pyrimethanil (Penbotec) has developed in *Penicillium expansum* populations in some packinghouses where the fungicide as a postharvest drench has been used annually for 4-5 consecutive years. In one packinghouse, over 90% of the isolates were resistant to pyrimethanil when Penbotec (pyrimethanil) was again used on 2010 crops, while on the fruit drenched with Scholar in 2010, resistance frequency was reduced to 4%. In another packinghouse where Penbotec was used during 2005-2009 but only Scholar was used on 2010 crops, and the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 (reported in 2010) to 1% in 2011. The results clearly demonstrated the benefit of rotation of postharvest fungicides for drench.

- In other three packinghouses, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in these three packinghouses. The findings support our recommendations on rotation of postharvest fungicides as a drench, and fungicide resistance management practices need to be implemented in the industry.

- The frequency of Pristine-resistant strains in apple orchards where Pristine had been used during 2005-1010 declined from 2010 to 2011 season. Fungicides used in these orchards and perhaps other factors such as competitive disadvantage of Pristine-resistant strains may affect the dynamic of Pristine-resistant populations. The results may suggest that Pristine can still be used and remain effective when the resistant populations decline.

- Reduced rates of tank-mixture of Pristine and Topsin M significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. Reduced rates of tank-mixture of BAS 703 and Topsin M significantly reduced incidence of gray mold caused by either pristine-sensitive or –resistant strain but was more effective against Pristine-sensitive strain. In 2011, we repeated the experiment with an emphasis on a tan-mixture of full label rates of Pristine and Topsin for control of Pristine-resistant strains. The results will be forthcoming.

- Although DPA is not a fungicide, TBZ-resistant isolates became sensitive to DPA and a DPA treatment significantly controlled gray mold caused by TBZ-resistant strains. Resistance to the AP fungicides compromised the efficacy of pyrimethanil as a postharvest treatment for control of gray mold. Fludioxonil was effective against all phenotypes. The results suggest that the use of AP fungicides in the orchards should be limited in order to minimize the risk of development of resistance to pyrimethanil.

- Preharvest applications of Serenade MAX or Sonata did not significantly reduce postharvest rots in comparison with the nontreated control.

Methods:

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

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

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

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

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

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

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

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

Results & Discussion:

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

In 2011, we monitored pyrimethanil resistance in *P. expansum* in the same five packinghouses we sampled in 2010. Blue mold-like decayed fruit from various grower lots from the five packinghouses were collected. In total, 410 *P. expansum* isolates were obtained (Table 1). In both Packinghouse A and B, Penbotec (pyrimethanil) was used as a postharvest drench from 2005 to 2009. On the 2010 crops, in packinghouse A, some lots were drenched with Scholar+DPA and some lots with Penbotec. Over 90% of the isolates were resistant to pyrimethanil, while on the fruit drenched with Scholar in 2010, resistance frequency was reduced to 4%. The packinghouse B switched to Scholar on 2010 crops, and the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 (reported in 2010) to 1% in 2011.

In packinghouse A, all isolates obtained from Penbotec-drenched fruit were resistant to TBZ, whereas 12.5% of the isolates from Scholar-drenched fruit were resistant to TBZ. TBZ-resistant strains were also present in other packinghouses but at a low level.

Some isolates showed reduced sensitivity to fludioxonil. As this was the first time that we found strains with reduced sensitivity to fludioxonil, we are currently re-testing these isolates to confirm whether the reduced sensitivity is stable.

Previously we reported the occurrence of pyrimethanil resistance in *P. expansum* in Packinghouses A and B as a result of annually repeated use of Penbotec as a postharvest drench from 2005 to 2009. Since 2010, packinghouses followed our recommendations on resistance management and started rotation of postharvest fungicides as drench. The data from these two packinghouses clearly indicated that switching to Scholar on 2010 crops significantly reduced the frequency of pyrimethanil resistant strains. In Packinghouses C, D and E, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in these three packinghouses. The findings support our recommendations on rotation of postharvest fungicides as a drench, and fungicide resistance management practices need to be implemented in the industry.

Table 1. Monitoring of resistance to postharvest fungicides in *Penicillium expansum* from apples in 2011

Packing house	**Drench Treatment**	**# isolates of *P. expansum***	**# isolates resistant to pyrimethanil**	**# isolates resistant to thiabendazole**
Packinghouse A	Scholar+DPA	48	2	6
Packinghouse A	Penbotec	119	113	119
Packinghouse B	Scholar or Scholar+DPA	99	1	6
Packinghouse C	Penbotec	56	0	3
Packinghouse D	Scholar	31	0	1
Packinghouse E	Penbotec	38	0	0

Monitoring Pristine resistance in B. cinerea in apple orchards

We monitored Pristine resistance in *B. cinerea* in five apple orchards. Pristine had been used for 5-6 years in these orchards. Except in orchard D, the frequency of Pristine-resistant strains in these orchards declined from 2010 to 2011 season. Fungicides used in these orchards and perhaps other factors such as competitive disadvantage of Pristine-resistant strains may affect the dynamic of

Pristine-resistant populations. The data we reported in the past year indicated that Pristine-resistant strains cannot compete well with Pristine-sensitive strains on apple fruit. The results may suggest that Pristine can still be used and remain effective when the resistant populations decline.

Table 2. Frequency of Pristine-resistant *B. cinerea* in 2011 from commercial Gala orchards where Pristine had been used

Orchard	Number of isolates	Frequency of Pristine-resistant isolates (%)	
		2010 season	2011 season
A	50	45.9	6.0
B	24	54.1	37.5
C	25	52.3	20.0
D	35	17.2	17.1
E	35	13.3	5.7

Control of gray mold caused by Pristine-resistant Botrytis cinerea

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

Pristine at 14.5 oz/A and the new fungicide BAS 703 at 4.11 fl oz/A significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. Reduced rates of tank-mixture of Pristine and Topsin M significantly reduced incidence of gray mold caused by the Pristine-sensitive strain but not the Pristine-resistant strain. Reduced rates of tank-mixture of BAS 703 and Topsin M significantly reduced incidence of gray mold caused by either pristine-sensitive or –resistant strain but was more effective against Pristine-sensitive strain. In 2011, we repeated the experiment with an emphasis on a tan-mixture of full label rates of Pristine and Topsin for control of Pristine-resistant strains. The results will be forthcoming.

Table 3. Efficacy of preharvest fungicide programs for control of Pristine-resistant strains of *Botrytis cinerea* on apple fruit

Treatment	Incidence of gray mold (%)	
	Pristine-sensitive strain	Pristine-resistant strain
Control: No Fungicide	100.0 a	98.8 a
Pristine 14.5 oz + Sylgard	41.3 cd	100.0 a
BAS 70301F 4.11 fl oz + Sylgard	58.3 bc	95.0 ab
Topsin 1 lb + Sylgard	68.8 b	72.5 c
Pristine 10.9 fl oz + Topsin .75 lb + Sylgard	32.5 d	98.8 a
BAS 70301F 3.08 + Topsin .75 lb+Sylgard	52.5 bc	81.3 bc

Values with the same letter in the same column are not significantly different based on the Waller-Duncan test ($P = 0.05$).

Sensitivity to DPA and control of Pristine-resistant strains of B. cinerea with postharvest fungicides and DPA

Sensitivity to DPA, TBZ, fludioxonil and pyrimethanil in Pristine-resistant isolates of *B. cinerea* was tested. All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. All isolates

remained sensitive to fludioxonil but some were resistant to pyrimethanil, likely because cyprodinil (Vangard) had been used in some of these orchards. The results indicated that Pristine resistance does not change the sensitivity of the isolates to DPA and that DPA may be able to control TBZ-resistant strains of *B. cinerea*.

An experiment was conducted during 2010-2011 storage season to evaluate postharvest fungicides and DPA alone or their combinations for control of gray mold caused by Pristine-resistant and/or MBC-resistant strains of *B. cinerea*. Although DPA is not a fungicide, MBC-resistant isolates became sensitive to DPA and a DPA treatment significantly controlled gray mold caused by TBZ-resistant strains (Table 4). Resistance to the AP fungicides compromised the efficacy of pyrimethanil as a postharvest treatment for control of gray mold. Fludioxonil was effective against all phenotypes.

Table 4. Effectiveness of DPA with or without postharvest fungicides for control of gray mold incited by various fungicide-resistant phenotypes of *Botrytis cinerea*

		Incidence (%)	
Phenotype[x]	Treatment	DPA -	DPA +
$MBC^{R}AP^{R}QoI^{R}SDHI^{R}$	Control	100 aA[y]	9.4 bB
	TBZ	100 aA	16.3 aB
	Fludioxonil	0 c	0 c
	Pyrimethanil	48.8 b	19.4 a
$MBC^{R}AP^{S}QoI^{R}SDHI^{R}$	Control	100 aA	13.8 aB
	TBZ	100 aA	16.3 bB
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^{R}AP^{S}QoI^{S}SDHI^{S}$	Control	100 aA	20.6 aB
	TBZ	100 aA	11.9 bB
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^{S}AP^{S}QoI^{R}SDHI^{S}$	Control	100 a	100 a
	TBZ	0 bB	5 bA
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c
$MBC^{S}AP^{S}QoI^{S}SDHI^{R}$	Control	100 a	100 a
	TBZ	2.5 bB	26.3 bA
	Fludioxonil	0 c	5.6 c
	Pyrimethanil	0 c	3.1 c
$MBC^{S}AP^{S}QoI^{S}SDHI^{S}$	Control	98.1 a	100 a
	TBZ	0 bB	6.9 bA
	Fludioxonil	0 b	0 c
	Pyrimethanil	0 b	0 c

[x] MBC = TBZ, thiophanate-methyl; AP = cyprodinil, pyrimethanil; QoI = pyraclostrobin; SDHI = boscalid.

[y] Values are the means of pooled data from the two runs of the experiment. Values followed by the same lowercase letter within a column in each isolate are not significantly different according to the ANOVA and LSD at $P = 0.05$. Values followed by the same capital letter within a row are not significantly different according to *t*-test at $P = 0.05$. Data were arcsine-transformed before analysis.

Preharvest biocontrol agents for control of postharvest fruit rots

An experiment was conducted in an organic Fuji orchard near Quincy. Biocontrol agents Sonata and Serenade were applied to the fruit 10 days and 1 day before harvest. Fruit were harvested and wounded with a finish-nail head to simulate puncture wounds. Natural inoculum was used in this study. Preharvest applications of Serenade MAX or Sonata did not significantly reduce postharvest rots in comparison with the nontreated control (Table 5).

Table 5. Efficacy of preharvest applications of Serenade and Sonata for control of postharvest fruits rots on organic Fuji apples

Treatment	**Rots (%)**
Nontreated	11.04 a
Serenade MAX at 10 and 1 day before harvest	6.88 a
Sonata at 10 and 1 day before harvest	6.98 a