2013 Apple/Apple Crop Protection Research Review

01/30/13 **APPLE COMMITEE**

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FINAL PROJECT REPORT
WTFRC Project Number: CP-10-105

Project Title: Sustainable postharvest decay control

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Cooperators: Selected packinghouses across central Washington State

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

Other funding sources:

Agency Name: Washington State Commission on Pesticide Registration
Amt. awarded: $11,247

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 History

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:

- 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 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 two of the three packinghouses, and 1.8% of the isolates from one packinghouse were resistant to pyrimethanil. 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. In 2011, we repeated the experiment with an emphasis on a tank-mixture of full label rates of Pristine and Topsin for control of Pristine-resistant strains. On 2011 crops, Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone. For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate.

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

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

- 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.

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

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

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

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

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

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

Results & Discussion:

Monitoring resistance of P. expansum to pyrimethanil and fludioxonil

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

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

In 2011, 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 pyrimethanil resistance in *Penicillium expansum* from apples in 2010

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

Table 2. Monitoring of pyrimethanil resistance 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	118	113	115
Packinghouse B	Scholar or Scholar+DPA	99	1	7
Packinghouse C	Penbotec	55	1	2
Packinghouse D	Scholar	31	0	1
Packinghouse E	Penbotec	40	0	0

Control of blue mold incited by pyrimethanil-resistant P. expansum

Postharvest fungicides were evaluated for control pyrimethanil-resistant strains on apple fruit. Penbotec at label rate (16 fl oz/100 gallon water) only partially controlled blue mold incited by a low-resistance strain (Table 3), and failed to control blue mold caused by strains exhibiting moderate or high resistance to pyrimethanil. Scholar was effective to control pyrimethanil-resistant strains regardless of pyrimethanil-resistant phenotypes (Table 3). Because all pyrimethanil-resistant strains also were resistant to TBZ, a postharvest treatment with TBZ did not provide satisfactory control of blue mold incited by strains that were resistant to both TBZ and pyrimethanil.

Table 3. Effectiveness of postharvest fungicides for control of blue mold incited by different phenotypes of pyrimethanil-resistant strains of *Penicillium expansum*.

Isolate	Phenotype	Treatment (%)	Decay Incidence %	Lesion size (mm)
8841	$TBZ^{R}Flu^{S}Pyr^{HR}$	Nontreated	95 a	28.4 a
		Pyrimethanil	93.3 a	26.8 a
		Fludioxonil	0 b	0 b
8818	$TBZ^{R}Flu^{S}Pyr^{MR}$	Nontreated	98.3 a	32.43a
		Pyrimethanil	92.5 b	26.7 a
		Fludioxonil	0 c	0 b
8873	$TBZ^{R}Flu^{S}Pyr^{LR}$	Nontreated	95 a	29.2 a
		Pyrimethanil	68 b	24.6 a
		Fludioxonil	0 c	0 b
8391	$TBZ^{R}Flu^{S}Pyr^{S}$	Nontreated	98 a	31.1 a
		Pyrimethanil	0 b	0 b
		Fludioxonil	0 b	0 b
8692	$TBZ^{S}Flu^{S}Pyr^{S}$	Nontreated	93 a	31.1 a
		Pyrimethanil	0 b	0 b
		Fludioxonil	0 b	0 b

TBZ=thiabendazole, Flu=fludioxonil, Pyr=pyrimethanil, R=resistant, S=sensitive, HR=high resistance, MR=moderate resistance, LR=low resistance

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 4. 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

Biological characteristics of Pristine-resistant strains of B. cinerea

Resistance stability and competitive ability of pyraclostrobin resistance and boscalid resistance in *B. cinerea* were studied. The results have been presented in the 2010 progress report. In summary, our results indicated that boscalid resistance and pyraclostrobin resistance in *B. cinerea* were stable. However, boscalid-resistant and pyraclostrobin-resistant strains had disadvantages in competing with fungicide-sensitive strains of *B. cinerea*, suggest that if the use of these fungicides is discontinued in the orchard, frequency of resistant populations will likely decline.

Control of gray mold caused by Pristine-resistant Botrytis cinerea

Field experiments were conducted on Fuji crops in 2010 and 2011. Fungicide treatments were applied one week or two weeks (for ziram-containing treatments) before harvest. The fruit were inoculated with Pristine-sensitive or Pristine-resistant strains of *B. cinerea*.

On 2010 crops, 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.

On 2011 crops, Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone (Table 6). For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate. New fungicide Merivon (not yet registered) also was effective for control of gray mold.

Table 5. Efficacy of preharvest fungicide programs for control of Pristine-resistant strains of *Botrytis cinerea* on apple fruit in 2010-2011

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 ($P = 0.05$).

Table 6. Efficacy of preharvest fungicide programs for control of Pristine-resistant strains of *Botrytis cinerea* on apple fruit in 2011-2012

Treatment	Incidence of gray mold (%)	
	Pristine-sensitive strain	Pristine-resistant strain
Control: No fungicide	100 a	100 a
Ziram 6 lb	75 b	61.25 ef
Merivon 4.0 oz + Ziram	23.75 e	52.5 f
Pristine 14.5 oz + Ziram	13.75 e	72.5 def
Merivon 4.0 oz	68.75 bc	88.75 bcd
Pristine 14.5 oz	56.25 cd	97.5 ab
Topsin 16 oz	66.25 bc	77.5 de
Merivon 4.0 oz+Topsin	48.75 d	61.25 ef
Pristine+Topsin	23.75 e	83.75 cd
Merivon 5.5 oz	58.75 cd	91.25 bc

Values with the same letter in the same column are not significantly different based on the Waller-Duncan test (P = 0.05). Merivon has not yet been registered for use on apple.

Sensitivity of P. expansum to Pristine

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

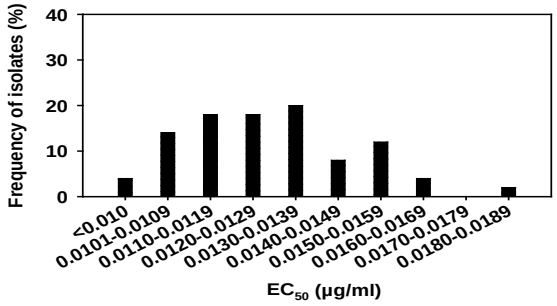

Fig. 1. Distribution of sensitivity of *Penicillium expansum* to Pristine.

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 7). 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 7. 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

Experiments were conducted in an organic Fuji orchard near Quincy in both 2010-11 and 2011-12 seasons. 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 8).

Table 8. Efficacy of preharvest applications of Serenade and Sonata for control of postharvest fruits rots on organic Fuji apples in 2010-11 and 2011-12 seasons

Treatment	**Rots (%) in 2010-2011 season**	**Rots (%) in 2011-2012 season**
Nontreated	11.04 a	4.48 a
Serenade MAX at 10 and 1 day before harvest	6.88 a	4.38 a
Sonata at 10 and 1 day before harvest	6.98 a	4.17 a

Executive Summary

This report is a summary of a two-year project conducted in 2010 and 2011. Part of the research was completed in 2012 because of the postharvest nature of the project. The objectives of the project were to monitor, characterize, and manage fungicide resistance in *Penicillium expansum* and *Botrytis cinerea*, two major postharvest pathogens of apples. The goal was to develop sustainable postharvest decay control programs.

Blue mold caused by *P. expansum* and gray mold caused by *B. cinerea* are major postharvest diseases of apples. In the present project, we monitored pyrimethanil resistance and fludioxonil resistance in *P. expansum*. Resistance to pyrimethanil (Penbotec) has developed in *P. 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, the frequency of pyrimethanil resistant strains was reduced from 7% in 2010 to 1% in 2011. In the other three packinghouses, neither Penbotec nor Scholar had been widely used before 2010. No pyrimethanil resistance was detected in two of the three packinghouses, and 1.8% of the isolates from one packinghouse were resistant to pyrimethanil. 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.

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

Pristine and Topsin mixture provided better control for Pristine-sensitive strain than Pristine or Topsin alone. For Pristine-resistant isolate, Pristine+Topsin mixture and Topsin alone provided better control than Pristine alone. Pristine+Ziram mixture was more effective than Pristine alone or Ziram alone for control of Pristine-sensitive isolate, but was less effective for control of Pristine-resistant isolate than for control of Pristine-sensitive isolate.

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

All Pristine-resistant isolates that were sensitive to TBZ were insensitive to DPA. However, Pristine-resistant isolates that were also resistant to TBZ became sensitive to DPA. The results indicated that Pristine resistance does not alter the sensitivity of the isolates to DPA but there is a negative cross resistance between TBZ and DPA. 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.

FINAL PROJECT REPORT **YEAR**: originally 2008-2009, (2011-2012)
WTFRC Project Number:

Project Title: Honey bee colony health

PI:	Walter S. Sheppard	**Co-PI(2):**	Richard S. Zack
Organization:	Wash. St. University	**Organization:**	Wash. St. University
Telephone/email:	509-335- 5180	**Telephone/email:**	509-335-3394
Address:	Dept. of Entomology	**Address:**	Dept. of Entomology
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:** Mary Lou Bricker; Adam Williams		
Telephone: 509-335-5180	**Email:** mdesros@wsu.edu; niehoff@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 the final $3635.75 was expended in 2012.

Recap original Objectives

The Apiculture program requested funds from the WTFRC 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 now submit colony samples to the WSU Honey Bee Diagnostic Laboratory for examination. Collection methods and details of shipment are available on the WSU Entomology Website. (http://entomology.wsu.edu/apis/diagnostic-lab/). Diagnostic results are usually 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 WSU 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 average 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 selection 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. In 2011 and 2012, aliquots of all semen samples were also cryopreserved in liquid nitrogen and deposited in the WSU germplasm repository.

Executive Summary

The funds requested from WTFRC in this proposal were part of a multi-source request to assemble funds to establish a honey bee diagnostic service at WSU, following major colony losses faced by Washington Beekeepers due to "Colony Collapse Disorder". The WSU Honey Bee diagnostic laboratory became operational in 2008 and has continued to provide diagnostic services for Washington beekeepers, funded with donations from individual beekeepers, local beekeeping organizations and some external grant funds (WSDA, WTFRC, USDA-NIFA Honey Bee CAP grant to WSS). Beekeepers from throughout Washington State continue to submit bee samples from their operations to be screened for 3 major honey bee parasites and pathogens: Varroa and tracheal mites and Nosema (a microsporidian pathogen). From a high of about 2500 sample submissions in 2009, sample submissions in 2011 and 2012 were 1540 and 800, respectively.

Significant colony losses for Washington beekeepers have continued since 2008, with current annual losses estimated to be around 30%. After 4 years of targeted research on Colony Collapse Disorder by a number of research groups nationwide, no single cause for CCD losses has been found. However, a number of potentially interacting factors have been reported to contribute to CCD, including sub-lethal pesticide exposure, nutritional limitations associated with placement on large monocultures, mite-virus interactions, moving "stress" in migratory operations, pathogen transmission in large "holding yards", microsporidian infections and others. Based on research at WSU, the interaction between sub lethal pesticide exposure and likelihood of infection with Nosema has been demonstrated.

The primary issue related to honey bee colony health continues to be the deleterious effects of parasitism with the Varroa mite. Current registered products for mite control available to commercial operations (fluvalinate, coumaphos) are no longer effective due to mite resistance. Alternative registered treatments (formic acid, hopguard) are less effective in commercial operations. The primary Varroa mite control for many commercial beekeepers is off-label use of Amitraz. Presently, a section 18 request for an Amitraz product in strip form is being considered for Washington.

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

FINAL PROJECT REPORT

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

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Cooperators: Dr. James McMurtry, UC Riverside, Emertus

Other funding sources

Agency Name: WTRC Technology Subcommittee
Amount awarded: $19,000
Notes: For development of application of lacewing eggs in a foam carrier

Budget History: Total $237,530

Item	Year 1:	Year 2:	Year 3:
Salaries	$51,244	$52,125	$53,041
Benefits	$10,621	$10,695	$10,772
Wages	$8,580	$8,923	$9,280
Benefits	$172	$178	$185
Equipment	$0	$0	$0
Supplies	$3,500	$3,500	$3,750
Travel	$3,000	$2,000	$1,500
Plot Fees	$2,000	$2,000	$0
Miscellaneous	$0	$0	$0
Total	$79,117	$79,649	$78,764

OBJECTIVES

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

   Managers of organic production for Zirkle Fruit Co. and Stemilt Growers Inc. were interviewed both in person and by phone to discover common practices their growers used to release predatory beetles, lacewings, and mites. Additional interviews with organic managers and onsite visits to ranches were conducted. Presentations made to interactions with attendees of the Wilbur Ellis organic growers meeting also identified common practices. These interactions showed high variability in practices used by growers. Use of predator release ranged from growers producing their own predator mites and making releases (ZIrkle) to releases of lady beetles when available. Four dominant vendors of lacewings, mites, and beetles were also interviewed by phone. They as well as Dr. Lynn LeBeck, representative of the National Association of Biological Control Producers, provided useful insights into availability issues. One specific problem was great variability in the availability of the Converse ladybeetle because it is a captive of the weather. Growers need it in spring but in some years with high snow pack in California, it cannot be collected. We dropped research on ladybeetles for this reason and general concerns of spread of disease into Washington from beetles in California. This objective is not discussed further.

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

   We found that we can tell Chrysoperla rufilabrus, the lacewing we were releasing, from native lacewings in both larval and adults stages. The convergent ladybeetle requires marking prior to release in order to differentiate them from the local beetles of the same species. Because we dropped work on the beetle, marking studies were not pursued. However, the discovery of two species of Galendromus predator mites in orchards, together with a diversity of other predators mites, became a major part of our research efforts in 2011-12.

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

   Releases of predator spider mites were conducted in all three years of the project and results are presented in more detail below. Releases of lacewing eggs were tested in 2010 and 2011 but persistent problems in persistence of eggs on trees lead to redirecting the studies to methods of application. See results and discussion..

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.

   Experimental sprays of lacewing eggs were conducted in experimental settings only to test organic adhesives. Galendromus occidentalis were released in a conventional orchard to test methods of evaluating efficacy of releases. Ladybeetles were not released in the field and feeding attractants were not tested. Significant efforts were devoted to the development of a liquid formulation as a carrier and adhesive for the application of lacewing eggs. Hatch rate

studies have continued and the use of foam as a carrier for egg application has been a focus and has been supplemented by funds from WTFRC Technology Subcommittee.

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

   Feeding capacity of purchased Chrysoperla rufilabrus was compared to native Chrysopa nigricornis using both Rosy apple aphid and Woolly apple aphid prey. The feeding capacity at different temperatures were compared in 2011 as this information may be critical for relating efficacy to release numbers in early season releases.

SIGNIFICANT FINDINGS

- ✓ Growers manually apply lacewing eggs glued to on paper pieces, a labor-intensive approach.
- ✓ Only Beneficial Insectary Inc. produces *C. rufilabrus,* all others are resellers.
- ✓ Hibernating ladybeetles collected in spring or fall and cold-stored vary greatly in quality and may be unavailable in early spring.
- ✓ Pesticide residues prevented predator mite establishment in field studies.
- ✓ Honeydew and waxes produced by Woolly apple aphid kill many small green lacewing larvae
- ✓ Purchased *C. rufilabrus* shows feeding capacity similar to native *C. nigricornis*.
- ✓ *C. rufilabrus* will hatch at temperatures corresponding to late March.
- ✓ Moe than 50% of lacewing eggs sprayed onto trees in a liquid carriers are lost on impact
- ✓ Large release experiments of both lacewing eggs (2011) and mites (2010-2012) showed no increase in predators and in the case of lacewing eggs, no released *C. rufilabris* were recovered.
- ✓ The dominant predator mite found in 5 orchards was *Amblydromella caudiglans* – a big surprise.
- ✓ We conclude there is little evidence supporting release of predator mites in apples and lacewing releases still need technological improvement to apply the eggs.

RESULTS & DISCUSSION

Objective 1 - Grower practices and needs

Organic managers for Zirkle and Stemilt outlined standard practices on the ranches they manage. Lacewings were released as eggs glued to strips of paper, which are hung in the canopy by workers on trailers. Mites mixed with corncob grit were dispersed into trees with a pollen blower or placed into the crotch of trees on infested bean plants. Ladybeetles were released in paper bags or boxes placed in orchard typically at night and after orchard irrigation. Both companies agreed there was a need to improve release methods and to evaluate efficacy of the releases.

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

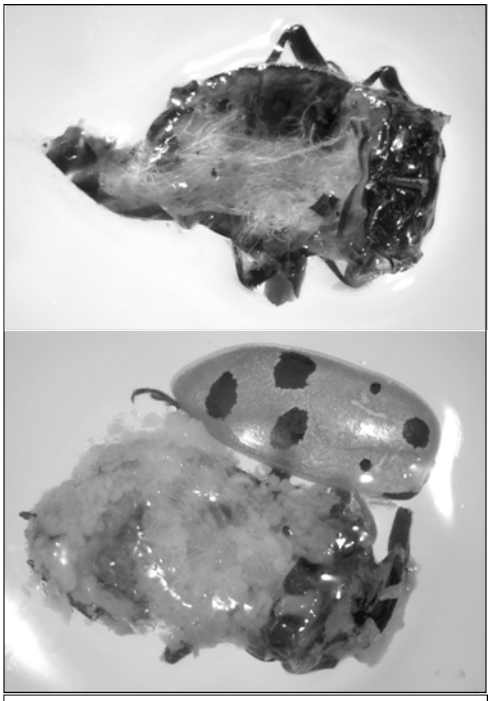

Figure 1. Convergent ladybeetle stored for 10 months (top) and for 2 months (bottom). Fat body completely obscures the strings of tracheae in beetle on bottom.

Objective 2. Differentiating species

Lacewings **-** Early in year 1 we found we could morphologically differentiate with some difficulty released *Chrysoperla rufilabrus* from endemic *C. plorabunda* as both adults and larvae and we could easily identify the abundant *Chrysopa nigricornis.*

Ladybeetles **-** Our plans to use protein marking to differentiate local *Hippodamia convergens* from released individuals were dropped when we found this species to be of unreliable quality in year 1 and unavailable in year 2. (See Objective 1)

Predator mites **-** We were able to identify phytoseiid females to species by slide-mounting mites in modified Berlese medium. Samples of 100 leaves were collected from different orchards or native plants throughout eastern Washington. These samples include apple, cherry, and wild blackberry leaves. Samples were taken from mid-June to early September. All phytoseiid mites were removed individually from the leaves using a paintbrush. The date, location, GPS coordinates, prey species available, and crop or plant species of the sample site were recorded. Identifications are in the process of being confirmed by Prof. James McMurtry (U.C. Riverside, emeritus).

Seven species of phytoseiids have been identified from surveyed locations to date: *Amblydromella caudiglans, Amblyseius andersoni, Euseius finlandicus, Galendromus flumenis, Galendromus occidentalis, Typhlodromina citri,* and *Typhlodromus pyri*. The majority of the individuals found were *G. occidentalis*, but *A. caudiglans* was

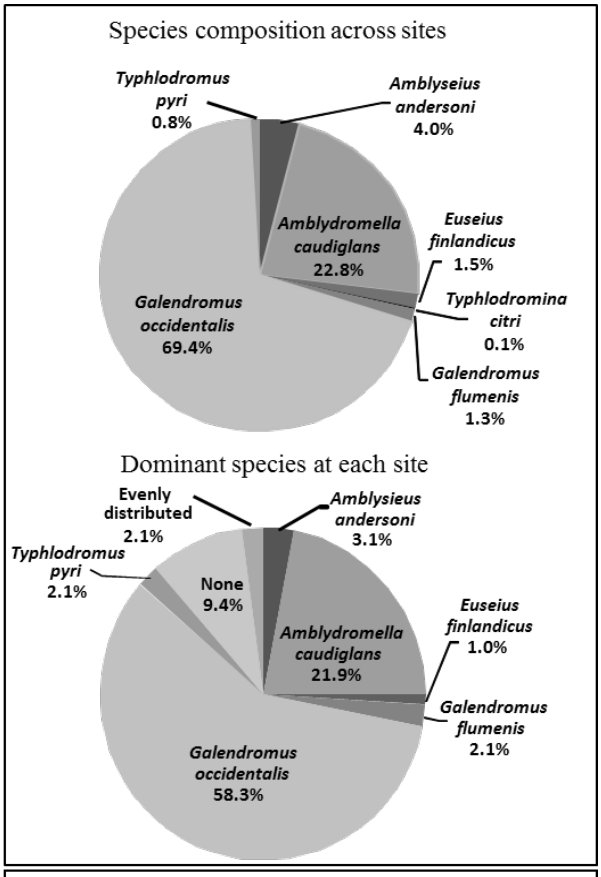

Figure 2. Phytoseiid species composition and dominance.

also present in significant numbers (Figure 2, top panel). Although *G. occidentalis* was also the dominant predator in the majority of sites, *A. caudiglans* was dominant at over 20% of the sites surveyed (Figure 2, bottom panel). The frequent occurrence of *A. caudiglans* was unexpected based on prior assumptions. However, it is possible that a shift in insect control programs, especially those for codling moth, could have resulted in the partial or complete replacement of *G. occidentalis* by *A. caudiglans*. *Galendromus occidentalis* has historically been shown to be highly resistant to pesticides (especially organophosphates or OPs) compared to other phytoseiid species. Implementation of softer programs could provide the impetus for the change in phytoseiid species composition. Additionally, European red mite (*Panonychus ulmi*) has replaced the McDaniel mite (*Tetranychus mcdanieli*) as the common outbreak pest-mite species in Washington apple orchards. While spider mites that spin copious webbing, like the McDaniel mite, are the preferred prey of *G. occidentalis*, *A. caudiglans* has difficulty moving through webbing and prefers spider mites such as *P. ulmi* that produce little webbing. Therefore, the transition to a new predominant pest mite species may have facilitated the increase in *A. caudiglans*.

Further research on *A. caudiglans* will facilitate the understanding of the role of this predator in our integrated mite management (IMM) programs. More selective pesticide use may promote the conservation of *A. caudiglans* as well as *G. occidentalis* reduce pest mite outbreaks. As a more generalized predator, *A. caudiglans* may be more efficient than *G. occidentalis* at maintaining higher densities because of its more omnivorous diet and thereby more reliably suppress pest mite populations, especially *P. ulmi*, below outbreak levels.

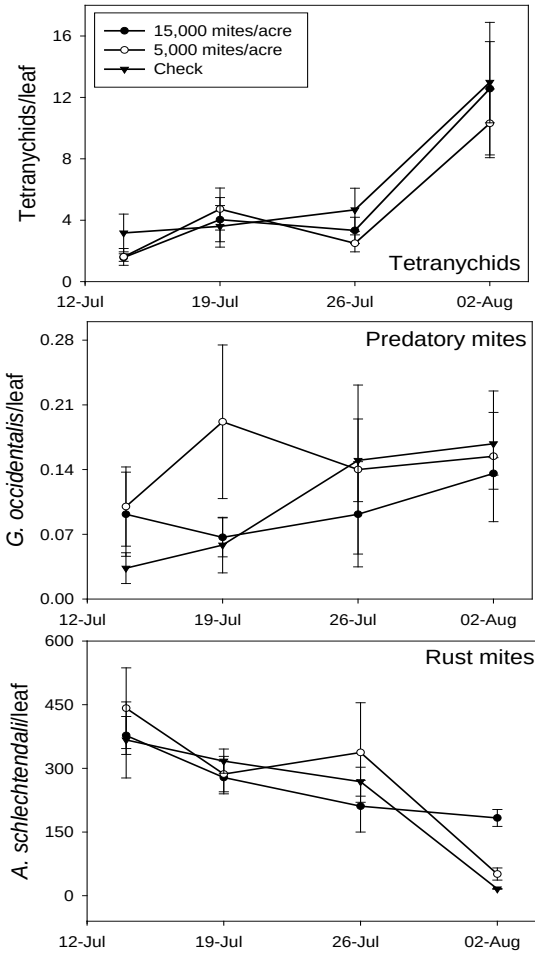

Figure 3. Mite densities in the 2010 releases.

Objective 3/4. **Releases in grower orchards**

Predator mites – <u>*Methods:*</u> In years 1 and 2, Western predatory mites, *Galendromus occidentalis* (Typhs) from the Sterling Insectary insectary were released in a mature blocks of 'Red Delicious' apples at a commercial orchard near Pasco, WA. Six plots of 9 trees per treatment. In year 1, predators were deployed onto the central release trees on 14 July at rates of 0, 5,000, and 15,000 mites/acre (= 0, 12, and 36 mites/tree). Adult female *G. occidentalis* were placed onto a bean leaf, and attached to the tree with a binder clip.

Higher release rates of 0, 15,000 and 50,000 predators/acre were used in year 2. This was done by placing an appropriate fraction of the leaf material from the insectary on each tree (0, 1, and 2.5 plant stems, respectively).

In year 3, mites were released in a mature block of mixed 'Red Delicious' and 'Golden Delicious' near Mattawa, WA. In this case, the effects of early (pest threshold of ~0.5 tetranychids/leaf) and late (three weeks later) releases were tested by releasing at a rate of 15,000 predators/acre or no release.

In year 1, the abundance of predator and prey mites were assessed first by visual counts in the field using OptiVisors and consisting of 15 leaves per tree without detaching the leaves. Only motile stages

of *Tetranychus urticae, Panonychus ulmi,* and *G. occidentalis* were counted. Second, we took five leaves from each of the four trees on the diagonal from the release tree standard and this 20-leaf sample was brushed onto sticky plates using a leaf-brushing machine. All stages of *T. urticae, P. ulmi, G. occidentalis, Aculus schlechtendali,* and *Zetzellia mali* were counted under a dissecting scope. On the final sampling date, *in situ* counts and leaf brush counts were made on the 9 sample trees, allowing us to compare the two sampling methods. In years 2 and 3, only brush counts were used to assess mite populations. Additionally, in these years, releases were performed on all trees within a treatment and a random sample of 100 leaves was taken from each replicate.

Each year, leaves from the release blocks were bio-assayed to determine if pesticide residues on the leaves affected the survival, fecundity of the commercially reared *G. occidentalis*. Release orchard leaves were compared to those from an untreated research orchard at WSU-TFREC. Leaf disks (2 cm diam) free of arthropods were placed on water-saturated cotton in small cups. Twenty female *T. urticae* were added to each leaf disk and allowed to oviposit for 24 h. After a sufficient number of eggs had been laid on each disk to provide the predators with food for the duration of the experiment, the *T. urticae* females were removed. One female *G. occidentalis* was placed onto each leaf disk. The bioassay was evaluated at 24 and 48 h for female mortality and fecundity. *G. occidentalis* females were removed after 48 h, and the position of each egg was marked with a felt-tip pen. On the fourth day after female removal, the number and status of eggs and larvae of *G. occidentalis* were counted.

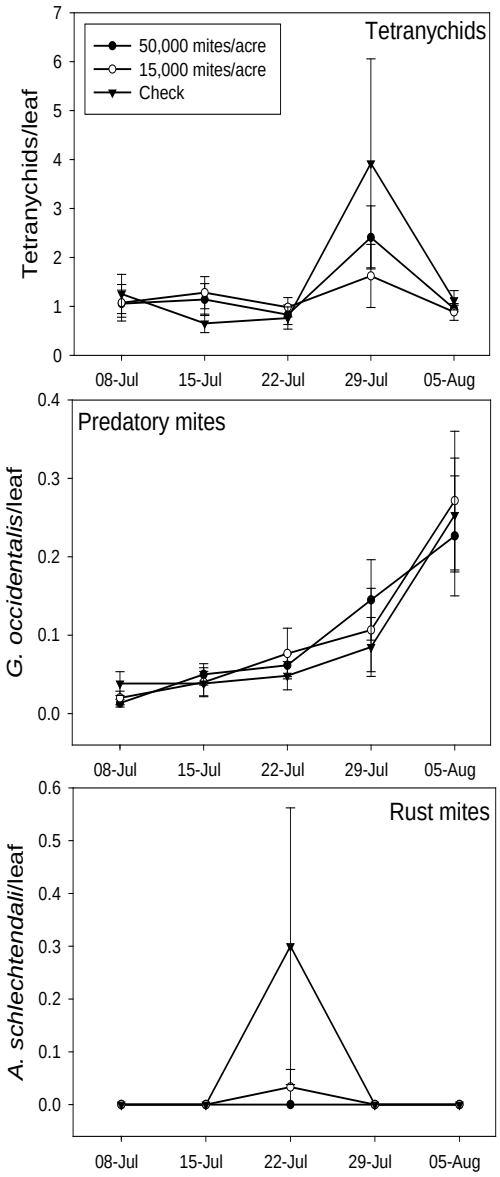

Figure 4. Mite densities in the 2011 releases.

<u>*Results:*</u> In year 1, counts of spider and rust mite were low at the time of predator release in mid-July. *Panonychus ulmi* was the dominant phytophagous mite species and it increased during the test despite an application of Zeal on May 24. Rust mite populations were moderate initially, but declined during July. There were no statistical differences between treatment means for any mite species or group on any date (Figure 3, above). These findings provide no evidence that the released predators became established or had any effect on pest mites. Similar results were found for years 2 and 3.

In year 2, *P. ulmi* was the dominant phytophagous mite species. Rust mite populations remained low throughout the sampling period. *Galendromus occidentalis* populations increased throughout the sampling period, but there were no differences in predator density between the three treatments (Figure4). There were also no differences in prey mite densities between the three treatments.

In year 3, *Tetranychus urticae* was the dominant phytophagous mite species. All phytophagous mite populations were low prior to releases. There were no differences between treatments in any of the

mite species sampled (Figure 5). A comparison could not be made between the early and late releases because sprays of Epi-Mek, Envidor, and oil were used to control the rising phytophagous mite populations. No post-release sample was collected for the later release. The *in situ* optiVisor mite counts (not shown) had consistently lower numbers than the leaf brush counts.

In situ P. ulmi counts had the best correlation with leaf brush counts, likely because their red color differentiated them from the leaf color. We had hoped the non-destructive *in-situ* samples would work but our inability to count all stages of *G. occidentalis* precludes this method. Because of these results, *in situ* counts were not used to monitor mite populations in years 2 and 3. In year 1, leaves from the sprayed grower block and those from the untreated block were similar in effects on mortality and fecundity of the insectary-reared *G. occidentalis* (not shown). However, there was significantly poorer egg hatch and numbers of live larvae on the leaves from the release plots, indicating some residues present on the leaves were sublethally toxic to the predators (Figure 6). Of the materials applied to the release block both carbaryl and thiacloprid are known to have some level of toxicity to predators, although it seems unlikely that the toxic effect could have persisted for several months. The effects of other materials applied (emamectin benzoate, etoxazole, trifloxystrobin, and *Bacillus thuringiensis*) are not known. In years 2 and 3, bioassays of the release site leaves did not negatively affect *G. occidentalis* mortality, fecundity, egg hatch, or larval survival (not shown).In all three years, releases of predatory mites failed to increase predator populations or decrease phytophagous mite populations in release areas. Pesticide applications toxic to *G. occidentalis* are attributed to the lack of success in year 1. However, in years 2 and 3, the leaves did not have toxic residues, thus other factors must be responsible. Another possibility for the lack of success is the predator:prey ratio established by releases. Insectary recommendations suggested 5,000 mites per acre early in the season and 15,000 mites per acre to control outbreaks. However, even at 50,000 mites per acre, we failed to see predator or pest population effects.

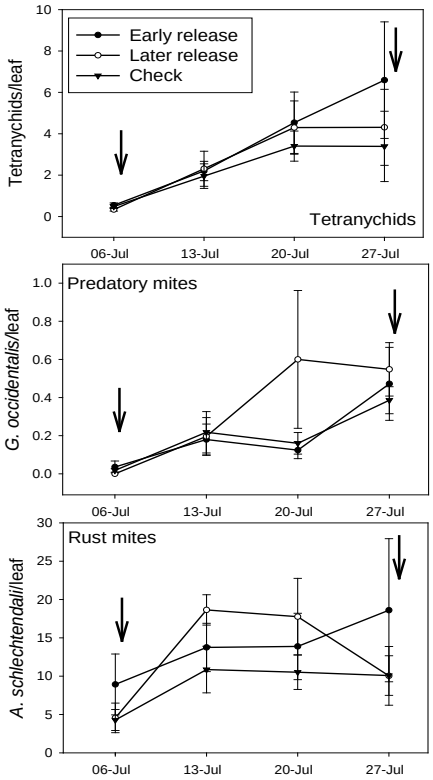

Figure 5. Mite densities in the 2012 releases; arrows indicate release dates

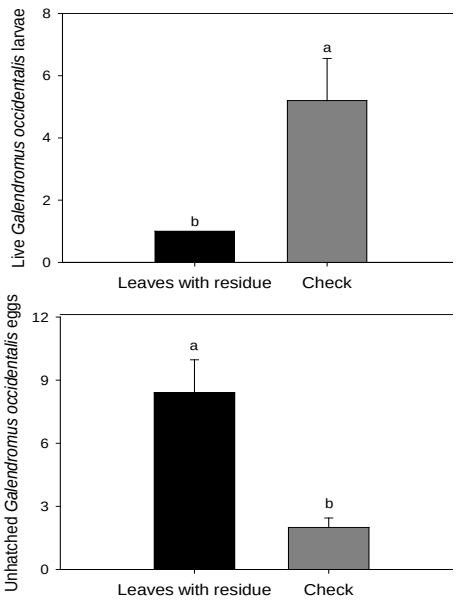

Figure 6. Effects of leaf residues on *G. occidentalis*, 2010.

Calculations were performed to determine the theoretical release rate needed to control a *P. ulmi* population of 3 mites per leaf. This value was chosen because at these levels, mite populations are noticeable but not damaging. Latham and Mills (2010) developed a method for calculating predator:prey ratios by using the following model: $N_{t+1} = N_t e^{rt} + gP/r(1 - e^{rt})$, where N_t and N_{t+1} represent prey population sizes at consecutive sampling dates, r is the growth rate of the prey, g is the

daily per capita consumption capacity of the predator and P is the predator density. If N_t is assumed to be zero, this can be rearranged to $P=rN_{t+1}/g$. When N_t is given the value of 3 (mites per leaf), P can be calculated using known values of g (1.97, Lee and Davis 1968) and r. A value for r (0.122) was calculated using life table information for *P. ulmi* provided by Herbert (1981) and the PopTools application for Excel. This gives P=0.186 or a ratio of 1 predator to 16.1 prey per leaf. When a conservative estimate of leaves per tree and trees per acre was obtained using information taken from Wunsche and Palmer (1997) and Ferree and Barden (1971), this ratio requires nearly 400,000 predators per acre to control 3 *P. ulmi* per leaf, at an estimated cost of $6,000 per acre. These calculations indicate that apple canopy volume is sufficiently large to make predator releases economically unfeasible. The success of releases in other cropping systems, such as strawberry, is likely in part due to much smaller leaf canopy volume.

The results of our releases, as well as our predator:prey ratio calculations, indicate that inundative releases of predatory mites are not a cost-effective solution to controlling pest mite populations in apples. An inoculative release may help speed re-establishment of a decimated predator population, but it should be combined with a long-term strategy of predator conservation through selective pesticide use. Non-target effects of new pesticides should be evaluated for effects on pest and predatory mites. This is especially relevant in light of our discovery of large populations of *A. caudiglans* in Washington apple orchards. If current IMM programs can be adapted to conserve all common mite predators, we may see better control of pest mite species in the future.

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

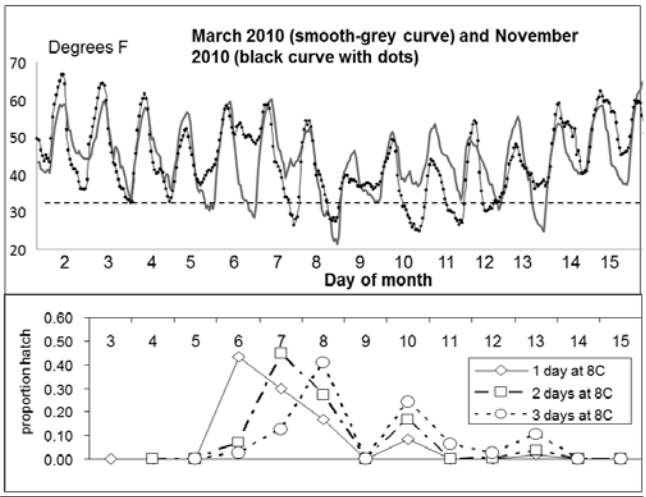

Figure 7. Pattern of lacewing egg hatch 1, 2, or 3 days after cold adaptation in Percival (46.4 F) outside in November temperatures

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.

Guar gum (0.04% was used as a sticker because it is organically approved for application in the field. ***Results:*** No *C. rufilabris* were detected in either study on any date indicating the need for a better sticker method and sent us back to the drawing board. These results were a surprise because we did have better evidence of egg adhesion in small studies at the Moxee Farm. From this failure, we moved to studies of the use of foam solutions to assist egg retention to trees. We have developed foaming agent that do cause eggs to adhere to foliage and tree bark. A significant portion of studies developing this foam was supported by additional funding from the WTFRC Technology subcommittee. Results from these efforts will be present orally.

Objective 5 – Feeding studies of insectary and native predators

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

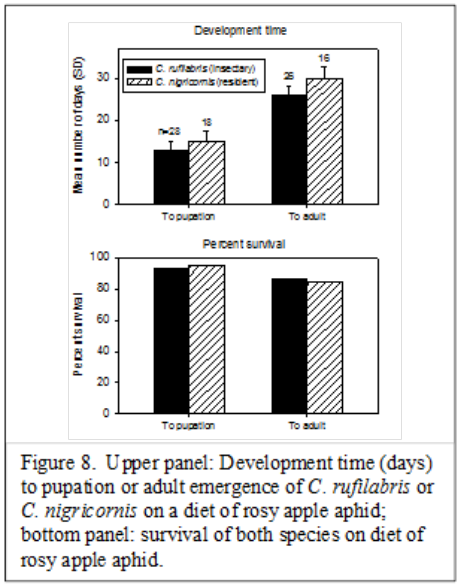

Figure 8. Upper panel: Development time (days) to pupation or adult emergence of *C. rufilabris* or *C. nigricornis* on a diet of rosy apple aphid; bottom panel: survival of both species on diet of rosy apple aphid.

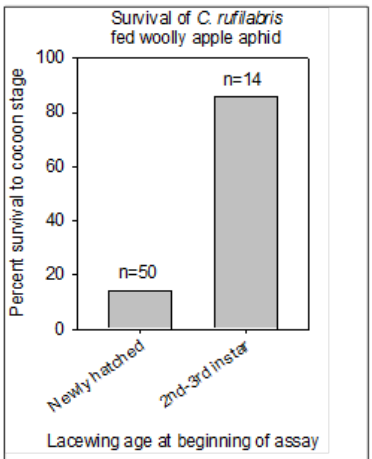

Figure 9. Survival of newly hatched or later instar *C. rufilabris* on diet of woolly apple aphid.

Woolly apple aphid. Our second study explored survival of the insectary-reared lacewing (*C. rufilabris*) on a diet of field-collected woolly apple aphid. In this study, we also explored how age of lacewing larvae affected survival, due to early observations suggesting large differences in success of small and large larvae on a diet of this aphid (see below). Methods were similar to those used in the trial with rosy apple aphid, except that *C. nigricornis* was not included for comparison (we could not find *C. nigricornis* eggs in the field). *Results*: We found that

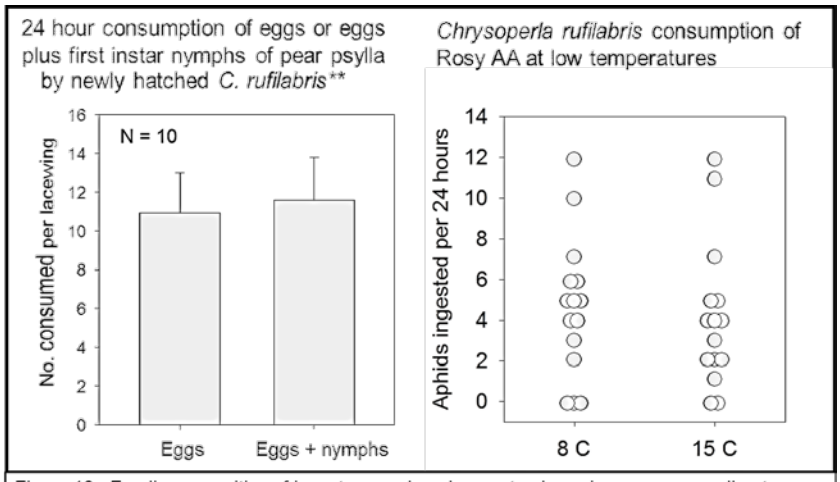

Figure 10. Feeding capacities of insectary produced neonates lacewings on pear psylla at room temperature and Rosy apple aphid at 8C (46.4 F) and 12 C (59 F)

newly hatched lacewings survived very poorly on a diet of woolly apple aphid (Figure 9), unlike what occurred in the previous study on a diet of rosy apple aphid (Figure 9 lower panel).

We discovered that mouthparts of newly hatched lacewings regularly became stuck in the aphid's waxy honeydew as the lacewing attempted to feed (not shown); over 80% of observed mortality was attributed to this honeydew factor. Conversely, large lacewing larvae (2^{nd} and 3^{rd} instars) were considerably more successful than newly hatched larvae, and showed excellent survival. Consumption rates of large larvae reached almost 25 aphids per day. These results suggest that releases of eggs or newly hatched larvae of lacewings may not be successful against woolly apple aphid, unless an alternative prey for hatchlings are also present in the trees.

Chrysopa rufilabris fed readily on Rosy apple aphid in reduced temperatures corresponding to conditions that would be experienced under typical early spring field releases (Figure 10). Neonates readily consumed pear psylla, which is of interest to Beneficial Insectary as a potential expansion of the market (Figure 10). Our conclusions from these studies are that *C rufilabris* is well fitted for release in both early spring and potential in late fall.

EXECUTIVE SUMMARY

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

Participants: Tom Unruh and Dave Horton USDA-ARS, Elizabeth Beers, WSU

OVERVIEW

The objectives of this research were to determine the needs of the grower community for better approaches to augmentative releases of beneficial insects for pest control. We found that almost 60% of organic growers use these practices and most of those that do would like to know if they are of value and if the process can be improved. Our conclusions from the studies described are that mite and ladybeetle releases are probably unwarranted. However, application of lacewing eggs appears to be a technological problem that may be close to solution. Also, during our studies of the mite predators in our orchards we encountered a much higher diversity of predator mite species that my underpin and much more stable form of biological control of spider mites in apple, warranting significantly more research

Species studied: We evaluated the three naturally enemy groups growers commonly released: the convergent ladybeetle, *Hippodamia convergens*, the green lacewing, *Chrysoperla rufilabris*, and the predator mite, *Galendromus occidentalis*. After the first year of studies, we dropped efforts on releases of the ladybeetle because of high variability of quality and unreliable availability of this species because it is harvested from the wild and is not always available or is in poor condition. In contrast, the predator mite and the lacewing are reared in insectaries.

Release studies: The predator mite predator, *G. occidentalis,* was experimentally released each year, but these experiments provided no evidence for the value of the releases. Two reasons were identified for this: first, the presence of pesticide residues prevented mite development in year 1; second, high abundance of predators in test orchards in years 2 and 3 caused releases to be of no value. These studies support one conclusion in the mite studies that there are no justifications for predator mite augmentation in the summer when spider mites can become abundant. However, we do not eliminate the possible value of early season inoculations of predator mites in orchards with chronic problems and free of insecticides. Releases of lacewings similarly provided no success but one reason alone seems responsible for this failure. Lacewing eggs applied in water solutions con do not readily stick on trees, with more that 60% loss on contact and additional loss over the day or two prior to hatch. However, due to additional funding by the WTFRC Technology Subcommittee this issue may be resolved in the near future.

Positive Discoveries: Washington apple orchards support a larger diversity of predatory mites that previously know and the second most abundant species, *Galendromus caudiglans,* is a more generalist species than is *G. occidentalis*. Its omnivorous eating habits (pollen and molds) make it more likely to persist at higher densities in the absence of a large number mite prey. There is a suggestion that they may be a superior predator in soft pesticide programs.

Studies of the lacewing, *C. rufilabris* show it to be capable of hatching very early in season (March temperatures) prior to bud break and before Rosy or Green apple aphids hatch; this augers well for early season releases (or autumn release). Similarly, laboratory studies show that the lacewing can consume aphids in relatively low temperatures that occur in that season. Finally, ongoing studies using foam for application of the lacewing eggs on tree trunks are highly promising.

Conclusion: We believe that studies of the predator mite complex in apple can provide useful insights into a biological control oriented management system. This is especially true with new, more selective approaches being used for key pests. Early and late season releases of releases of lacewing eggs still remains a potential approach to improving spring aphid control, particularly in organic production.

FINAL PROJECT REPORT
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	**Co-PI (2):**	Amit Dhingra
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Other funding sources
None

Total Project Funding: $60,098

Budget History:

Item	Year 1:	Year 2:
Salaries	12,766.80	3,100
Benefits	1,688.51	949
Wages		
Benefits		
Equipment		
Supplies	13,593.69	16,000
Travel		
Plot Fees		
Miscellaneous 454 Sequencing	12,000	
Total	40,049	20,049

ORIGINAL OBJECTIVES

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

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

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)

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

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.

Year 2

- Transcriptome of RNA extracted from codling moth larval, pupal and adult heads was completed
- A codling moth neuropeptide F receptor has been cloned and sequenced
- Codling moth neuropeptide F receptor was cloned into a mammalian expression vector and is currently being selected for expression in a mammalian cell line
- Expression of codling moth neuropeptide F has been detected in RNA extracted from adult male and female antennae

RESULTS AND DISCUSSION

Hormones are an organism's chemical messengers and a specific class, peptides (compounds consisting of two or more amino acid residues, the building blocks of proteins), regulate most every physiological function. Neuropeptides are peptides produced by cells in the brain and are released into the hemolymph (insect blood), sending signals to different tissues in the body. Because the hemolymph bathes virtually every cell in the insect body, circulating neuropeptides have the potential to come into contact with all tissues. Specific receptor sites form the connection between a circulating neuropeptide and particular target cell. When the neuropeptide interacts (binds) with its specific cell surface receptor, a signal is initiated causing the cell to perform a specific function. They work slowly, over time, and affect many different processes, including growth and development, metabolism, sexual function, and reproduction. Neuropeptides and hormones in general, are

powerful. It takes only a tiny amount to cause big changes in cells or tissues which is why too much or too little activity can cause serious affects. Because of critical functions of insect neuropeptides, they have been of interest for their use in pest control. Our overall goal was to generate a transcriptome from codling moth brain tissue, which is a known source of cells that express neuropeptides and neuropeptide receptors.

Cloning codling moth neuropeptide F receptor

The Neuropeptide F (NPF) family of peptide hormones regulates feeding and digestion in insects. For Drosophila, disruption of the NPF receptor (NPFR) inhibits larval food seeking and feeding behaviors. We used oligonucleotide primers designed from conserved amino acid sequences of known NPFRs in PCR reactions to clone a fragment of the gene transcript encoding codling moth NPFR. The nucleotide sequence of the NPFR fragment was determined and we then designed specific oligonucleotide primers that were used in PCR reactions to obtain the full length codling moth NPFR. The NPFR protein encoding region of the gene transcript is 1383 nucleotides in length and encodes a protein of 461 amino acids. The cDNA encoding NPFR has been cloned into a mammalian expression vector and has been used to transfect Chinese hamster ovary cells to generate a cell line that will be used in upcoming functional assays. These cell lines will be used in the future assays to discover compounds that may have antagonistic effects on receptor function perhaps leading to new codling moth control agents.

Codling moth neuropeptide F receptor is expressed in male and female antennae

Recently, it has been determined that a NPFR is expressed in Drosophila antennae, and it has been proposed that this receptor may signal the insect to express odorant receptors that have a role in detecting host or food seeking odorant cues. Based on these findings, we wanted to determine if codling moth NPFR is expressed in male and female antennae. Using oligonucleotide primers specific for codling moth NPFR, we performed PCR reactions with cDNAs prepared from RNA extracted from male and female antennae. In figure 1, we show that PCR amplifies products of the size expected for the codling moth NPFR protein encoding portion of its gene transcript (~1400 nucleotides). To confirm that this PCR product contains the NPFR protein encoding region, we cloned the PCR product and through sequence determination found it to indeed be a transcript encoding the codling moth NPFR.

Transcriptome generation and analysis

The “genomics revolution” has provided powerful new tools and information that might be used to discover novel protein targets for insect control. Our goal is to generate a transcriptome from codling moth heads, the site of neuropeptide and peptide hormone synthesis, in an effort to identify potential targets for the development of new compounds for codling moth control. RNA was extracted from heads dissected from all codling moth life stages and converted to cDNA for sequencing. The sequencing is now complete and the information is currently being analyzed. Analysis of the codling moth sequence information is not complete as of January 4, 2013 but will be continued until finished. As soon as we compile all the data and analysis is completed, we will send an addendum to Kathy Coffey so that she can forward it out to the Commissioners and committee members.

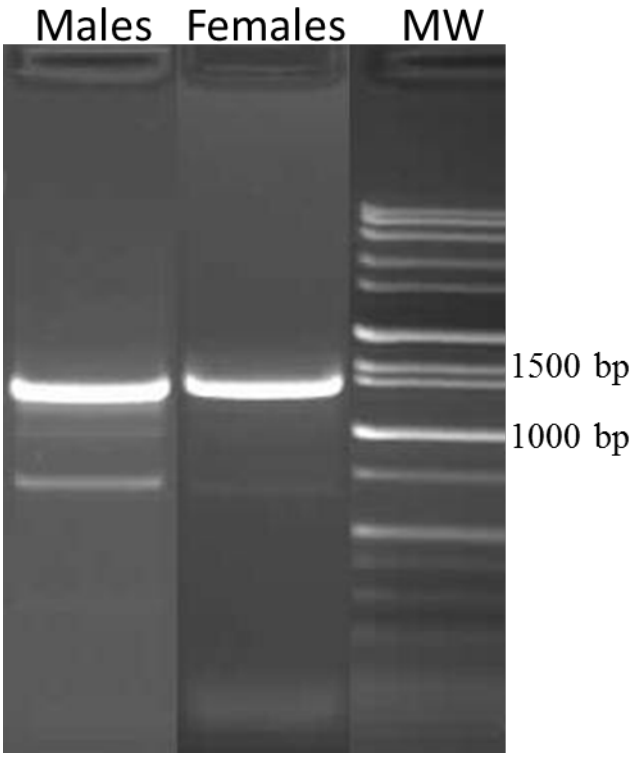

Figure 1. PCR amplification of codling moth NPFR from cDNA prepared from RNA extracted from male and female antennae. PCR was performed with primers specific for the codling moth NPFR protein encoding region of its gene transcript. The bright band at approximately 1500 base pairs in the male and female panels is the codling moth NPFR.

EXECUTIVE SUMMARY

Resistance to chemical insecticides used to control codling moth in the orchard is a major concern and is potentially costly to orchardists. Because many insecticides target proteins are produced by the brain and nervous system, the main goal of our project was 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) from heads of various larval and adult stages of codling moth. Analysis of the transcriptome will be useful in identifying new protein targets that may be important in developing novel classes of compounds with unique modes of action that can be used in future resistance management programs to control codling moth. A second goal of this project was to initiate studies to identify cDNA transcripts encoding neuropeptides and receptors involved in the regulation of feeding and reproduction of spotted winged Drosophila (SWD), an emerging pest of tree fruit in the Pacific Northwest. This will provide basic information that can be used by other researchers in attempts to control this insect pest.

For SWD, we cloned gene transcripts encoding sex peptide and its putative receptor, as well as transcripts that encode for neuropeptide F, short neuropeptide F, and their putative receptors. These targets were chosen based on their roles in regulating reproduction (sex peptide) and feeding (neuropeptide F and short neuropeptide F). These clones have been made freely available to the research community in efforts to further characterize the neuropeptide/receptor interactions with the hope of developing control agents that disrupt their physiological function.

A codling moth neuropeptide F receptor was also identified and cloned as part of this project. Neuropeptide F regulates feeding and digestion and is expressed in codling moth neonates. Future work will be to fully characterize the interactions of this receptor with its neuropeptide ligand in attempts to exploit this system as a potential target for control of codling moth neonates. Interestingly, we detected the neuropeptide F transcript expressed in codling moth adult male and female antennae. Because neuropeptide F is involved in regulation of feeding, it is hypothesized that activation of this receptor in antennae turns on expression of gene transcripts encoding for receptors involved in host plant seeking or feeding behaviors. We will be examining this hypothesis in future studies addressing the codling moth olfactory system.

A transcriptome has been generated from RNA extracted from codling moth heads dissected from larvae and adults. The transcriptome annotations are being completed and we will be analyzing this data as it becomes available. It is anticipated that we will have a plethora of new information regarding proteins produced in the codling moth brain. This information will be used in the future to identify novel targets that may be used in the development of new compounds that can be useful in insect control efforts.

FINAL PROJECT REPORT

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

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Cooperators: Bill Lingren, Trécé Inc., Adair, OK

Other Funding Sources: $22,000, Trécé Inc.

Total Project Funding: $52,500

Budget History:

Item	2011	2012
Salaries	19,255	19,450
Benefits	2,145	2,200
Wages		
Benefits		
Equipment		
Supplies	2,500	2,600
Travel	2,100	2,250
Plot Fees		
Miscellaneous		
Total	26,000	26,500

ORIGINAL OBJECTIVES

The overall objective of this two year project was to develop and assess the use of a combination lure to monitor both codling moth and leafrollers within a single trap. Studies were conducted with both Pandemis and oblique banded leafrollers in apple and pear. The first specific objective was to use a standard lure loaded with the sex pheromone of codling moth in combination with a host plant volatile and a second lure loaded with acetic acid. We tested a number of potential host plant volatile attractants for their relative contribution to the combination lure. The final specific objective was to assess the correlation of leafroller adult captures in traps baited with the most effective multi-species lure with local infestations of leafrollers.

SIGNIFICANT FINDINGS

- ✓ The addition of an AA lure (TRE3321) to the sex pheromone-pear ester combo lure-baited traps significantly increased codling moth catches, especially of female moths.

- ✓ A commercial acetic acid plastic cup lure, Pherocon AA, was developed by Trécé Inc. for use with the CM-DA Combo lure for codling moth as a result of this research.

- ✓ The optimal daily release rate of acetic acid from lures required to be effective for leafrollers was found to be higher than for codling moth. A second lure (TRE0421) was developed for eventual commercial use by growers to monitor both codling moth and leafrollers.

- ✓ Studies showed that the cardboard lure holder developed to hold both the CM-DA Combo lure and the acetic acid cup lure significantly reduced catches of codling moth and this device was discontinued by Trécé Inc. Instead, the acetic acid lure is placed horizontally on the sticky surface of the liner.

- ✓ Studies conducted with five host plant volatiles in addition to pear ester combined with codling moth's sex pheromone and used with an acetic acid lure found these lures all performed similarly in traps for Pandemis and oblique banded leafrollers. However, pear ester provided the highest catch of codling moth, especially of female moths.

- ✓ A new attractant (International patent pending) developed in New Zealand was found to be significantly more (2 to 7-fold) attractive than pear ester when used with acetic acid for both leafroller species and the eye-spotted bud moth.

- ✓ Field studies with both species of leafrollers found that the single trap baited with codling moth pheromone, pear ester, and acetic acid provided useful information about the presence of local infestations of leafrollers.

- ✓ Several factors were found to be of significant concern with the use of this monitoring approach.

    - o 'False negatives' where the trap fails to catch adult leafrollers and larvae were detected occurred in a few sites with the presence of overwintering larvae and no subsequent adult catches. This was likely due to the use of control tactics against the spring generation of leafroller larvae which eliminated the subsequent emergence of the summer generation adults in the orchard. No cases occurred where traps failed to catch moths and larvae from the subsequent generation were detected. The occurrence of 'false negatives' also appeared to have occurred in some pear blocks

where the eye-spotted bud moth was present and injured fruits were misclassified as oblique banded leafroller damage.

- 'False positives' where the trap catches leafroller adults but no larvae are found was more common and always occurred in blocks with adjoining cherry blocks. Due to the immigration potential of leafroller adults from cherry these catches are considered to be useful information for apple and pear growers to assess their risk. Growers need to sex moths to ascertain if females are moving into the orchard.

- In the great majority of orchards the use of the CM-DA Combo lure with acetic acid caught one or more leafroller adults when leafroller pressure was ranked as moderate to high (based on the presence of larvae or injury); and traps failed to catch any adult leafrollers when the pest pressure was rated low to nonexistent.

RESULTS & DISCUSSION

1. Benefit of Adding AA to traps with the CM Combo lure

The positive effect of adding an acetic acid lure to codling moth traps baited with a CM-DA Combo lure has been clearly shown in both conventional and sex pheromone-treated orchards. During 2011 we evaluated this effect in a collaborative project including 21 orchards with Dr. Diane Alston at Utah State University, Rick Hilton at Oregon State University, and several consultants in Washington (Table 1). Both the total number and number of female moths caught per trap was significantly higher with the addition of the acetic acid lure. The nearly 4-fold increase in female moth catches was of particular interest. A precision management program has been developed that uses action thresholds based on female and total moth catches. The development of a more sensitive monitoring tool for female moths could be a useful addition to this program. Further studies are required to determine if the current threshold of a single female moth should be increased with the adoption of this more powerful combination lure.

Table 1. Codling moth catches with the Pherocon CM-DA Combo lure with and without the addition of a Pherocon AA lure in 21 orchards in Washington, Oregon, and Utah, 2011.

	Mean (SE) moth catch per trap		
Lure	Male	Female	Total
Combo	18.1 (3.8)	3.0 (1.4)	21.1 (4.1)
Combo + AA	28.1 (7.3)	11.4 (3.8)	39.5 (8.7)
ANOVA	$F_{1, 40} = 1.70$ $P = 0.20$	$F_{1, 40} = 6.59$ $P < 0.0001$	$F_{1, 40} = 4.53$ $P < 0.0001$

2. Optimal AA loading for lure

The use of an acetic acid lure was developed during a four year project with Trécé Inc. to improve the CM-DA Combo lure for codling moth. Various trials were conducted to assess the optimal emission rate of acetic acid required to synergize pear ester. This work led to the Pherocon AA lure which has now been added to their commercial catalogue. However, our studies in 2011 found that the Pherocon AA lure is not optimal for catching leafrollers (Table 2). A higher emission rate is required and thus we were forced to replace all of the Pherocon AA lures in early summer of 2011 with a vial with a 3.1 mm hole. This vial was also used during 2012. Meanwhile, we have been testing larger cup lures for their effectiveness with both leafroller species and codling moth (Table 2). The new lure tested in 2012 (TRE0691) has a 10-fold higher emission rate than the Pherocon AA and appears to perform similarly in catching leafroller adults as the 3.1-mm vial. This acetic acid dispenser may not be completely optimized for leafrollers and codling moth, but a similar high emission prototype should be available from Trécé Inc. in 2013 for further testing by consultants.

Table 2. Moth catches of Pandemis leafroller and oblique banded leafrollers and weight loss from acetic acid lures in three trials with traps (N = 10) baited with the Pherocon CM-DA Combo lure plus one of several AA co-lures.

	PLR				OBLR	
	June – July 2011		Aug. – Sept. 2011		August 2012	
AA co-lure	Lure wt loss (mg/d)	Moth catch	Lure wt loss (mg/d)	Moth catch	Lure wt loss (mg/d)	Moth catch
Vial, 3.1 mm hole	40	11.5	55	3.0	53	0.6
Vial, 1.7 mm hole	17	9.3	20	4.3	-	-
Pherocon AA	3.5	1.6	4	1.4	3.8	0.0
TRE0421	-	-	12	3.9	-	-
TRE0691	-	-	-	-	40	0.4

3. 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 in 2011 and four volatiles for codling moth and oblique banded leafroller in an apple block in Medford in 2012 (Table 3). In both tests the different host plant volatiles were equally effective in catching leafroller adults when combined with the AA lure. Beta ocimene, farnesol, and nonatriene lures all caught good numbers of codling moths, but no lure outperformed pear ester, especially in the catch of female moths. Because pear ester is already commercialized it seems that the use of the CM-DA Combo lure with an acetic acid lure similar to TRE0691 would be an effective approach going forward.

Table 3. Comparison of moth catches of codling moth and Pandemis leafroller (PLR) in Yakima and codling moth and oblique banded leafroller (OBLR) in Medford 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.

	Yakima - 2011				Medford - 2012			
	Codling moth		PLR		Codling moth		OBLR	
Host plant volatile	Total	Females	Total	Females	Total	Females	Total	Females
Pear ester	0.9	0.3	9.4	3.4	6.2	1.1	0.6	0.6
Beta ocimene	0.8	0.3	12.0	4.6	2.7	0.1	0.7	0.6
Nonatriene	0.9	0.4	9.0	4.2	1.4	0.7	0.8	0.6
Farnesol	0.5	0.1	10.1	3.3	3.2	0.5	0.9	0.6
Beta farnesene	0.0	0.0	8.6	2.8				
Butyl hexanoate	0.4	0.1	8.5	2.5				

4. New Attractant for Leafrollers

We have been testing lures with Dr. Ashraf El-Sayed from HortScience in New Zealand for several years to allow us both to utilize the reverse growing seasons. During 2012 in one of these trials we found that the B3 volatile in combination with acetic acid caught greater numbers of both sexes of Pandemis and oblique banded leafroller adults (Table 4). However, B3 was not effective for codling moth. The combination of pear ester with B3 plus acetic acid provided the highest catches of both codling moth and leafrollers. The use of B3 with acetic acid was also an interesting bisexual lure for the eye-spotted bud moth. Dr El-Sayed has found that this volatile is effective for a number of important pest species and has applied for an international patent to protect his intellectual property. Further testing of this volatile is planned for 2013, including its use in attract and kill studies for OBLR and eye-spotted bud moth.

Table 4. Evaluation of three new attractants for leafrollers including pear ester (PE) and acetic acid (AA).

	Mean moth catch (Male / Female) per trap				
	Yakima 2012		Medford 2012		
NZ lures	CM	PLR	CM	OBLR	ESBM
B1 + PE + AA	11.2 / 18.4	2.0 / 1.2	0.0 / 0.4	0.0 / 0.4	0.0 / 0.0
B2 + PE + AA	13.0 / 15.2	1.0 / 0.2	1.0 / 1.0	0.0 / 0.6	0.0 / 0.0
B3 + PE + AA	4.6 / 4.8	3.8 / 1.4	0.4 / 0.2	1.2 / 3.4	0.4 / 0.6
B3 + PE	0.2 / 1.2	0.2 / 0.2	0.4 / 0.0	0.0 / 0.4	0.0 / 0.0
B3 + AA	0.4 / 0.4	4.8 / 2.6	0.0 / 0.0	1.2 / 4.6	0.2 / 0.6
B3	0.4 / 0.0	0.4 / 1.0	0.0 / 0.0	0.0 / 0.0	0.0 / 0.0
PE + AA	14.2 / 19.2	1.0 / 2.0	2.2 / 2.6	0.4 / 0.4	0.0 / 0.0

5. Effect of the cardboard lure holder on moth catches.

During the course of the season it became obvious that the use of the cardboard lure holder for the septum and the acetic acid cup lure provided by Trécé Inc. was negatively impacting moth catches (Fig. 1). To be sure we conducted a specific experiment to compare moth catches when the lures were placed in the cardboard hanger, pinned to the roof, or placed on the sticky liner (Table 5). A similar study was also repeated by collaborators in Chile. These trials showed that the acetic acid lure needs to be placed horizontally on the center of the trap's sticky liner to avoid this repellency. Trécé Inc. has discontinued this holder as a result of this study. This finding also suggests that the data in Table 1 might have been impacted, and the benefit of adding the acetic acid co-lure was likely underestimated as the holder was used in all 21 sites.

Table 5. Effect of Trécé Inc. lure holder on moth catches of codling moth with the Combo lure and the Pherocon AA lure.

		Yakima 2012	Chile 2012
CM-DA Combo lure	AA lure	Mean Male / Female catch	Mean Male / Female catch
In holder	In holder	1.4 / 0.2	6.2 / 0.4
On liner	On liner	4.8 / 1.0	-
Pinned to roof	On liner	-	24.6 / 0.8
In holder	On liner	-	14.0 / 0.0

6. 2011 correlation of moth catches with local leafroller populations

Studies were conducted with both Pandemis and oblique banded leafrollers in apple orchards near Brewster, Quincy, Wenatchee, and Yakima, WA in 2011 (Table 6). Sites 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 CM-DA plus AA-baited traps in 11 orchards. No signs of leafroller larvae were found in these orchards except that spring larvae were sampled in the Wenatchee2 site which was also nearby known infested blocks. Low levels of leafroller adults ($\leq$ 1 moth) were 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 near the orchard. The most interesting block in this category was Naches1 that had very high levels of leafroller adults without any injury occurring. 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 that

were not sampled. Traps in all blocks in which leafroller fruit injury was detected caught leafroller adults.

Table 6. Summary of moth catches and leafroller infestations in orchards monitored during 2011 with the Pherocon CM-DA Combo lure plus a vial with a 3.1 mm hole loaded with AA.

	Mean catch PH trap		Mean catch Combo + AA trap		Infestation
Orchard	PLR	OBLR	CM	LR	presence or potential
Naches1	294	81	61	24	Nearby injury & hosts
Naches2	66	9	86	1	No
West Valley	23	35	30	0	No
Wiley City	42	31	22	0	No
Moxee1	2	18	41	0	No
Moxee2	19	123	23	1	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	9	0	No
Quincy1	-	217	6	4	High fruit injury
Quincy2	-	228	6	1	Some fruit injury
Quincy3	-	42	21	1	Nearby hosts
Quincy4	-	52	13	4	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	?

7. 2012 correlation of moth catches with local leafroller populations in apple

During 2012 a portion of this project was conducted with cooperation from consultants in the Orondo and Quincy area to assess if CM-DA+AA lure baited traps would capture OBLR and if these captures were reflective of OBLR densities in monitored orchards (Table 7). Codling moth and oblique banded leafroller were monitored at 12 locations. At each location traps baited with CM/DA+AA lures and traps baited with OBLRW lures were used to monitor CM and OBLR. Monitoring traps were placed in orchards in late May and checked through August. The traps were checked weekly and number of CM and OBLR counted and removed in the CM/DA+AA traps and OBLR in the OBLRW traps. The risk rating for each orchard was determined by consultants who monitored the orchards for presence of OBLR larvae, pheromone trap captures, and injury.

The capture of OBLR in CM/DA+AA lure-baited traps seemed to be a good predictor of OBLR pressure in the first flight in blocks in the Quincy area and in the Orondo 1-4 blocks (Table 7). In the Quincy area orchards those blocks classified as high pressure both captured some OBLR in the first flight, the block classified as moderate pressure caught only one OBLR moth, and in the block

classified as low pressure no OBLR moths were captured. In the Orondo 1-4 blocks, all classified as low pressure, there was only one OBLR moth captured, in the second flight period.

There was not as good of relationship between OBLR capture in CM/DA+AA lure-baited traps and OBLR pressure classification in the Orondo 5-6 sites. The one block, Orondo 6, classified as high due to the presence of several overwintering larvae, the trap did not capture any OBLR moths in the first flight, One Orondo site caught OBLR in the CM-DA baited trap in the first flight (Orondo 8) and these were all caught on one date. Orondo 8 was close to a sweet cherry orchard which could have harbored an OBLR population but this orchard was not monitored nor sampled for presence of OBLR in the spring. OBLR moth captures in the CM/DA + AA traps were higher in the second flight in the Orondo blocks and this matched a higher capture of OBLR in traps baited with the OBLR-W lures.

Table 7. Summary of results from the Wenatchee area over both OBLR generations, 2012.

	Mean catch	Mean catch with Combo + AA		
	OBLR PH lure	CM	OBLR	
Orchard	1^{st} / 2^{nd}	1^{st} / 2^{nd}	1^{st} / 2^{nd}	Rating risk
Orondo 1	9 / 29	1 / 3	0 / 1	Low
Orondo 2	N.A.	4 / 0	0 / 0	Low
Orondo 3	12 / 24	0 / 0	0 / 0	Low
Orondo 4	N.A.	0 / 0	0 / 0	Low
Orondo 5	1 / 0	2 / 0	0 / 4	Low
Orondo 6	<1 / 4	1 / <1	0 / 2	High
Orondo 7	1 / 0	0 / 0	0 / 5	Low
Orondo 8	8 / 2	2 / 0	4 / 1	Low
Quincy 1	183 / 21	<1 / 0	3 / 0	High
Quincy 2	86 / 34	0 / 0	1 / 0	Mod
Quincy 3	144 / 17	1 / 0	3 / 0	High
Quincy 4	0 / 0	0 / 0	0 / 0	Low

8. 2011-12 correlation of moth catches with local leafroller populations in pear

Studies were conducted in pear blocks in Medford over both years of the project (Table 8). These blocks were selected based on an expected moderate to high pest pressure from OBLR. OBLR counts in the pheromone traps were high in both years. Counts of CM in pheromone traps were more variable among orchards. Orchards ranged from organic to conventional and generally received few sprays during 2012. OBLR adults were captured in all but one orchard in 2011 and two orchards in 2012. Fruit injury in 2011 from leafrollers was found in four blocks. The Medford 7 block did not have fruit injury but leafroller larvae were sampled in June. The two other blocks had no injury and no signs of larvae and had either 0 or 1 leafroller adult caught in traps.

Results in 2012 were somewhat more difficult to interpret. Considerable injury purportedly from leafrollers was found in two blocks. Counts of OBLR were low in all blocks in the CM-DA + AA baited traps with no evident pattern. However, we discovered that the eye-spotted bud moth was present in high numbers in some of these blocks. Field scouts in the spring generally ignored the large number of larvae found in developing buds because they were not oblique banded leafrollers and efforts to rear and identify them failed. Later in the season while testing the lures previously mentioned it became obvious that these larvae were likely eye-spotted bud moth and orchards had an unmanaged population of a new pest. Unfortunately, fruit injury by OBLR and the bud moth are nearly identical and there was no way to differentiate the injury. We believe this confusion may have been responsible for the poor correlation that occurred in 2012 and not in 2011. These new findings

have stimulated further research into the attractant from New Zealand and the potential to develop attract and kill tactics for both pests simultaneously. Studies are planned for pear in Medford in 2013.

Table 8 Summary of results from Medford pear blocks

	Mean catch in PH trap		Mean catch Combo + AA trap		Infestation presence or potential
Orchard	CM	OBLR	CM	LR	
		2011			
Medford1	31	298	55	6	Some fruit injury
Medford2	81	260	126	5	Some fruit injury
Medford3	2	320	1	1	No
Medford4	0	57	0	0	No
Medford5	2	335	4	4	Some fruit injury
Medford6	97	149	122	4	Some fruit injury
Medford7	37	408	26	8	June larvae
		2012			
Medford 1	4	174	17	0	No injury
Medford 8	0	376	1	1	6% injury
Medford 5	1	262	2	2	No injury
Medford 3	161	233	95	1	No injury
Medford 6	0	373	0	2	7% injury
Medford 9	118	203	17	0	No injury

DISCUSSION

The use of traps baited with CM-DA plus acetic acid lures to monitor both codling moth and leafrollers appears to be a promising new tool for pest managers. These traps provide useful information at a minimal cost and training. Implementation of action thresholds based on moth catches for codling moth and use of higher densities of traps can allow growers to use less insecticide and target their valuable resources to treat 'hot-spots'. The detection of leafrollers in these traps alerts the farm manager to a potential problem. Control actions can then be taken based on this information as well as the orchard's pest history, other monitoring data, and grower's risk preferences.

In general, these traps when placed in commercial orchards will catch < 10 leafroller adults per season. The capture of one or more leafroller adult suggests that a local infestation of leafrollers is present either in the block with the trap or in adjacent blocks. Unfortunately, the catch of leafrollers without the occurrence of local injury can be relatively high depending on the proximity and severity of the infestation. Pome fruit orchards adjacent to cherry blocks are at the greatest risk from female moths immigrating and laying eggs during the season. The CM-DA Combo plus acetic acid lure catches both sexes of leafrollers and data interpretation (as with codling moth) would likely be improved if moths were sexed. Female adults of both leafroller species can be readily identified by their larger size, female genitalia, and the greenish hue of their abdomen due to the presence of eggs.

The second major consideration when using this trap is that monitoring the adult stage occurs at a different time period than other sampling protocols used for larvae and larval injury of the fruit. Thus, the detection of overwintering larvae in the spring may not always correlate with adult captures in orchards where subsequent curative treatments are applied. Also sprays applied for codling moth and other pests can impact leafroller larval density; and levels of parasitism can be very high in some orchards which would also disrupt this correlation. Larval populations in the summer and/or fruit

injury in our study generally occurred where traps previously caught leafrollers. Populations developing in cherry after harvest can build up and adults can then move into pome fruit. Thus more temporal information is needed to assess the specific correlations of trap counts (each sex) with summer and fall larval populations. These types of data proved to be very difficult to collect from sprayed commercial orchards.

Figure 1. Trap with both codling moth and leafroller adults.

Figure 2. The cardboard lure holder developed by Trécé. Inc.

EXECUTIVE SUMMARY

Studies were conducted to develop the use of acetic acid with the sex pheromone of codling moth and pear ester for monitoring codling moth and leafrollers in a single trap. The concept is important because monitoring is expensive and traps baited with sex pheromone lures of leafrollers do not provide a useful measurement of pest pressure for orchardists.

First we optimized the acetic acid lures that would be effective for both codling moth and leafrollers. We encouraged Trécé Inc. to develop a commercial acetic acid lure, Pherocon AA, for codling moth. We found that codling moth is attractive over a wide range of emission rates of acetic acid but that both leafrollers require a higher emission rate. A similar lure with a higher emission rate for leafrollers will be available for testing in 2013.

Field studies found that the novel lure holder developed by Trécé Inc. to hold both the combo septa and the acetic acid lure interfered with moth catch. Instead, we showed that the acetic acid lure must be placed on the trap liner's adhesive in the middle of the trap. Trécé Inc. has adjusted its label to reflect this finding.

Pear ester is widely used in a combo lure with codling moth sex pheromone and its attractiveness is synergized by acetic acid. Studies were conducted with alternative host plant volatiles for both codling moth and leafrollers. Several compounds other than pear ester were found to be similarly attractive for leafrollers when used with acetic acid. However, pear ester remains the most attractive plant volatile in combination with acetic acid for codling moth, especially for female moths.

A new host plant volatile was discovered in tests with Dr. Ashraf El-Sayed from New Zealand. This compound is attractive for a number of species. Patent protection for this compound has been submitted. Further studies are planned to use this volatile in 'attract and kill' studies of oblique banded leafroller and eye spotted bud moth in 2013.

Studies showed that the use of a single trap for codling moth and leafrollers can provide useful management information. Traps failed to catch leafrollers in orchards where leafrollers were not present, except in some orchards adjacent to cherry blocks. These catches provide some indication of the orchard's risk from immigrating moths and are useful data. It is important to sex the leafrollers caught in traps and establish a threshold based on female moth catches as well as total catch of leafrollers. In a few cases, overwintering larvae were sampled in orchards in which local traps did not later catch moths. Due to the use of insecticides it is possible that this can occur and does not discount these results. More importantly, no cases were found in which traps failed to catch adult leafrollers but leafroller larvae were detected during the subsequent generation. Correct identification of fruit injury and alternative monitoring of rare pests are both important for this approach to be reliable.

In summary, the numbers of leafroller adults caught in traps baited with codling moth pheromone, pear ester, and acetic acid are low in most commercial orchards, but any catch of leafroller adults appears to be closely correlated with local pest pressure. Thus, growers at no additional cost while monitoring codling moth can also obtain additional information about their potential need to treat for leafrollers.

FINAL PROJECT REPORT
WTFRC Project Number: CP12-106A

Project Title: Optimizing attract and kill to enhance control of apple pests

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Cooperators: Peter McGhee, PhD student, Michigan State University

Other funding Sources: *None*

WTFRC Collaborative expenses: *None*

Total Project Funding: **Year 1:** $60,000

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	2012
Salaries [1] Technical assistant	18,334
Benefits [2] Technical assistant	6,398
Wages (temporary labor) [3]	3,520
Benefits [3]	610
Equipment	0
Supplies [4]	638
Travel [5]	500
Plot Fees	0
Miscellaneous	0
Total	30,000

Footnotes:
[1] Technical Assistance TBN (0.5 FTE for 8 months).
[2] Technical Assistance TBN (34.9%).
[3] Temporary labor ($11/h, 40h/wk, 8 wks); benefits at 17.3%.
[4] Includes monitoring supplies, rearing materials for colony, sterile moths.
[5] Within State Travel.

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

Item	**2012**
Salaries	14,000
Benefits (38%)	5,320
Wages	0
Benefits (7.5%)	0
Equipment	0
Supplies	680
Travel	0
Miscellaneous	0
Total	20,000

Budget 3:
Organization: USDA-ARS, Wapato **Contract Administrator:** Jim Harris
Telephone: (509)454-6565 **Email:** James.harris2@ars.usda.gov

Item	**2012**
Salaries	0
Benefits	0
Wages	7,500
Benefits (10% of labor)	750
Equipment	0
Supplies	1,750
Travel	0
Miscellaneous	0
Total	10,000

Project objectives:

1. Develop commercially viable attract-and-kill technologies by optimizing moth attraction, moth contact, and the within-orchard spatial distribution of technologies.

Significant Findings:

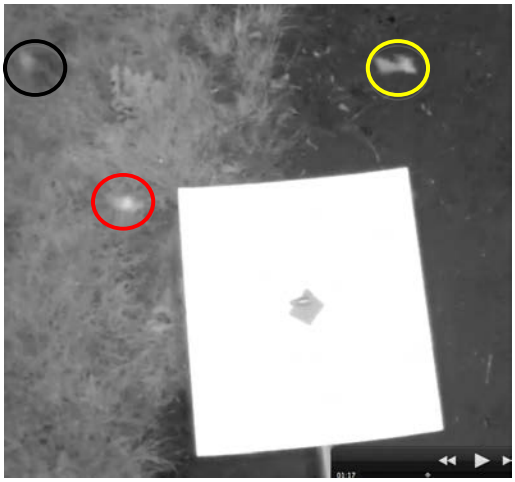
1. Purchase of a new video recording system provided much more detailed images and flexibility with managing images over the previous system.
2. Attempts to construct a trap that mimicked the complexity of foliage and provided multiple landing areas did not prove successful.
3. As part of the effort to construct a complex trapping surface we discovered that dry sticky liners were about 10% as efficient at capturing codling moth (CM) as a standard polybutene liner.
4. In a wind tunnel OBLR moths were attracted to and made contact with the pheromone source that was associated with a flat platform more frequently than to a pheromone lure alone.
5. The increase of OBLR pheromone load into grey rubber lures increased capture of male moths up to 10 mg, for both a three- and four-component pheromone blend.
6. In wind tunnel studies the commercial product SPLAT with the lowest CM pheromone concentration attracted the most CM and accounted for the most contacts with the source.
7. When moths contacted the SPLAT containing 3% cypermethrin 70-80% were knocked down, that is were unable to continue directed flight activity.
8. Field studies showed that there was strong support for interaction between N-butyl sulfide (NBS) and acetic (AA) in capture of CM.
9. When NBS and AA were combined with pear ester (PE) the capture of CM was higher that NBS+AA or AA+PE.

Results and Discussion:

A key aspect of developing an attract-and-kill (A&K) technology for either codling moth (CM) or leafrollers is to determine the impact of design on moth behavior, especially making contact with the technology so that intoxication occurs. In the past we have evaluated different types of A&K designs that were aimed at optimizing attraction to and contact with, or capture in, the technologies. With CM we found that a high pheromone release rate from lures attracted moths to the source but inhibited entry into a trap and/or contact with the attractant source. We have used simple home security video cameras to record and analyze behaviors of moths, but these simple and cheap systems had several limitations associated with transferring videos into formats that could be easily analyzed and the resolution was limited, especially for night active moths. This year we invested in a video recording system (not funding by the commission grant) that provided high-resolution digital images of moth activity (Fig. 1). The cameras were of megapixel quality allowing for a wide angle of view but with the capability to zoom in on close range behaviors, i.e. contact with attractant sources. We recorded images from four stations focusing primarily on CM behavior, as leafroller populations in the study site, Sunrise Research Orchard, were too low to provide sufficient responses to attractants.

Fig. 1. Switch and hard-drive recorder with fan inside shelter and camera mounted above platform with attractant source.

We initially recorded behaviors to platforms (non-sticky trap liners) with two attractant sources of different strength, a 1 mg or 0.1 mg lure (Fig. 1 – and image at right). In the image at right there are three moths attracted to the pheromone source associated with the platform. For each day we captured and saved sections of video from each camera that contained moth activity. Unfortunately as of the writing of this report we are still in process of analyzing data from these videos to classify behaviors. One observation that led us to test different A&K designs (see below) was that moths approaching a flat platform were observed to predominantly approach the pheromone source from beneath the platform and fly under it, thus loosing the plume of the attractant. Moths were seen repeatedly moving from side to side under the platform. A video will be shared at the final report on this project showing this behavior.



We evaluated four different A&K technology designs, each baited with a 0.1 mg lure. These models included a badminton racquet, a whiffle ball, a shuttle cox, and a wooden dowel (Fig. 2). The different designs would allow not just attraction to but hopefully contact with a toxic surface. The alternative A&K designs were an attempt to modify the approach of moths so that they would contact a surface as they were approaching the attractant source. Of course in an A&K design that surface would be coated with a toxicant.

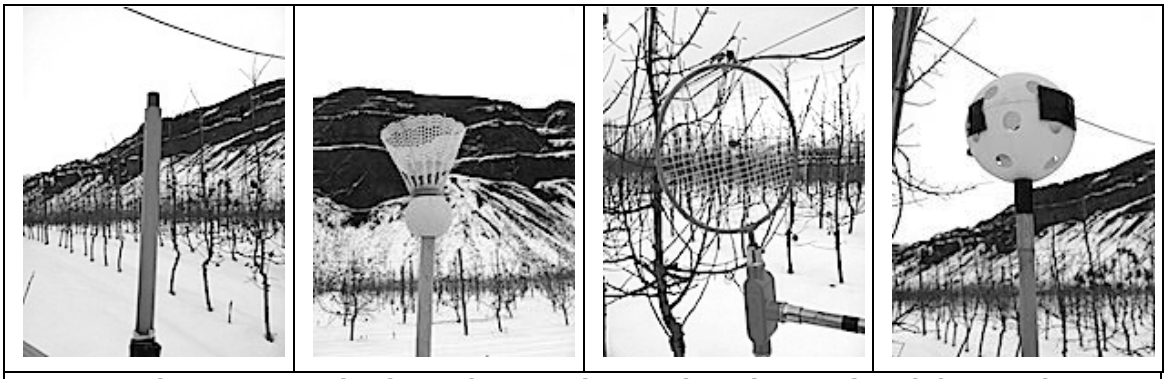


Fig. 2. Four devices associated with CM pheromone lures used to video record CM behaviors when approaching or contacting surfaces.

We also conducted some trapping designs that were intended to mimic more complex structures and optimize moth attraction to and contact with various surfaces. We constructed two models, a multi-layered circular and panel trap (Fig. 3), each of which had sticky upper surfaces only, lower surfaces only, or both were sticky. We used dry sticky trap liners (bottoms) to construct these traps as it made it easier to put them together. We compared captures in the different trap designs with a standard delta trap baited with either a dry sticky liner or a polybutene liner, which is more typical of monitoring traps for CM and leafrollers. Traps were baited with a CM L2 pheromone lure. The multi-layered traps caught very few moths, but were captured most on the upper sticky surface, 86%, compared to the lower sticky surface. These data are confounded by the discovery that the dry sticky surface was not a good capture substrate for CM adults. Average moth captures in the delta traps with the dry sticky liners was 2.7 ± 1.5, about 10% of the capture in delta traps with the standard polybutene sticky liners, 22 ± 11.2, respectively. The multi-layered traps captured large numbers of non-target insects, especially leafhoppers and flies,

indicating that the dry sticky surface was efficient in retaining some kinds of insects. Further studies should be conducted to determine if different dry sticky trap liners have limited capacity to capture CM and other pest moths, as some of these trap liners are sold for use in monitoring traps in orchards for moth pests.

Fig. 3. Trap designs tested in 2012 for capture of CM and leafrollers.

Obliquebanded leafroller (OBLR) males were flown in a wind tunnel to assess behavior of males exposed to pheromone lures (1 mg of a three-component blend). OBLR males were flown to a lure associated with either a vertical or horizontal card or to a lure only (Fig. 4). OBLR males made more upwind flights toward, 90%, and more source contacts, 80%, with a card present than when only the lure was present, 60% and 24%. In addition, OBLR males spent nearly twice the time searching around the lure when it was associated with a card. These data show that some structure, vertical or horizontal, in association with a pheromone attractant is important in enhancing OBLR searching time and source contact.

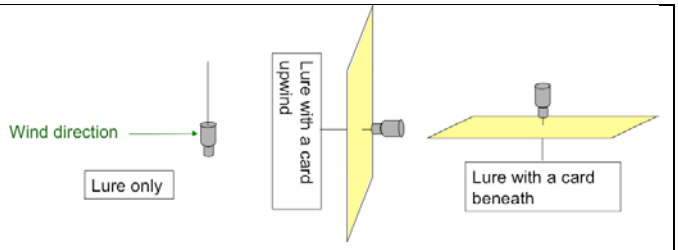


Fig. 4. Three design set-ups compared in wind tunnel studies attracting OBLR to pheromone lures.

As a follow up to the above study a piece of polyester fabric (10 x 28 cm) was treated with deltamethrin, a synthetic pyrethroid. A 1 mg pheromone lure was then associated with the fabric, either treated with deltamethrin of untreated. OBLR moths were flown in a wind tunnel towards the fabric, similar to the vertical card shown in Fig 4. Behavior (wing fanning, upwind flight, source contact, and no response) of 20 OBLR moths was recorded. We found there was no difference in any of the behavioral parameters measured for moths flown to the treated or untreated fabric with source contacts being between 60-70%. This showed that the pesticide was not a repellent to OBLR moths. In addition, moths that contacted the fabric in each treatment were recaptured and held in a small plastic container and mortality recorded. After 1h 100% of the moths contacting the deltamethrin treated fabric were knocked down (inability to manage controlled activity) and after 24h 100% of these moths were dead while only 8% of moths contacting the untreated fabric were dead. If these behaviors could be replicated under field conditions there is promise that a simple A&K product could be developed for OBLR.

Three- and four-component blends of chemicals previously reported as OBLR pheromones were loaded into grey rubber septa at rates of 0.01, 0.1, 1, 10 and 20 mg per lure. These lures, along with commercially available OBLR lures from Trécé Inc., were placed in pheromone traps and OBLR captures recorded. The number of OBLR males captured increased with increasing load rate up to 10 mg, with no difference in captures between 10 and 20 mg lures, and these captured more moths than the Trécé lures (Fig 5). There was a slightly higher capture of OBLR in the three-component pheromone blend, especially at lower load rates, than the four-component blend. These data show that increasing load rates can increase moth captures in pheromone traps, but does not mean that increased load rates will increase OBLR moth contact with a pheromone source associated with an A&K device.

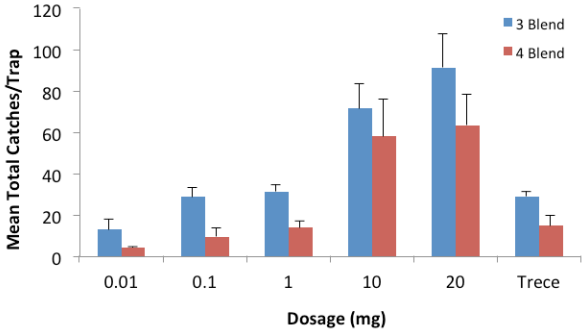


Fig. 5. Average number of OBLR males captured in traps baited with different loads of two pheromone blends.

Male CM were flown in a wind tunnel to different SPLAT™ formulations containing varying concentrations of CM pheromone plus 3% cypermethrin, a synthetic pyrethroid. Moths were also flown to a lure loaded with 0.1 mg of CM pheromone. SPLAT formulations and the CM lure were aged for 7, 14 and 28 days prior to testing in the wind tunnel. The size of the SPLAT dollop was held constant at 0.1 ml for each treatment. Source contact was highest to the SPLAT formulations containing the two lowest CM pheromone concentration and equal to the 0.1 mg lure. There was no difference in moth behaviors to different aged SPLAT formulations or 0.1 mg lure.

The formulation of SPLAT containing the lowest concentration of CM pheromone was used in another wind tunnel study where moths that contacted the SPLAT, about 55%, were recaptured and evaluated for knock down. Seventy percent of the moths were knocked down on the day of recapture and this increased to 80% on the second day after contact.

Preliminary field trials with a SPLAT CM A&K formulation (with cypermethrin) and two SPLAT CM pheromone only (mating disruption) formulations showed an advantage of the A&K formulation over the pheromone only formulations.

Based on previous research four chemicals, benzyl ether, N-butyl sulfide (NBS), acetaldehyde, and acetic acid (AA) were evaluated for their co-attraction of male and female CM. There was no or little evidence for a positive interaction between benzyl ether and AA or acetaldehyde and AA, but there was strong support for interaction between NBS and AA (Fig. 6). Further studies showed no advantage of adding either acetaldehyde or benzyl ether to AA+NBS, nor did a combination of all four chemicals increase moth capture over AA+NBS. Increasing the release rate of NBS by changing the hole size in the release vial from 1 to 12 mm showed no difference in CM captures when the release rate of the AA lure was held constant. There was also no difference in CM captures when AA and

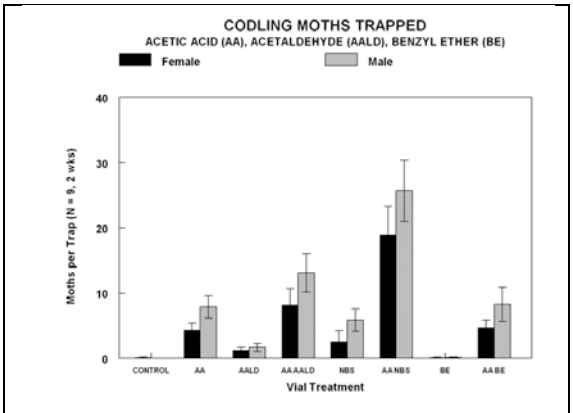


Fig. 6. Average number of CM captured in traps baited with different combinations of kairomone attractants.

NBS were released from separate, vials each with a 3 mm hole, or when the two chemicals were mixed in the same vial with the 3 mm hole.

The number of CM captured in traps baited with AA+NBS lures was lower than traps baited with AA+PE (pear ester), but captures of CM were higher still when all three chemicals were combined (Fig. 7).

Further studies evaluating a tube-type A&K design showed a significant increase in male but not female captures as tube length varied over the range of 5.5 to 10 cm. Changing the width of the tube significantly increased capture of both male and female CM over a range of 1.75 to 3.0 cm.

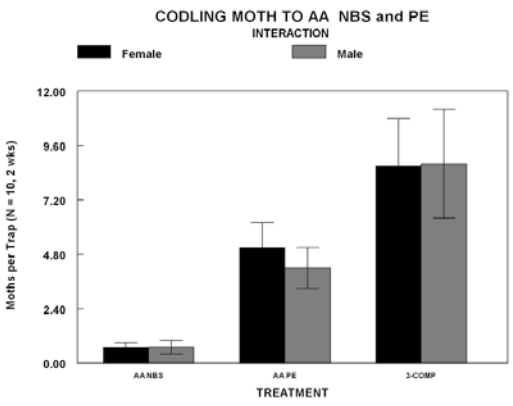


Fig. 7. Mean number of CM moths captured in traps baited with AA+NBS, AA+PE or AA+NBS+PE.

Executive Summary:

This project involved collaborative research of three institutions examining issues of codling moth and obliquebanded leafroller behavior as it relates specifically to the development and design of attract-and-kill technologies. At its core the development of an attract-and-kill technology must deal with attraction to and contact with a source or surface in order to have success. Attraction without source contact, as we have observed on many occasions, does not achieve the killing component the attract-and-kill concept and such technologies typically end up as a weak form of mating disruption. In this study we utilized a high quality field video camera system to capture behavior of codling moth to different sources as a basis for evaluating structures that enhance source contact. Various kinds of trapping systems were also evaluated for codling moth and obliquebanded leafroller and some key parameters were identified that would need to be incorporated into a trap-out attract-and-kill design. One unanticipated consequence of our research was the discovery that dry sticky trap liners were not efficient in capturing codling moth, though they did capture many other kinds of insects. This phenomenon needs further investigation as dry sticky trap liners are sold for use in monitoring traps for pest moths. Wind tunnel results showing differential behavior of obliquebanded leafroller moths with or without a flat surface associated with attraction to and contact with an attractant source demonstrate the need for some kind of structure in combination with a pheromone source to enhance an attract-and-kill design. The high knock down rate for obliquebanded leafroller moths flown to a pheromone lure associated with a fabric panel treated with deltamethrin was an encouraging step towards the development of an attract-and-kill device for this insect. Wind tunnel studies with codling moth that showed highest source contact to a SPLAT attract-and-kill formulation with the lowest pheromone concentration confirmed previous studies, however, the failure in the field of the SPLAT attract-and-kill formulation to increase suppression of codling moth male captures in pheromone monitoring traps over a SPLAT formulation with pheromone only was a repeat of previous experiences and suggested that moths are not contacting the attract-and-kill product in sufficient frequency to add value to a mating disruption effect. Pear ester has been the best kairomone found for attracting codling moth. Recent research has shown that combining acetic acid with pear ester increases attraction and capture of codling moth. The search for additional kairomones that attract codling moth have been frustrating, but the discovery that N-butyl sulfide combined with acetic acid is attractive to codling moth was encouraging. In addition, when N-butyl sulfide and acetic acid were combined with pear ester, codling moth capture in field traps was greater than in traps baited with pear ester and acetic acid. Results from this one-year project point to promising lines for further investigation in the development of attract-and-kill technologies for codling moth and leafrollers, but sources of funding other than the commission will be sought for this work.

CONTINUING PROJECT REPORT 2012 **Year** 1 of 3

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

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Total Project Request: **Year 1:** $74,266 **Year 2: $79,287** **Year 3:** $82,378

Other funding sources
We have submitted a new grant ($21,438, one year) to the Washington State Commission on Pesticide Registration to leverage some of the work being done on this grant. That grant is "Evaluating low dose insecticide residues on codling moth flight and behavior".

WTFRC Collaborative expenses:

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

Footnotes: [1]WTFRC Collaborative expenses for spraying plots

Budget 1

Organization: WSU-TFREC Contract Administrator: Carrie Johnston

Telephone: 509 335-4564 Email: carriej@wsu.edu

Item	2012	2013	2014
Salaries [1]	46,783	49,233	51,202
Benefits [2]	18,429	19,317	20,090
Wages	3,200	4,500	4,680
Benefits [3]	554	437	454
Equipment	0	0	0
Supplies [4]	2,500	2,600	2,704
Travel [5]	800	1,200	1,248
Plot Fees (WSU Sunrise: 2 acres@$1,000/acre)	2,000	2,000	2,000
Miscellaneous	0	0	0
Total	74,266	**79,287**	82,378

Footnotes:

[1] Tawnee Melton (0.7 FTE for 7 months), Angela Gadino (1.0 FTE for 7 months)

[2] Tawnee Melton (49.2%), Angela Gadino (33.7%)

[3] 9.7%

[4] Includes bioassay and field supplies needed for objectives 2 & 3

[5] Within State Travel

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

Significant Findings:

- A laboratory colony of *C. nigricornis* has been established from field-collected adult lacewings. Although rearing has been successful through three generations, we are currently optimizing our methods for efficiency and to ensure colony stabilization. We hope to start gathering the life history data needed to develop the models in the next four months.
- Residue mortality bioassays for OBLR were conducted this past season using leaves from an apple orchard treated with seven pesticides. The data will be used this winter in the demographic models for this species.
- We have performed preliminary tests comparing leafroller bioassays done on fresh leaves and those using leaves vacuum packed and frozen. We found no significant differences in the results, which will allow us to extend the time over which bioassays can be performed.
- Tested preliminary bioassays using neonate CM larvae on leaf disks to determine residue mortality. Initial bioassays testing CM on apple fruit experienced high mortality in the control tests leading us to develop alternative methods for these experiments.
- The model for codling moth has been completed and only needs the residue data.
- The OBLR model will be considerably more complicated than those for CM or the lacewings because OBLR has some individuals that go through five larval instars and some that go through six. This requires two models that run independently and then combine their data after the simulations are done. We have finished the simulation of phenology and will start working on the pesticide effect model in the next few months.
- A demographic model for the lacewing *Chrysoperla carnea* is nearly complete, only requiring the initial stage distribution that will allow the model to mimic the field phenology. Phenology of *C. carnea* was only recently completed in the SCRI biological control project.
- The model for *Chrysopa nigricornis* will be started when the laboratory studies are completed, but the basic model of *C. carnea* will be modified as needed for *C. nigricornis.*

Objective 1

Methods: Live adult *C. nigricornis* were collected from orchards in Quincy and at WSU Sunrise during the summer months and transported to the laboratory to start the colony. Male and female adults were placed together in 10-gallon glass cage aquariums to allow mating. Eggs oviposited by adult females are clipped from the rearing cages and new cohorts of *C. nigricornis* are started once a week in plastic snap top containers with 2-4 grams of *Ephestia kuehniella* (Mediterranean flour moth) eggs as a food source. Larvae develop through three instars, pupate and emerge as adults in individual cups. As new adults emerge, they are released into adult rearing cages. Over the course of the past five months, we have offered an artificial raw beef and hen's egg diet (adults) or *E. kuehniella* eggs (larvae and adults) in order to determine the optimal resources needed for *C. nigricornis* development, survival and reproduction. This particular species of green lacewing is carnivorous in both the adult and larval stages, so we have had to develop new methods to successfully rear this insect on artificial diets.

Fig. 1. Lacewing eggs oviposited on artificial apple in an adult rearing cage (inside rectangle, white dots).

Results: At present the colony consists of approximately 175 individual adults all reared from the original field collected adults. Egg production for reared females has been inconsistent ranging from about 50-100 (lowest) to 500-600 (highest) eggs per week. Currently, egg production is averaging 150-200 eggs per week and is increasing. We have been working to maximize reproduction by testing a number of different rearing strategies to ensure optimal nutrition, assess mating behavior, and improving food delivery and oviposition surfaces (Fig. 1). For adult lacewings, it appears that the artificial diet increases egg production compared to feeding on *E. kuehniella* eggs alone. We have also observed that the period of time from adult emergence to egg laying is longer than expected (> 2 weeks) suggesting that the larval diet should also be supplemented with the artificial diet. We are in the process of developing methods that will allow us to make this addition to the larval rearing. Another issue we are addressing is the predation of newly oviposited eggs by adult lacewings in the rearing cages, which reduces the number of eggs for collection. We are currently observing *C. nigricornis* adults during the dark photo-phase, when they are most active, to determine the patterns of oviposition and egg predation events in order to adjust our methods and limit this issue.

Plans for 2013: As the colony population increases and becomes stable we will begin to gather the life history information needed to develop the demographic models. We are expecting to be able to start our evaluations between February and April. We hope to have most of the life history information gathered and analyzed by early summer. Once this step is complete, the data will be used to begin development of the models needed for population projections.

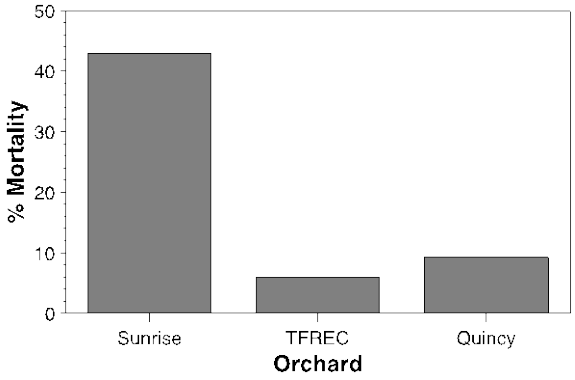
Objective 2

Methods: OBLR and CM bioassays were conducted during early and late summer (OBLR only) this past year to determine the mortality versus residue age curves needed for the demographic models. We applied the maximum recommended field rate for Altacor, Assail, Delegate, Entrust, Proclaim, Rimon and Warrior. These materials were applied on June 1 (WSU Sunrise research orchard) for the first set of bioassays and on August 23 (non-bearing orchard Quincy, WA) for the second set. Control plots remained untreated for all experiments. At Sunrise, leaves or fruit were collected every 4-7 days until sampling was discontinued due to unexpected high mortality in the control plots (*see results*). For the second set of bioassays, leaves were sampled every 4-7 days up to 55 days after pesticide applications. Extra leaves were also collected for each treatment on all sample dates and brought back to the laboratory where they were vacuum packed and frozen. We have started testing these leaves using OBLR bioassays and so far, we have not found significant differences in the mortality between using fresh and frozen leaves. This will allow us more flexibility to conduct bioassays beyond the field season and to better use the colonies and labor. We will be validating these fresh versus frozen assays for all three insects this winter.

Residue bioassays were conducted in the laboratory using leaves (OBLR) or fruit (CM) containing the field-aged residue for each sample day. First instar OBLR larvae (0-4 days old) were confined to

four leaf discs (2cm diameter) in a plastic petri dish. Neonate (<24h old) CM larvae were transferred to apple fruit contained in a plastic deli cup. A total of 150 CM or OBLR larvae were evaluated for each pesticide and residue sample day. Larvae were checked for mortality at seven days after exposure to each residue sample. These data were then used to develop the mortality versus residue age curves.

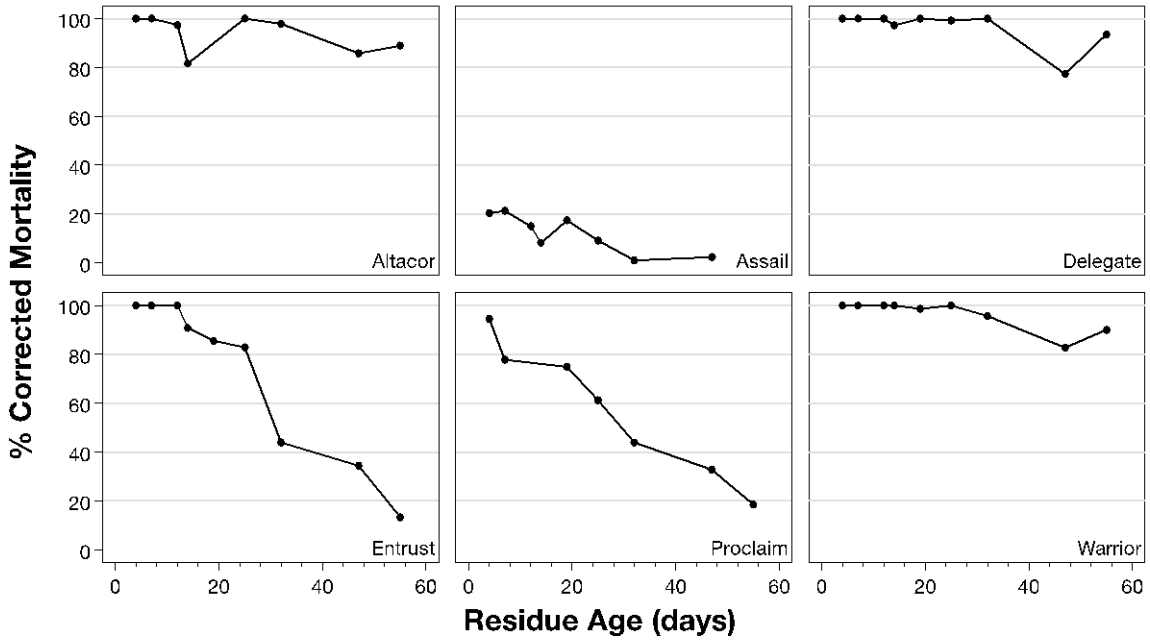
Fig. 2. Comparison of OBLR larval mortality on untreated leaves from three orchards.



Results: The first set of bioassays using leaves (OBLR) or fruit (CM) collected from Sunrise were discontinued earlier than expected due to high mortality levels in the control assays. To investigate this, we compared OBLR larval mortality on a series of untreated leaves from different orchards. We found larval mortality was >40% in the control plots at Sunrise, while it was <10% at the other two orchards (Fig. 2). To solve this problem, we relocated the field experiments to a large acreage, non-bearing orchard in Quincy, WA that was under a minimal management program with limited possibility for drift contamination. Initial tests with untreated leaves found low OBLR mortality levels allowing us to proceed with our second set of bioassays.

In evaluating the residual longevity data for all pesticides, we found unusually low mortality in the bioassays for Altacor on day 19 and for Proclaim on days 12 and 14. These assays are currently being repeated using our frozen leaves and are not shown in figure 3. After correcting for the natural mortality occurring in the control assays, we found that 55 day-old residues of Altacor, Delegate and Warrior continued to show activity with >80% OBLR larval mortality (Fig. 3). Mortality for Entrust and Proclaim residues remained above 50% until it was 32 days old, but was <20% by day 55. Assail, a pesticide not commonly used against leafrollers, did not show average OBLR mortality greater than

Fig. 3. Corrected mortality of OBLR larvae on leaves with different field aged residues of six different pesticides. Data from Altacor (day 19) and Proclaim (day 12 and 14) are not shown and being re-tested using frozen leaves because of unusually low mortality.



30% at any point during the assays and was equal to the control by the time residues were 32 days old. Leafrollers exposed to Rimon residues did not show a systematic pattern of residue decline with age, but instead varied erratically from 30-80%, probably because of the mode of action.

Since we conducted the late season experiments using a non-bearing orchard block, we did not have fruit available to sample for our CM bioassays. This may not present a problem for future bioassays as we are currently evaluating the potential to use leaves instead of fruit for these assays. Previous research has shown that CM neonates can develop through their first instar on apple leaves. Our preliminary assays using untreated leaves look promising with 15% larval mortality after 5 days. Another benefit to using leaves instead of fruit is that we do not have to be concerned with CM infestations from the field that could contaminate the assays.

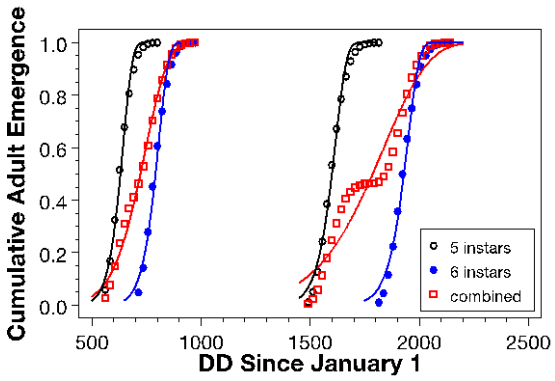
Plans for 2013: During the winter months we will work on assessing the use of frozen leaves to conduct the bioassays for OBLR, CM and *C. nigricornis*. We will begin planning for the field sprays in April by locating suitable non-bearing orchards where we can apply the pesticide treatments. We will apply similar pesticide treatments (except Rimon) and rates as last year and conduct bioassays for the three species during the field season in addition to collecting and freezing leaves for future assays.

Objective 3

Methods: The CM model has been developed and only needs the residue longevity data before validation can begin. We also developed a model for the lacewing *Chrysoperla carnea* because it is the second most common lacewing predator in our orchards, literature data on its life history were already available, and because the model would need only minor changes to be adapted to *Chrysopa nigricornis* once the information in objective one is available. The model for *C. carnea* still needs to mimic the phenology we see in the field, but that should take only a small amount of time, because we have recently completed the phenology model in our SCRI grant. We expect that we can use the residue longevity data developed for *C. nigricornis* to give us a rough approximation of the effects on *C. carnea*, so that we will get more value out of the residue studies done in objective 2.

Results and discussion: We have started the model for OBLR, focusing initially on making the model mimic the phenology we observe with OBLR in the field. After several days working on the project, it became apparent that a single model would not be able to estimate phenology. This is because OBLR has a proportion of its population that undergoes five larval molts and a portion that undergoes six molts. The additional molt adds about 180 DDF (100 DDC) time to each generation and, in retrospect, explains the variability that we see in phenology in the field. The reasons for the differences in life history are not clear, but the effects are rather dramatic on phenology. We can fit the emergence curves of the first five instars in the overwintering generation, but the pupal and adult phenology is shifted by ≈180 DDF from the two different groups, leading to a much different prediction than if we have groups that are only five or six generations. The differences cause our models to underestimate the emergence rate of the portion of the population with five instars and overestimate that with six instars (Fig. 4). This effect is amplified in the pupal and adult emergence of the summer generation, because the shift is doubled to 360 DDF (200 DDC), and the

Fig. 4. Effect of OBLR having five or six larval instars on phenology of the adult stage. "Combined" is similar to what we see in the field.



first-fifth generations are shifted 180 DDF. This makes it appear that the summer generation is protracted compared to the overwintering larvae.

The OBLR model is still possible, but it will require two separate models, one for larvae undergoing five instars and one for larvae undergoing six instars. We can then combine the output of the models to achieve our best estimate of what is happening in the field (see Fig. 4); essentially, we come up with three predictions. This will undoubtedly make the model more complex, but should help us better understand not only pesticide effects, but also phenology. I expect this may also change our management recommendations and those will be updated on DAS once we have evaluated this further.

Plans for 2013: The full OBLR models will be developed this coming year and the *C. nigricornis* model will be developed when the developmental studies are complete. All the models will be validated when the residue studies are far enough along and that component can be added to the models.

CONTINUING PROJECT REPORT Year 2 of 3
WTFRC Project number: CP-11-102

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

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Cooperators: Jay Brunner, WSU-TFREC

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

Other funding sources: USDA-SCRI grant (Enhancing BC in Western Orchard Systems) $2.25M. Approx. $60K from that grant will be used for this project.

WTFRC Collaborative expenses:

Item	2011	2012	**2013**
Salaries	0	0	0
Benefits	0	0	0
Wages	0	0	0
Benefits	0	0	0
RCA Room Rental	0	0	0
Shipping	0	0	0
Supplies	0	0	0
Travel	0	0	0
Miscellaneous	4,000	4,200	4,410
Total	4,000	4,200	**4,410**

Footnotes: WTFRC Collaborative expenses for spraying plots

Budget 1
Organization: WSU-TFREC Contract Administrator: Carrie Johnston, Kevin Larson
Telephone: 509-335-7667/509-663-8181 x221 Email: carriej@wsu.edu / kevin_larson@wsu.edu

Item	2011	2012	**2013**
Salaries[1]	55,000	55,000	55,000
Benefits [2]	18,920	17,820	17,186
Wages	13,440	14,112	18,000
Benefits[3]	2,016	2,117	3,042
Equipment	0	0	0
Supplies[4]	4,600	6,997	7,306
Travel [5]	2,940	3,087	3,241
Plot Fees	0	0	0
Total	96,916	99,133	**103,775**

Footnotes:
[1] Post-doctoral Research Scientist (Andrea Bixby-Brosi)
[2]Benefits 32%
[3]Benefits 16.9%
[4]Includes trapping supplies (nearly 6,000 traps are used in objectives 1 and 2) that cost $5,550 in 2011. Other supplies are field supplies, and any lab supplies needed.
[5] Within-state travel

Objectives:

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

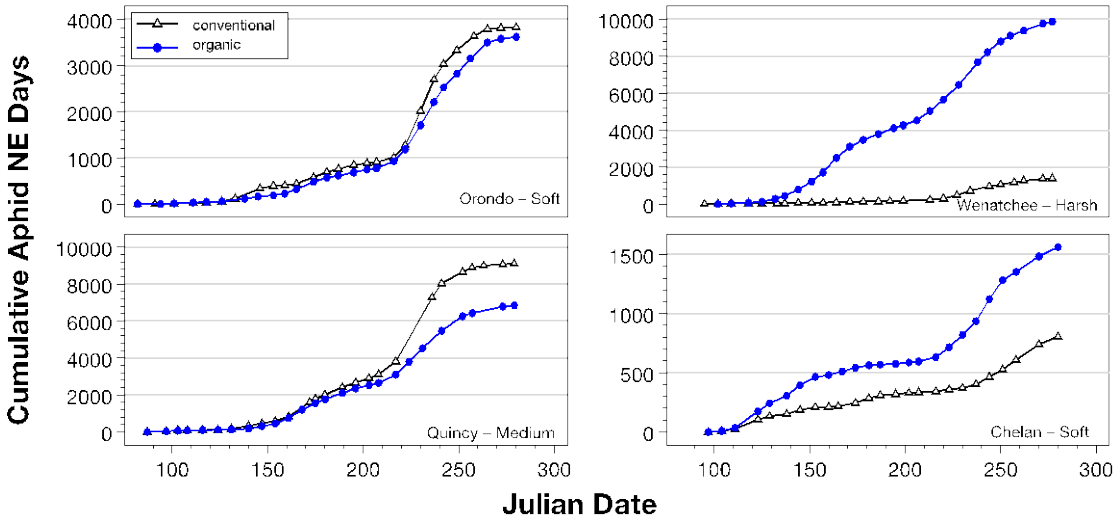
Significant Findings:

- Aphid damage was consistently higher in conventional orchards than in organic orchards.
- The lacewing *Chrysopa nigricornis*, the woolly apple aphid parasitoid *Aphelinus mali*, and syrphid flies appear to respond quickly to prey numbers in orchards with soft or medium harsh management programs. The harsh management pair showed >5 fold reduction in natural enemy populations levels in the conventional treatment compared to the organic treatment.
- Earwig populations were also typically higher in organic blocks than conventional blocks, which probably contribute to the lower aphid numbers in organic blocks.
- The data from the low rate Delegate (10% normal field rate) trial showed no significant differences in codling moth, leafroller, or San Jose scale infestations compared to the full rate Delegate or organic treatments. Effects on natural enemies varied depending on the specific natural enemy and to some extent on the aphid infestation differences.
- Protein marking showed that lacewings moved easily up to 200 feet from marked areas with no pattern of drop-off with distance, suggesting that they are more mobile than previously thought.

Objective 1.

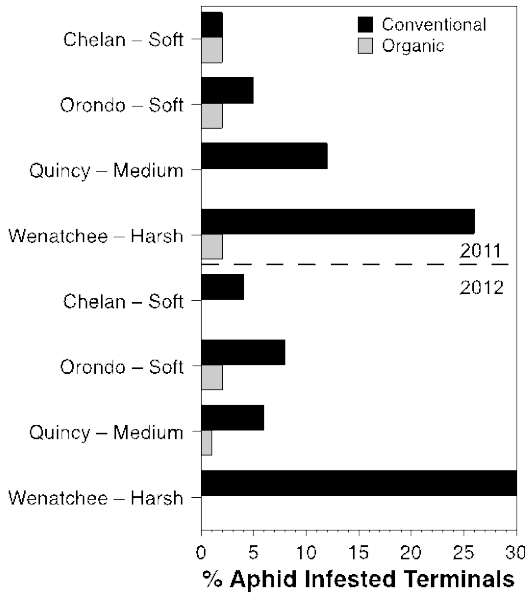
Methods: We have four pairs of orchards separated by <0.5 mile that are sampled intensely for differences in natural enemy (NE) populations using our HIPV traps and earwig (cardboard) bands. These orchards are classed in terms of program harshness to NEs: two pairs are very soft with few differences between the management programs between the conventional and organic treatments (e.g., virus + oil were used in both pairs of orchards), in one pair both conventional and organic had harsh programs (large number of disruptive treatments), and in the final pair both conventional and organic had medium programs (one to several harsh treatments and a few softer materials used at other times in the season). We have completed processing all the 2012 traps, except for traps from

Fig. 1. Cumulative aphid NE days over the 2011 season in 4 pairs of conventional and organic orchards.



July (we skipped July and did August and September and are going back to finish July). We also have not yet received all the spray records for 2012, so the program classifications are based on the 2011 spray records at this point. The discussion is focused on the aphid NEs for brevity. The data suggests that we need to focus on the woolly apple aphid (WAA) parasitoid *Aphelinus mali*, the lacewing *Chrysopa nigricornis*, the lacewing *Chrysoperla plorabunda*, the European earwig (*Forficula auricularia*), the predatory bug, *Deraeocoris brevis*, and several species of syrphids, especially *Eupeodes fumipennis*. To simplify the analysis and presentation, we use a "natural enemy-day" analysis, which is calculated by taking the number of average NE density of the six natural enemies between two sample dates multiplied by the number of days between samples. This gives us an estimate of the amount of potential predation and can be accumulated over the season to give overall differences between orchards. One caution is that each of the NEs eat different amounts of prey and the generalists may eat more than just aphids, thus this is a simplification and the data is skewed by the most common aphid feeding NEs (*C. nigricornis, C. plorabunda,* and the European earwig). Aphids monitored included WAA, green apple aphid (GAA), and rosy apple aphid (RAA).

Fig. 2. Percentage terminals infested by aphids at the end of the season 2011-2012



Results*:* In 2011, the Orondo orchard (soft spray program) had very similar populations in the conventional and organic blocks, but slightly higher damage and NE population levels were found in the conventional block (Figs. 1-2). The Chelan orchards (soft programs) had roughly twice the potential predation in the organic block, with no differences in the aphid infestation in either block. In the Quincy orchards (medium programs), the WAA populations were higher in the conventional block and the composition of the natural enemies shifted to compensate with a nearly five-fold increase in the *A. mali* and a slight increase in the *C. nigricornis* levels, especially later in the season. Populations of *Deraeocoris*, *E. fumipennis*, and *C. plorabunda* were higher in the organic blocks at Quincy, which helped make the overall cumulative NE days closer. In the Wenatchee blocks (harsh programs), the overall NE population levels were >6 fold higher in the organic block, and in the conventional block 30% of the terminals were infested with green apple aphid. Much of the difference in the harsh blocks was the number of *C. nigricornis* (608 versus 4253), but all of the other NEs mentioned above were also considerably more common in the organic block.

The results in 2012 follow similar trends, although the total number of NEs caught was much higher (even though we have not yet processed all the samples yet). In three of the orchard pairs, the number of NEs in conventional blocks did not change significantly, but the numbers in organic pairs were 2-3 fold higher than in 2011 in two of the three pairs. The Quincy pair (classified as having a medium harsh spray program) had a very high WAA population in the conventional block (40% terminals infested in July), with only 2% terminals infested in the organic block at the same time. This resulted in a doubling of *A. mali* populations from 2011, and which was nearly 10x higher than in the organic block. The *C. nigricornis* populations also were nearly 2 fold higher (3510 versus 1864 caught) and the *E. fumipennis* population was similarly nearly 3x higher in the conventional block. The only major NEs in higher numbers in the organic blocks were *Deraeocoris* and the European earwig. The earwig numbers were highest at the end of the year, but added up to similar NE days, indicating that they might be a key NE for cleaning up aerial colonies of woolly apple aphid.

Our results over the two years show some NEs (e.g., *C. nigricornis, A. mali*) respond rapidly and aggregate in the areas with the highest aphid density. However, other species (e.g., *C. plorabunda, Deraeocoris*) do not appear to respond as quickly, are poor dispersers, or are impacted by the pesticides applied to a greater extent in the conventional blocks. Overall, organic orchards had a larger and more diverse NE complex in both years, and the differences in the NE complex between the management programs were magnified in the medium and harsh program pairs of orchards. The medium harsh blocks still allowed response of *A. mali, E. fumipennis,* and *C. nigricornis*, but the European earwig (which has a very long lifespan and only one generation per year) was nearly non-existent in the conventional block with high WAA populations until the start of August. In the blocks with harsh management programs, the organic blocks had roughly 6x higher NE populations and considerably fewer aphids at the end of the season.

Other pests: We also collected data on leafrollers, San Jose scale, codling moth, and mites. There were no significant differences in damage or abundance for any of those groups (mites are not yet tabulated) between the orchard pairs.

Work next year: This coming year, we will continue the study and, with the larger data set, start to investigate the importance of each NE in terms of each pest. We will take more frequent aphid samples to better define the role of NEs in aphid suppression. These steps will allow us to assess the most important NE targets for conservation BC.

Objective 2.
Methods: The evaluation of low dose pesticide applications to minimize NE impacts while maintaining low pest density was continued for the second year. The entire block is under mating disruption, and all plots had a single 1% horticultural oil spray applied at ≈375 DD to allow us to use delayed first cover application of other materials. The organic treatments were Cyd-X HP applied

Fig. 3. The cumulative natural enemy days for the woolly apple aphid parasitiod (*A. mali*), the mirid bug predator *Deraeocoris brevis,* and two lacewing species. The solid diamonds represent spray timing of the organic materials and reduced field rates of Delegate®, the open diamonds when the full rate of Delegate® was applied in the conventional plots. The very first application is oil in all blocks.

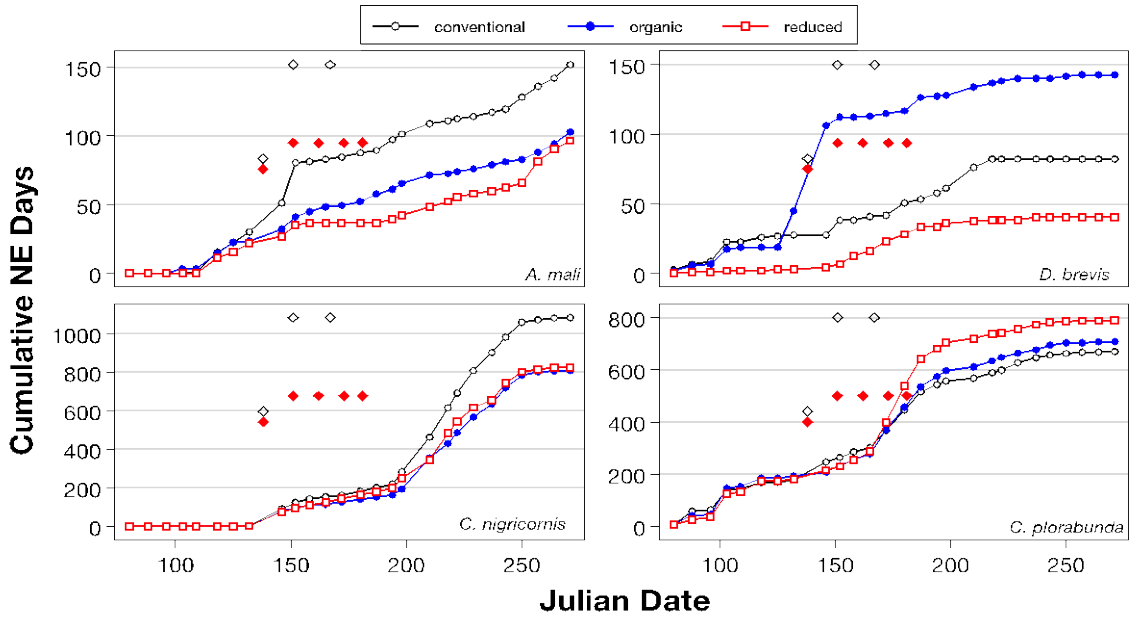
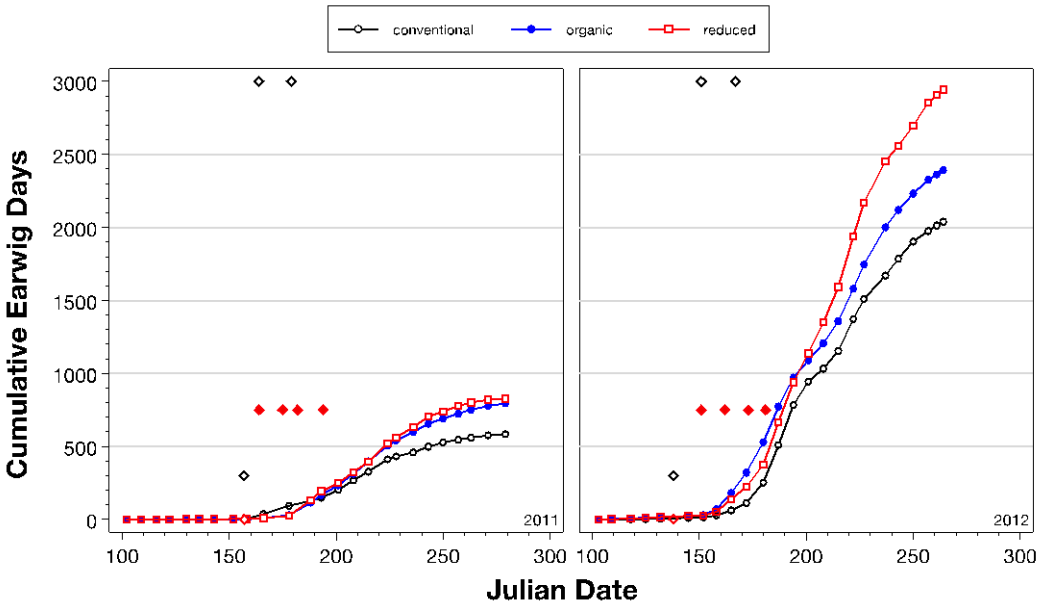


Fig. 4. Cumulative earwig-days occurring at the Sunrise orchard in 2011-12. Open diamonds are the spray timings for the conventional treatments, and solid diamonds are the spray timings for organic and reduced rate applications. The very first application is oil in all



four times in the first generation starting at 540 DD and repeated at ≈10 day intervals. The reduced rate material was a 10% field rate of Delegate (0.7 oz) applied at the same times as the organic treatments. The conventional treatment was a full rate of Delegate (7 oz) applied at 540 DD and then 15 days later. No treatments were applied in the second generation and no other treatments were applied in the block in either year.

Results: No significant differences in damage from codling moth, leafroller, or San Jose scale were found either within the season or at the end of the season. In early July we did see 6% of the terminals in the conventional treatment infested with woolly apple aphid, versus 2.75% and 2.5% terminals infested in the organic and reduced rate treatments, respectively. However, by seasons end, the WAA population on terminals was virtually non-existent (<0.25 infested terminals, in the reduced treatment, none in the other two).

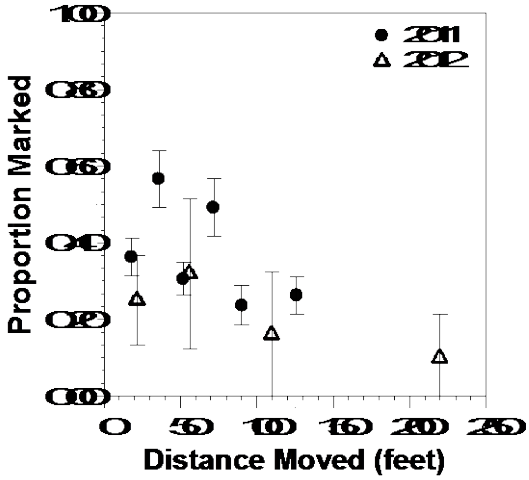
Evaluating the NE days associated with the major aphid predators, it is clear that *C. nigricornis* was more common in the conventional treatment as was *A. mali*, while *C. plorabunda* was more common in the reduced rate treatments, and *Deraeocoris brevis* was most common the in organic block (Fig. 3). This is similar to the results we saw with the conventional–organic paired blocks (objective 1), where areas with the highest density of WAA had the greatest *C. nigricornis* levels, but *C. plorabunda* and the other aphid predators did not respond as quickly. In all cases, it appears that the natural enemy populations were somewhat suppressed by the sprays (the curves tend to be flat in the time frame when the sprays were applied).

We also sampled earwigs (a WAA & generalist predator) using rolled cardboard traps, and the earwig predator days were considerably higher this year than last (Fig. 4). There appeared to be no real effects of the different applications on the accumulation of earwig-days (*i.e.*, compare the shape of the organic curves to the other two), but in both years the reduced rate treatment ended up with the highest level of potential predation (earwig days) from the earwigs. By comparing figures 3 and 4, it is apparent that *A. mali* and *D. brevis* accumulate fewer natural enemy days (again, a measure of potential predation) than the two lacewings or the earwigs. However, this difference may be of minor

importance and simply be a function of how attracted *A. mali* and *D. brevis* are to our traps compared to the other species. The important consideration is the differences in the population trends between the three different treatments, not the absolute magnitude of what we recovered.

Work next year: Next year we will change the spray program to include second-generation sprays so that we can evaluate effects of harsher programs on NE diversity and abundance. We will also focus more on the aphids, taking samples every 2-3 weeks throughout the season to assess potential disruption of the natural enemies and outbreaks of the WAA.

Fig. 5. Proportion of lacewings at different distances from the treated area testing positive for egg protein in 2011 and 2012

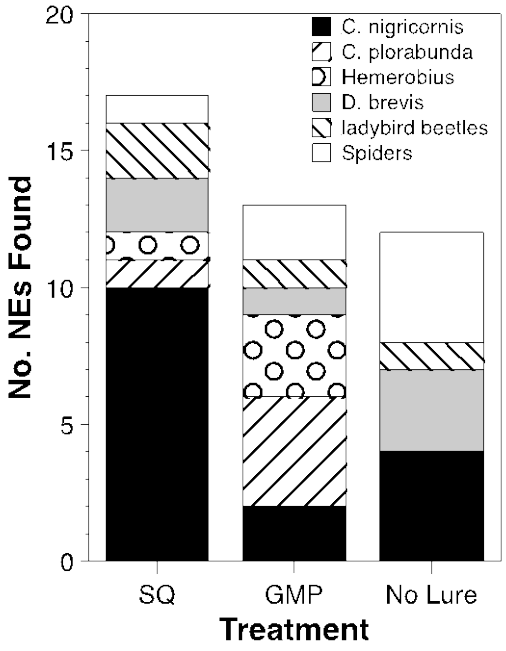


Objective 3.
Methods: *Measuring NE movement*
Large-scale field studies were conducted in grower fields (>50 acres). We used the egg-white based protein marking system to track NE movement patterns and how the lures affect them. In 2011, we sprayed two rows (× 2200 ft.) of trees in the center of our experiment block, and lure/trap combinations were placed at various distances away from marked trees across rows (18-126 ft.). In August 2012, we focused on distances traveled by NEs within rows by treating single trees with egg whites using a backpack sprayer, then hung traps and lures at various distances away (22-220 ft., within the same row).

Results*:* In 2011 and 2012, green lacewings (GLW), *C. nigricornis*, and *C. plorabunda* made up the bulk of NEs caught in traps. In 2011, positively marked GLWs were found in traps at all distances away from marked trees. There were no large differences in the proportion of marked moths at the different distances (Fig. 5), suggesting that GLWs are active fliers and are much more mobile than we had previously thought. In 2012, overall numbers of NEs captured was lower and the proportion testing positive for the mark was slightly lower, but similar trends occurred.

Methods: *Biological control contribution of NEs responding to HIPVs*
In July 2012, a field experiment was conducted to evaluate NE response to HIPV lures. Small aphid colonies (25 aphids/colony) infesting 36 small potted Jonagold apple trees were used as indicators of predation. We hypothesized that aphid disappearance due to predation would occur at a greater rate on trees with an HIPV lure as opposed to trees without lures.

Fig. 6. Number of NEs caught or observed on trees with and without lures.



Results: The day after treatments were placed in the field, many of the aphids had already disappeared. Out of 36 trees, 15 contained less than five aphids each, while three trees contained colonies that were mostly intact (≈25 aphids). All three trees with intact

colonies had no HIPV lures. Aphid predators observed resting on trees and in traps, included *C. nigricornis, C. plorabunda, Deraeocoris brevis, Hemerobius* sp. (brown lacewings), ladybird beetles, and spiders. The most abundant NE, *C. nigricornis*, was found more often on trees with lures, and brown lacewings and *C. plorabunda* were only found on trees with lures (Fig. 6). Our results suggest that NEs responded to aphid cues and/or HIPV lures very quickly. We observed more NEs on trees with HIPV lures than without, and considering that intact aphid colonies were found only in no-lure treatments, it is likely that HIPV lures do contribute to aphid suppression. Experiments where we placed a lure in the tree and then observed how long it took NEs to respond showed the lures recruited NEs within 30 minutes of placement in the field, providing greater confidence to our supposition that response by NEs to HIPV lures can be quite rapid.

Work next year: The focus next year will be to evaluate not only NE aggregation, but to also evaluate the area of influence of the lures. This will be done by choosing locations with aphid infestations, counting the infestation on the adjacent 10 trees in each direction within a row. Two minute NE counts will then be done on each tree and recorded. We will then place a lure on the central tree and follow the aphid infestations and NE trends (using the timed samples) on each tree for 2-3 weeks. This will be replicated in a large non-bearing block, and every 2-3 weeks we will break down the experiment, move to a new area, and set up new experiment. This should give us information as to the scale of the effects of lures not only on NE numbers, but also on the aphid infestations and how the NE counts vary over time.

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

Project Title: Integrating codling moth granulovirus into conventional orchards

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Organization: USDA, ARS
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City/State/Zip: Wapato, WA 98951

Cooperator: Dr. Mike Dimmock, Certis USA

Total Project Request: **Year 1:** $21,000 **Year 2:** $21,000

Other funding sources

Agency Name: California Pear Advisory Board
Amt. requested: $10,500

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

Item	2012	2013
Salaries		
Benefits		
Wages	17,454	17,454
Benefits	1,546	1,546
Equipment		
Supplies		
Travel	2,000	2,000
Plot Fees		
Miscellaneous		
Total	21,000	**21,000**

OBJECTIVES

The overall objective of this project is to develop effective feeding stimulants for codling moth larvae that can significantly improve the performance of selective insecticides. Selective insecticides are materials that are toxic for codling moth and whose use will not disrupt the biological control of mites, aphids, or other secondary pests. These include the microbial insecticides, CpGV and Bt's, and synthetic compounds such as Intrepid and Altacor. Specific objectives include conducting laboratory bioassays with possible feeding stimulants including naturally occurring yeasts isolated from codling moth larvae, commercial bread yeast, monosodium glutamate, L-aspartate, and Monterey Insect Bait. The second specific objective of this project is to evaluate a promising subset of the most effective feeding stimulants characterized in the laboratory bioassays with and without the addition of a microencapsulated formulation of pear ester, a known larval attractant that stimulates non-host finding activities.

The goals for the second year of the project include both the further development of effective feeding stimulants through bioassay screening and continued field testing of the most effective materials in field plots. Laboratory bioassays are continuing until May. Field trials begin in late May. The anticipated accomplishments include the demonstration of improved efficacy of one or more selective insecticides with the addition of one or more feeding stimulants for control of codling moth.

SIGNIFICANT FINDNGS

- A *Metschnikowia* sp. yeast collected from codling moth larvae was shown to enhance larval survivorship and accelerate larval development within surface-treated apples compared with sterilized fruits.
- The addition of the *Metschnikowia sp.* yeast with cane sugar significantly improved the efficacy of a CpGV insecticide in laboratory bioassays.
- Three additional species of yeast were isolated from codling moth field-collected larvae and were found to also improve the performance of CpGV in laboratory bioassays..
- Other materials, such as active bread yeast with sugar, the amino acid, L-aspartate with sugar, and the Monterey Insect Bait were found to be very effective in improving the activity of the CpGV in laboratory bioassays. MSG with or without sugar was not very effective in similar bioassays.
- Adjuvants with yeasts cannot be used with Bt insecticides.
- Field trials with *Metschnikowia* sp. yeasts and bread yeast both with sugar significantly increased larval mortality and reduced fruit injury in a season-long virus spray program. The addition of MSG or L-aspartate without sugar enhanced the kill of larvae, but did not add any protection of the fruit from codling moth injury.
- The addition of the microencapsulated pear ester formulation did not improve the efficacy of CpGV with or without bread yeast and sugar added.
- Fruit injury from San Jose Scale was significantly reduced with the addition of the yeasts plus sugar.

METHODS

In the laboratory a standardized bioassay has been developed (Fig. 1). Bioassays are conducted with field-collected unsprayed apples stored at 2 ^{o}C. Fruits are rinsed, soaked in 10% bleach, air-dried, dipped into solutions, and allowed to air dry. . Solutions of yeasts with or without brown sugar are added to insecticides at rates of 1-3 lbs per 100 gallons. MSG and L-aspartate have been tested at rates form 0.1 – 3 lbs. Monterey insect Bait has been tested at 2 qts. Blossom Protect was tested at 1.25 lbs. Apples dipped in distilled water are the untreated controls. Insecticides are tested at low rates to allow ca. 30-50% survivorship without the addition of the adjuvants. Gelatin capsules are glued to the upper rim of fruits and a single black-headed codling moth egg is placed inside each capsule. Eggs are provided by the USDA insect rearing facility. After 14 d at 25 ^{o}C the gelatin

capsules are removed from the fruit and the apple is cut to retrieve the larva. Larvae are scored as dead or alive, and the instar and depth of fruit penetration is measured.

Field trials are conducted at the USDA Research Farm on several cultivars of apples and in pears beginning in 2013. The experimental design is the use of randomized single-tree plots (N = 10) spaced 10 m apart and sprayed with a hand-gun to deliver 100 gpa. Studies in 2012 evaluated the use of adjuvants to improve the CpGV insecticide, Cyd-X. Similar studies will continue in 2013 and will test the use of the Monterey Insect Bait, L-aspartate with sugar, a dry bread yeast formulation with sugar, and the new species of *Cryptococcus* yeast isolated from codling moth larvae with sugar. Three additional studies will compare a CpGV plus Entrust program with or without an organic bread yeast and sugar, a CpGV (1st generation) plus Bt (2nd generation) program with or without the Monterey Insect Bait added to each spray, and a conventional program using selective materials. The structure of this insecticide program will be determined based on the results of the laboratory bioassays this spring.

RESULTS

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

Not all the laboratory data included in this report was available prior to the start of the 2012 field season so four materials were selected based on limited information (Tables 2 and 3). Both the *Metschnikowia* and bread yeast were applied with 3 lbs of sugar per 100 gallons. Both MSG and L-aspartate were applied at a 1 lb rate without any sugar. The PE MEC was tested with the bread yeast at 12 ml per acre.

The addition of the yeasts with sugar significantly reduced codling moth fruit injury compared with the virus alone. The virus applied at the low rate of 1 oz per 100 gal was not very effective in preventing fruit injury under these high pressure conditions. Unexpectedly, the use of the yeasts with sugar significantly reduced fruit injury from San Jose scale. Levels of leafroller injury were also lower with the addition of the yeasts compared to the untreated controls. The use of PE MEC with CpGV provided no additional control. Both MSG and L-aspartate significantly increased larval morality but levels of fruit injury were not reduced compare with the virus alone. The addition of all adjuvants except PE MEC significantly increased the proportion of dead larvae and decreased the proportion of live larvae in or exiting the fruits in fruits (Table 3). The insecticide program with Altacor and Delegate was much more effective than the virus programs in protecting the fruits. However, the proportion of live and dead larvae were similar among the insecticide and the virus plus adjuvant programs (Table 3).

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

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

Materials in shaded rows were tested in 2012 field trials.

Fig. 1 Gel capsule bioassay.

Table 2. Summary of insect-caused fruit injury in four replicated (N = 10) apple studies.

		Mean (SE) % fruit injury		
Orchard	Treatment [a]	CM	LR	SJS
1	UTC	39.7 (7.1)a	36.0 (3.6)a	44.7 (6.2)a
	CpGV	33.3 (3.4)b	21.8 (3.7)ab	33.8 (6.7)ab
	CpGV + MY3 + S3	21.1 (2.70c	9.7 (2.0)bc	15.4 (4.3)bc
	Insecticides [b]	1.5 (0.4)d	5.0 (5.0)c	8.9 (6.5)c
	ANOVA	$F_{3, 26} = 90.39$ $P < 0.0001$	$F_{3, 26} = 13.68$ $P < 0.0001$	$F_{3, 26} = 6.57$ $P < 0.01$
2	UTC	48.9 (5.1)a	28.0 (3.3)a	34.3 (5.2)a
	CpGV	38.3 (3.1)ab	14.7 (3.6)ab	25.7 (7.1)ab
	CpGV + PE	33.1 (3.8)bc	16.4 (3.4)ab	21.4 (4.7)abc
	CpGV + BY3 + S3	21.7 (2.4)c	12.1 (3.0)b	12.0 (3.7)bc
	CpGV + PE + BY3 + S3	25.7 (2.4)bc	14.0 (2.30ab	8.7 (2.1)bc
	Insecticides [b]	1.9 (0.010d	4.0 (2.8)c	9.1 (4.7)c
	ANOVA	$F_{5,}$ 54 = 38.54 $P < 0.001$	$F_{5, 54} = 9.41$ $P < 0.0001$	$F_{5, 54}$ = 5.71 $P < 0.001$
3	UTC	28.1 (0.2)a	-	-
	CpGV	19.2 (2.7)b	-	-
	CpGV + MSG1	23.0 (2.8)ab	-	-
	CpGV + ASP1	16.6 (1.3)b	-	-
	Insecticide[b]	1.3 (0.1)c	-	-
	ANOVA	$F_{4, 45} = 45.24$ $P < 0.0001$	-	-

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

[a] CpGV was applied as 1 oz of CpGV in 100 GPA. Virus was applied on ten dates during the season.

[b] The standard insecticide program was three sprays of Rynaxypyr (4 oz per 100 gpa) in the first generation and three sprays of spinetoram (6.7 oz per 100 gpa) in the second generation.

DISCUSSION

Tremendous progress was made in this project during 2012 in developing feeding adjuvants to enhance the toxicity of insecticides for codling moth. This is the first study to use live yeasts to enhance insecticides. The activity of several wild yeasts isolated from codling moth was characterized as high in combination with CpGV. However, an inexpensive and readily available yeast, *Saccharomyces cerevisiae* was also found to exhibit significant activity. Previous studies reporting minimal activity from adding sugar alone to CpGV were confirmed. The addition of PE MEC with virus provided no additional activity. The level of activity from adding MSG was shown to be low, but the amino acid L-aspartate looked promising with sugar. Laboratory trials suggest that the new material, Monterey Insect Bait is a promising adjuvant for codling moth and should be evaluated in field trials. Studies during the second year of the project will evaluate the most promising and cost effective adjuvants with a larger number of selective insecticides, including the use of Bt, insect growth regulators such as Intrepid, and Altacor from the diamide insecticide class.

Table 3. Characterization of fruit injury in four field trials with replicated apple plots (N = 10) following a seasonal spray program evaluating various materials to codling moth granulosis virus (CpGV).

		Mean (SE) proportion CM injury					
		Dead larvae			Live larvae		
Orchard	Treatment [a]	Sting	Entry	All	Entry	Exit	All
1	UTC	0.14 (0.03)b	0.00 (0.00)b	0.14 (0.03)b	0.41 (0.03)a	0.45 (0.04)a	0.86 (0.03)a
	CpGV	0.39 (0.03)ab	0.04 (0.01)b	0.43 (0.04)b	0.19 0.02)b	0.38 (0.03)a	0.57 (0.04)a
	CpGV + MY + S	0.61 (0.03)a	0.20 (0.03)a	0.81 (0.02)a	0.04 (0.01)c	0.15 (0.01)b	0.19 (0.02)b
	Insecticides [b]	0.48 (0.18)ab	0.00 (0.00)b	0.48 (0.18)ab	0.30 (0.10)ab	0.22 (0.10)b	0.52 (0.18)ab
	ANOVA	F = 5.18	F = 29.43	F = 10.98	F = 15.12	F = 8.55	F = 10.98
	df = 3, 26	P < 0.01	P < 0.0001	P < 0.001	P < 0.0001	P < 0.001	P < 0.001
2	UTC	0.17 (0.02)d	0.02 (0.01)b	0.18 (0.03)b	0.45 (0.03)a	0.37 (0.04)a	0.82 (0.03)a
	CpGV	0.38 (0.04)bcd	0.03 (0.01)b	0.41 (0.04)b	0.21 (0.03)b	0.38 (0.03)a	0.59 (0.04)a
	CpGV + PE	0.37 (0.02)cd	0.05 (0.01)b	0.42 (0.02)b	0.20 (0.02)b	0.38 (0.02)a	0.58 (0.02)a
	CpGV + BY + S	0.64 (0.03)ab	0.17 (0.02)a	0.81 (0.02)a	0.05 (0.01)c	0.14 (0.01)b	0.19 (0.01)b
	CpGV + PE + BY + S	0.56 (0.02)abc	0.18 (0.02)a	0.74 (0.02)a	0.09 (0.01)bc	0.18 (0.02)b	0.27 (0.02)b
	Insecticides [b]	0.62 (0.10)a	0.07 (0.03)b	0.69 (0.11)a	0.18 (0.06)bc	0.13 (0.06)b	0.31 (0.11)b
	ANOVA	F = 9.57	F = 14.13	F = 13.40	F = 14.27	F = 13.12	F = 13.24
	df = 5, 54	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
3	UTC	0.09 (0.02)d	0.01 (0.01)b	0.10 (0.02)c	0.43 (0.02)a	0.47 (0.02)a	0.90 (0.02)a
	CpGV	0.45 (0.04)c	0.02 (0.01)b	0.47 (0.04)b	0.25 (0.02)b	0.28 (0.03)b	0.53 (0.04)b
	CpGV + MSG	0.70 (0.02)ab	0.12 (0.01)a	0.82 (0.02)a	0.07 (0.01)c	0.10 (0.02)c	0.18 (0.02)c
	CpGV + ASP	0.69 (0.02)b	0.13 (0.02)a	0.81 (0.02)a	0.06 (0.01)c	0.13 (0.01)c	0.19 (0.02)c
	Insecticide[b]	0.80 (0.09)a	0.02 (0.02)b	0.82 (0.09)a	0.08 (0.04)c	0.10 (0.05)c	0.l8 (0.09)c
	ANOVA	F = 37.20	F = 22.22	F = 43.36	F = 30.33	F = 24.09	F = 43.27
	df = 4, 43	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

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

[a] *CpGV* was applied as 1 oz of CpGV in 100 GPA. The *Metschnikowia* and bread yeast and cane sugar were added at 3 lbs per 100 gallons. Virus sprays were applied on ten dates during the season.

[b] The standard insecticide program was three sprays of Rynaxypyr (4 oz per 100 gpa) in the first generation and three sprays of spinetoram (6.7 oz per 100 gpa) in the second generation.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-12-101

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

PI: Stephen F. Garczynski
Organization: USDA-ARS
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City/State/Zip: Wapato, WA 98951

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

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

Other funding sources

Agency Name: **National Institute of Food and Agriculture (NIFA)**
Amt. requested: **$ 426,000**
Notes: A preproposal based on this WTFRC proposal was submitted to the AFRI program supported by NIFA and received an invitation for submission of a full proposal (due 2/19/2013)

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

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

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

OBJECTIVES

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

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

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

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

SPECIFIC OBJECTIVES FOR YEAR 2

1) Expression of odorant binding protein and odorant degrading enzyme clones in bacteria
2) Purification of proteins expressed in bacteria
3) Flight tunnel behavioral studies with codlemone based parapheromone
4) Studies to determine if parapheromone disrupts mating
5) Cell-based assays to identify odorant receptors that bind codlemone
6) Quantitative PCR studies to determine relative abundance of proteins that interact with codlemone

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS)

- Cloned gene transcripts encoding six candidate codlemone odorant binding proteins
- Cloned gene transcripts encoding four candidate odorant degrading enzymes
- Cloned three additional candidate pheromone family receptors
- Generated antibodies that recognize with two different odorant binding proteins
- Codlemone-based parapheromone was synthesized and used in a preliminary (very preliminary) trap trial

METHODS (PROJECT APPROACH)

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

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

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

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

4) Flight tunnel studies using ODE inhibitors and parapheromones. A variety of chemical inhibitors of ODEs are commercially available. We will treat codling moth with these inhibitors to disrupt ODE function, and then use flight tunnel studies to determine the effects on codling moth

attraction to codlemone. We will score moth response to codlemone with and without inhibitor treatments and positive effects will be either disruption of codling moth attraction or enhanced responses to the pheromone source. In a recent collaboration, a chemical modification of the navel orangeworm sex pheromone made this new semiochemical more potent than the original pheromone. We will have a modified codlemone parapheromone synthesized to determine if this modification will produce a more potent attractant. To test the potency, flight tunnel studies will be used as above. If this parapheromone is attractive to codling moth, we will proceed with a limited field trial to determine if it is more effective than codlemone for attracting males.

RESULTS AND DISCUSSION

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

Cloning gene transcripts encoding for proteins involved in the regulation of codlemone signaling

In previous WTFRC funded projects, we have been able to identify several codling moth gene transcripts encoding proteins related to those in other moths that are thought to play important roles in sex pheromone signaling. Using nucleotide sequence information derived from codling moth antennal transcriptomes, we first designed oligonucleotide primers specific to each gene transcript for use in PCR based cloning strategies. This accomplishes two purposes; one to verify the presence of these gene transcripts in codling moth antennae and secondly to confirm the transcriptome nucleotide sequence data. We focused our cloning efforts on gene transcripts encoding for odorant binding proteins, odorant receptors and odorant degrading enzymes because of their proposed role in pheromone signaling (Fig.1).

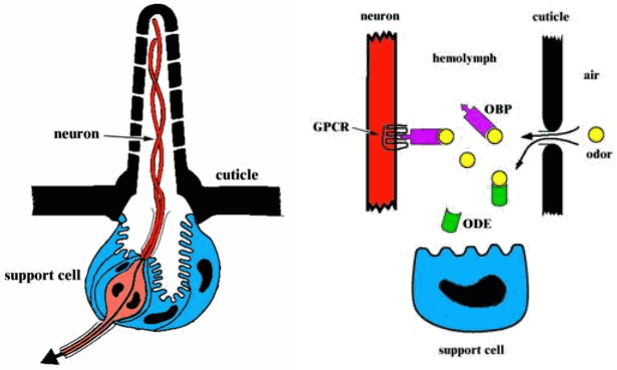

Fig. 1. Schematic of an olfactory trichoid sensillum and a generalized pathway of odor reception. ***Sensillum Structure*** (left illustration). An olfactory sensillum includes 2-3 neurons whose olfactory dendrites/cilia project up the fluid filled lumen of a cuticular hair. The sensillum lumen is isolated from hemolymph by a cellular barrier provided by 3 support cells. ***Perireceptor Events*** (right illustration). Hydrophobic odor molecules enter the aqueous sensillum lumen via pores penetrating the cuticular hair wall. Hydrophilic Odorant Binding Proteins (OBPs) are proposed to bind and transport odors to receptor proteins located in the neuronal membranes. Odorant Degrading Enzymes (ODEs) in the sensillum lumen are proposed to degrade these odor molecules. Figure created from components found at http://www.inscent.com/chemosensory_system.php.

Odorant binding proteins (OBPs) are small, soluble polypeptides and are thought to have key roles in perireceptor events leading to chemoreceptor signaling events whereby they bind hydrophobic odorants and transport them through the aqueous lymph of olfactory sensillum and presenting the odorant to an appropriate odorant receptor. Pheromone binding proteins (PBPs) and general odorant binding proteins (GOBPs) are defined as subfamilies within the clade of OBPs. As with other members of the OBP protein family, PBPs and GOBPs also have a tertiary structure that is coordinated by the formation of three disulfide bridges. PBPs and GOBPs are mainly expressed in antennae, with PBPs usually localized to trichoid sensilla and GOBPs usually to basiconic and trichoid sensilla. Both PBPs and GOBPs have been shown to bind pheromones, and GOBP2 has also been shown to bind plant volatiles suggesting a possible role in host finding. While much is known about the structure and ligand binding of PBPs and GOBPs, their roles in pheromone and host plant reception remains incomplete. In the past year, we have cloned gene transcripts from codling moth antennae encoding for three PBPs and three GOBPs. Interestingly, only two GOBP transcripts have been found in other moths. In the next year we will use the clones encoding for PBPs and GOBPs to produce these proteins for codlemone interaction assays.

Odorant receptors (ORs) are seven transmembrane domain proteins that are present in the membranes of olfactory neurons. When an odorant binds to its OR, it causes nerve firing which sends a signal to the brain leading to a behavioral response. Some ORs belonging to the pheromone receptor subfamily have been shown to interact with sex pheromones. For codling moth, we have previously identified five gene transcripts encoding for ORs that are members of the pheromone receptor subfamily. In the past year, we have identified three additional codling moth gene transcripts encoding for ORs of the pheromone receptor subfamily, bringing our total to eight candidate codlemone receptors. We have cloned one putative codlemone receptor into a mammalian expression vector and stable cell lines expressing this receptor have been generated for use in cell-based assays to determine if it binds codlemone. Preliminary results from the cell based assay indicate that this receptor (called OR11) does indeed interact with codlemone.

Odorant degrading enzymes (ODEs) are present in the sensillum lymph and their role is to degrade odorants, which is thought to be important for regulation of nerve firing and to clear the lymph of excess odorant molecules. Antennal carboxylesterases have been shown to degrade sex pheromones in other moth systems. We have cloned four gene transcripts encoding carboxylesterases expressed in codling moth antennae which will be used to produce these proteins for use in codlemone degradation assays.

Generation of antibodies that recognize GOBPs

The deduced amino acid sequences from the cloned gene transcripts encoding for codling moth GOBPs were used to generate antibodies that can be used to identify these proteins. Because codling moth GOBPs share much amino acid similarities, we had to generate antibodies using peptide fragments unique to each GOBP. The antibodies were generated by a company (GenScript, Piscataway, NJ) that synthesized peptides based on GOBP amino acid sequences and used these peptides to generate a polyclonal antibody pool in rabbits. These antibodies were then used to detect GOBPs present in protein extracts from codling moth antennae. We had success with two of the antibodies, those generated to detect codling moth GOBP1 and GOBP2. In the future, these antibodies will be used in immunocytological studies to localize the antennal sensillum where these proteins are present, in studies to determine basal levels GOBPs, and to determine if protein amounts increase in response increased concentrations of odorants.

Synthesis of a codlemone-based parapheromone and a very preliminary field trial

Through a collaboration with a synthesis chemist, Dr. Jocelyn Millar (University of California, Davis), we had a codlemone-based parapheromone synthesized. Alan Knight (USDA,

Wapato) set up traps with codlemone, the parapheromone, or a combination of codlemone and parapheromone for field catch studies. In this initial study, moths were attracted to traps containing only codlemone, but no moths were caught in the parapheromone trap. Most interesting from this limited study was that traps containing equal amounts of both codlemone and the parapheromone did not attract codling moth. We hypothesize that the parapheromone may have repellent activity and we will be testing this in the upcoming year through flight tunnel behavioral assays, mating assays and further trapping studies.

Summary

In year one of this project we have cloned gene transcripts encoding proteins thought to play a critical role in sex pheromone signaling in codling moth. These clones will be used to produce the proteins that they encode so that we can proceed with the assays to determine their roles in codlemone binding, signaling and behavioral response. We have also worked on developing a reliable, reproducible and sensitive cell-based binding assay to examine OR/codlemone interactions. Furthermore, we have a codlemone based parapheromone in hand that appears to have a potential repellent activity which will be determined in flight tunnel, mating and field studies to determine if it may be useful in control of codling moth in the orchard. Thanks to WTFRC support of this project, a preproposal submitted to USDA National Institute of Food and Agriculture (NIFA) Agriculture and Food Research Initiative (AFRI) has been invited for a full proposal submission!

CONTINUING PROJECT REPORT **YEAR**: Years 2 of 3
WTFRC Project Number: CP11-103

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

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Cooperators: Keith Granger, WSU TFREC, WA.

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

Other funding sources: NONE
WTFRC Collaborative expenses: NONE

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

Item	2011	2012	2013
Salaries (grad student) [1]	22,901	23,817	26,055
Benefits [1]	1,895	1,970	2,207
Wages [2]	10,094	15,840	15,120
Benefits [3]	514	2,772	1,466
Equipment	0	0	0
Supplies [4]	1,500	6,500	6,000
Plot fees [5]	0	2,000	2,000
Travel [6]	1,000	500	500
Miscellaneous	0	0	0
Total	37,904	53,399	**53,348***

Footnotes:
**** The 2013 budget is approximately $11,000 higher than initially anticipated. This is due to the cost labor required to conduct bioassays with leafroller and codling moth. This cost was expected to decline in 2013, however, projected work in this area will remain as the level as in 2012 in order to meet project objectives.***
[1] Joseph Schwarz, PhD student
[2] Temporary summer labor – three people at $11/h @ 40h/wk for 12 weeks
[3] 9.7%
[4] Rearing supplies, leafroller colony rearing supplies
[5] Maintenance fees for two 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. A whole-leaf bioassay for OBLR, developed in year one (2011) was utilized to assess differences in larval survivorship, development time, pupal weight, and adult fecundity for different genotypes of *Malus*.
2. For some genotypes (e.g., Cox's Orange Pippen), resistance was expressed as a function of the plants' phenology (spring, summer, fall).
3. The expression of resistance for only a short time during the year could be valuable in suppressing a pest like leafroller and could reduce that possibility of the target pest would overcome the plant's resistance.
4. Some apple genotypes (e.g., Antonovka 1.5) appeared to disrupt normal hormone development in OBLR larvae, which inhibited the completion of pupal development, suggesting plant-produced juvenile hormone analogs might be involved.
5. Other mid-eastern genotypes (i.e., KAZ 96-07-06) expressed high mortality to OBLR larvae. No larvae survived to pupate. More importantly, prior to death, most larvae showed signs of hemorrhaging, suggesting the action of plant proteases on the digestive system of OBLR.
6. Oil as a residue on foliage was shown to be highly toxic to young OBLR larvae while the codling moth virus had no effects.

Methods:

Identify and characterize leafroller resistance.
The bioassay method developed in 2011 provided leaf quality over time to measure key developmental parameters in 2012. The bioassay involved using a whole leaf placed in a large Petri dish (94 mm X 16 mm) with the leaf petiole placed inside an Eppendorf vial (2.0 ml) that contained water. The Eppendorf vial was inserted through a hole in the side of the plastic Petri dish and sealed with Teflon tape to prevent larval escapes – 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 emergence was recorded, and adults were placed inside a mating/oviposition cage and the number of egg masses deposited was recorded. Egg masses were placed individually in a small plastic Petri dish (with diet) and the number of larvae per egg mass was recorded.

Figure 1. Whole leaf bioassay method developed for OBLR.

OBLR feed on apple foliage at three distinct periods: in spring as overwintered larvae, in summer as a new generation of larvae, and again in fall as young second-generation larvae prior to entering overwintering hibernacula. Since resistance may be variably expressed across the plants' phenology, our research in 2012 explored development performance of OBLR in the spring, summer, and fall

periods. We evaluated foliage from 26 *Malus* genotypes in the first two periods. Although OBLR overwinter as second or third instar larvae, to capture the expression of resistance in *Malus* during the fall phenology 11 of the 26 genotypes were also evaluated in that period. About 33 OBLR larvae were used for each *Malus* genotype evaluation in each of the three periods: spring, summer and fall.

A bioassay was developed for CM in 2011. Fruit from different *Malus* genotypes are exposed to newly hatched CM larvae by transferring them to individual fruit in a plastic container. Because the development of CM larvae cannot be observed, the number of mature larva that emerge from each apple and time required to emerge are recorded, as well as the time to adult emergence. Adult CM are placed in a mating/oviposition chamber and the number of eggs laid and number that hatch is recorded.

Localize genes for resistance **-** see original proposal for methods.

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

Results and Discussion:

Characterization of Malus resistance to OBLR. Data on the development time and mortality of OBLR larvae reared on an artificial diet was collected as an independent standard to compare with OBLR larvae reared on leaves of various *Malus* genotypes. Previously reported work showed that under controlled temperature conditions (22% RF; 23°C; 16:8 LD), OBLR larvae were primarily in the second instar after seven days, in the fourth instar after 14 days, in the fifth and sixth instars after 21 days and in the pupal stage after 28 days. These development data provided a time line on which to evaluate OBLR development on leaves, and when to change leaves with minimal disturbance to larvae. 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 the sensitive molt period can increase mortality.

Based on the data generated from 2011, we evaluated 11 *Malus* genotypes (accession number) from the "diversity map set" at Sunrise in 2012 for possible OBLR resistance as part of the phenology study: Antonovka 1.5 (107196), Yellow Transparent (588859), Northern Spy (588872), Viking (589434), Keepsake (589894), Cox's Orange Pippin (5888853), Jonafree (589962), Trent (589490), Lady (589053), and Florina (588747), and Granny Smith (588880). Additionally we evaluated 14 ancestral genotypes from the core diversity set from the Mideast region: KAZ form 181 (613969), sieversii UZB GMAL 3265 (596280), KAZ 95-05-01P-22 (633918), KAZ 96-09-05 (633920), KAZ 96-07-06 (613994), KAZ 95-08-06 (613976), sieversii TAJ GMAL 3244 (596282), sieversii TUR GMAL 2251 (594104), sieversii KYR GMAL 3158 (590043), KAZ 96-05-05 (633919), sieversii KAZ GMAL 3310 (596283), sieversii KYR GMAL 1750 (589380), KAZ 96-09-02 (614000), and KAZ 96-07-03 (613991).

In 2012 OBLR development was observed over three distinct phenology periods (spring, summer and fall) in order to determine if resistance was expressed differentially in each period for a specific *Malus* genotype. Here we report on mortality, development rate, pupal weight, and fecundity parameters of OBLR for twelve *Malus* genotypes, ten that showed variability across two time periods. We then show data for OBLR reared on Cox's OP in all three time periods in which responses were highly variable. We show only mortality data for the 14 Mideast genotypes and then address two varieties of special interest, KAZ 96-07-06 and Antonovka 1.5, that each displayed an interesting form of resistance against OBLR. Finally, we present information in a summarized format by indexing the response of *Malus* genotypes against the most susceptible genotype in each time period for mortality and developmental parameters.

One measure of larval development is the average days required to complete development, that is, to reach the pupal stage. Fig. 2 shows the average days to pupation for male and female OBLR for two different time periods, spring and summer (fall data not shown). The days to pupation (larval longevity) are shorter for males than females. For example, it takes an average of 25 days for males

and 30 days for females to pupate when reared on artificial diet. Longer development times signal that a genotype is less favorable for larval development. For example, long development time means longer exposure of larva to the elements and to predators. Longer development time is also correlated with smaller pupal weight (data not shown). In the spring, larval development on certain genotypes took considerably longer (e.g., Keepsake, Viking, Cox's OP and Trent) than on others (e.g., Northern Spy). Similar trends are shown for the summer period on certain genotypes. For example, male development times were especially long on Cox's OP, Viking, and Yellow Transparent.

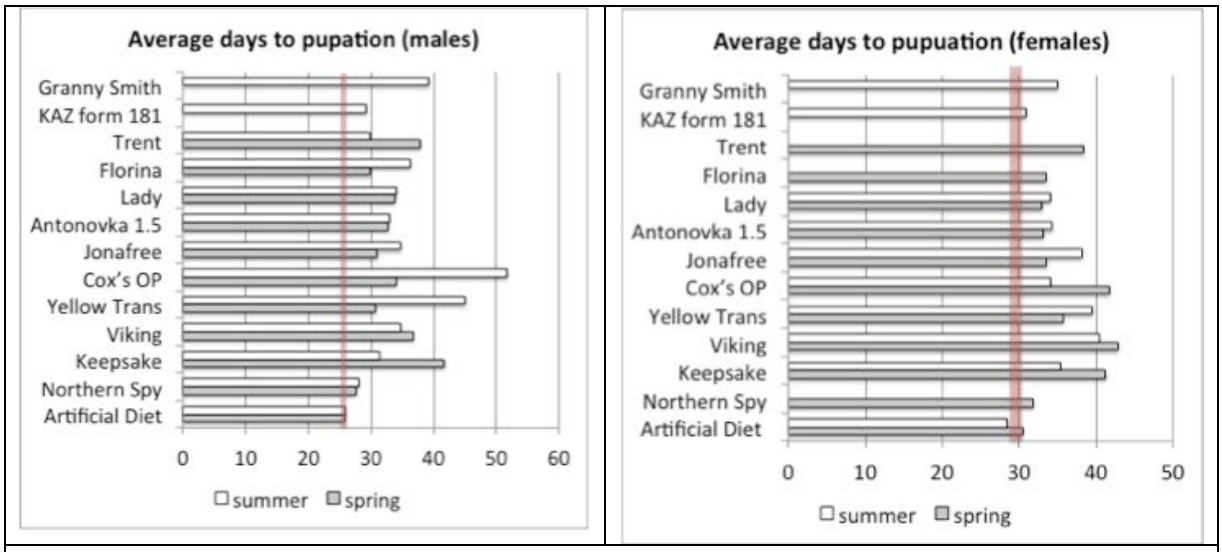

Figure 2. Average days to pupation for male and female OBLR reared on different apple varieties.

Another measure of the impact of different apple varieties on OBLR larvae is the size, or weight of pupae. When reared on diet, male OBLR pupae weigh less than female pupae, about 100 mg verses 170 mg, respectively (Fig. 3). Lower pupal weight signals negative impacts on development and suggests resistance. For example, a smaller insect has a greater exchange with the environment, which can increase metabolism and desiccation. Moreover, increased metabolism requires increased

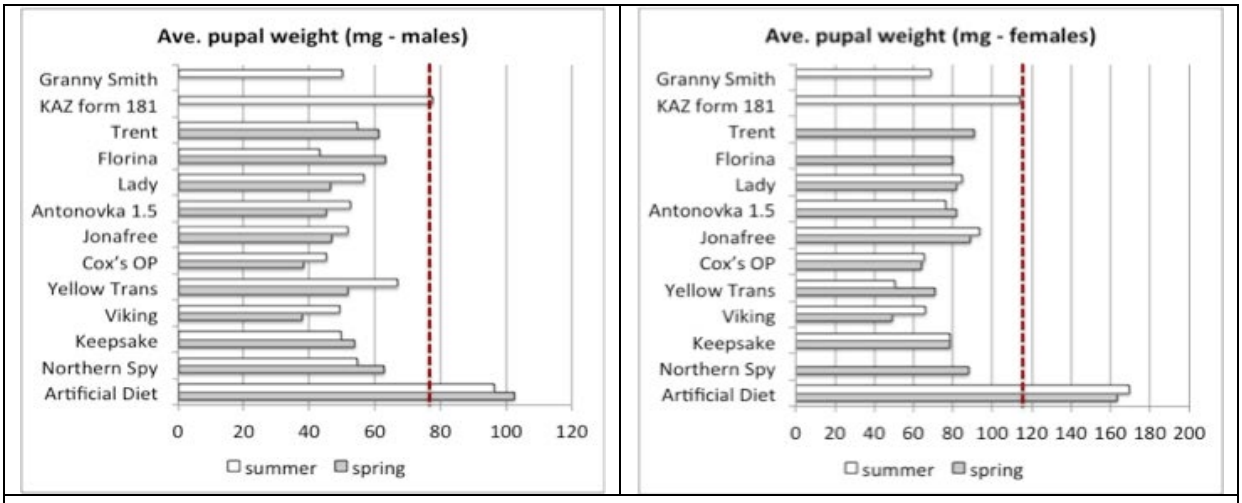

Figure 3. Average pupal weight for male and female OBLR reared on different apple varieties.

energy demands, and therefore, increased foraging time and exposure to elements and predators. Small size is also correlated with low fecundity (data not shown). No data are shown for females

(during summer) reared on Florina or Trent as no female larvae survived to the adult stage. In general, pupal weights were much lower when reared on *Malus* foliage than reared on artificial diet. The exception is KAZ form 181, which had the highest OBLR pupal weight. Using this genotype as an index of susceptibility (dotted line) shows the relative impact of other genotypes on OBLR pupal weight.

Survival of OBLR larvae or pupae on different *Malus* genotypes is one of the most important parameters in assessing resistance. Figure 4 shows the overall percent mortality experienced by OBLR reared on different *Malus* genotypes and on the artificial diet. There was very low mortality, 10%, of OBLR reared on the artificial diet. When reared on *Malus* genotypes, most of OBLR mortality, 80-90%, occurred in the larval stage (Fig. 4 - white bars). Jonafree, Antonovka and Northern Spy had relatively low mortality in the spring but Antonovka and Northern Spy showed high mortality along with most of other *Malus* genotypes in the summer period. There were no data for pupal mortality in the summer period for Northern Spy and Lady (Fig. 4) because only one and two larvae, respectively, survived to the pupal and adult stages. The much higher overall OBLR mortality in the summer compared to the spring period suggests that resistance mechanisms may be up-regulated in this period when the summer generation of OBLR would be developing.

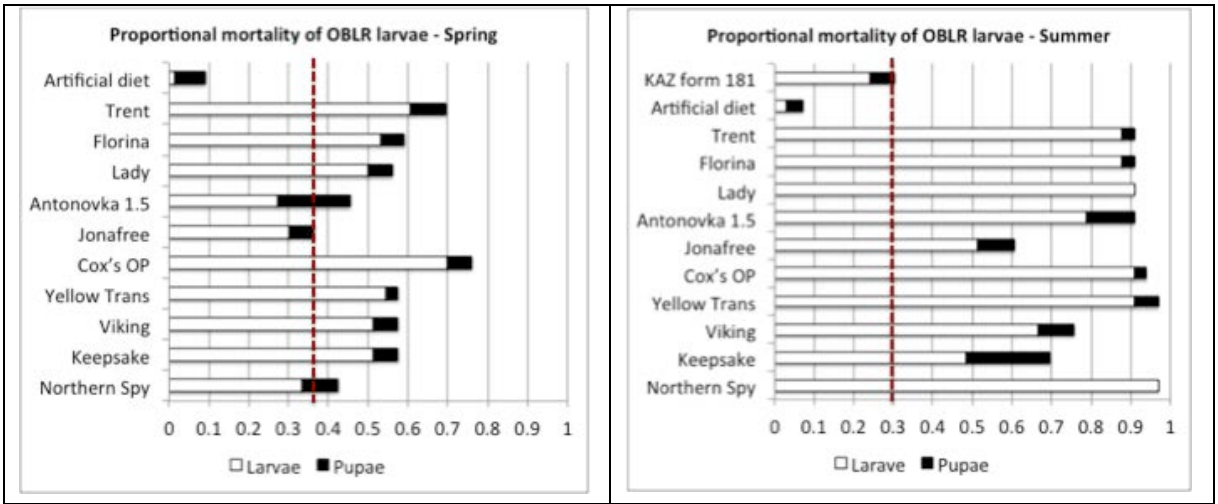

Figure 4. Percent of overall mortality, stacked bars of larval and pupal mortality of OBLR reared on *Malus* genotypes.

The reproductive assessment of OBLR adults showed that when reared on most *Malus* genotypes fecundity was significantly reduced or non-existent (Fig. 5). There were no offspring produced for many of the *Malus* genotypes because larval+pupal survival was so low that no adults were produced, or numbers of adults were so low that no mating occurred or no viable eggs were laid. This was especially true for OBLR reared on *Malus* genotypes in the summer period. Of the few *Malus* genotypes that did produced OBLR adults in summer, most or all were males. If a strong bias towards the production of males were consistent it would result in a dramatic impact on population dynamics of OBLR. Therefore, just because OBLR adults are produced from a genotype does not mean that genotype does not have some level of resistance.

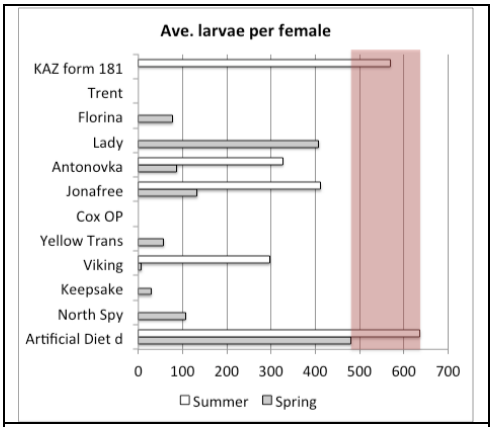

Fig. 5. Average larvae per OBLR female reared on different Malus genotypes.

Example of genotype variation across all periods – OBLR larvae were exposed to a limited number (11) of *Malus* genotypes in the fall period. Limitations were due to the lack of availability of leaves on many genotypes and human resources required to follow OBLR through development to the adult stage. Cox's OP is used as an example of how developmental parameters vary between time of year. Figure 6 shows larvae per female, pupal weight (male and female), development time (male and female), and percent mortality for the spring, summer and fall period. There were no offspring produced from OBLR larvae reared on Cox's OP in the spring or summer (top graph) but high numbers of offspring were produced when OBLR were reared on Cox's OP in the fall. Pupal weights were lower and development times longer in the spring and summer compared to the fall. Mortality was also higher in the spring and summer than in the fall. These data show that factors influencing resistance to OBLR can be differentially expressed in foliage of *Malus* genotypes at different times of the season.

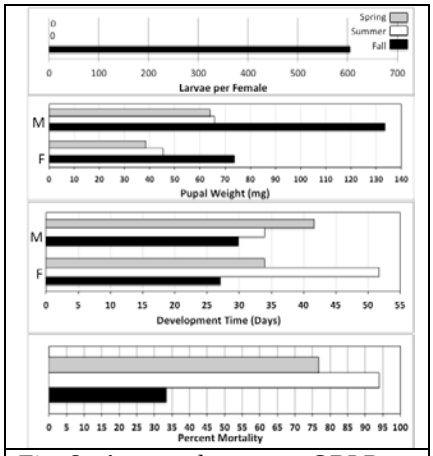

Fig. 6. Average larvae per OBLR female reared on different Malus genotypes.

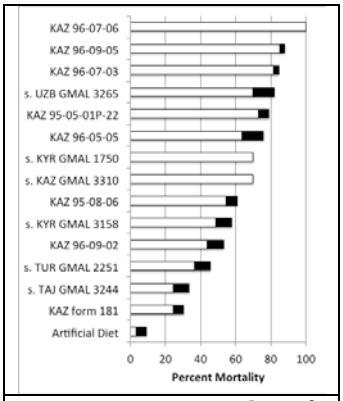

Fig. 7. Percent mortality of OBLR reared on mid-eastern genotypes.

KAZ genotypes – Because we were not able to rear OBLR on commercial cultivars (pesticide overspray issue) we focused some additional efforts in the summer period on selected *Malus* genotypes originating from mid-eastern genotypes. One of the genotypes, KAZ form 181, appeared to be highly susceptible, as did a few others (Fig. 7), but the majority showed high levels of mortality (over 50%). As in our other bioassays most or all of the mortality observed was in the larval stage. One interesting note is that KAZ 181 showed higher levels of resistance to OBLR when reared on its foliage in the fall (data not shown).

Antonovka 1.5 - We reported some interesting effects of the genotype Antonovka 1.5 on OBLR larvae in 2011. We evaluated the effects the same genotype on OBLR in 2012. When OBLR larvae were reared on leaves in the spring of 2012 overall mortality was low, less than 30%. However, in both years when OBLR was reared on leaves from the summer period there was high mortality, about 80%. Of the OBLR larvae that survived to the pupal stage in the summer of both years, 50-60% were larval/pupal intermediates (pupation was incomplete; see image at right) while there were very few larvae in the spring that showed these abnormalities, less than 5%. These larval/pupal intermediates suggest a disruption of the normal hormonal regulation of development, similar to a leafroller larva intoxicated by a juvenile hormone mimic, like the pesticide Esteem. None of the other *Malus* genotypes expressed these kinds of developmental abnormalities to the degree observed in Antonovka 1.5; however, some mid-eastern genotypes did (data not shown).

Resistance Indices – To summarize all the impacts on OBLR from exposure to different *Malus* genotypes we developed separate indices for larval and pupal mortality and development parameters (development rate and pupae weight). Instead of using the artificial diet we used the least resistant *Malus* genotype in each time period as the basis of relative comparison. For mortality (higher values show more resistance), we subtracted the genotype with the lowest mortality value (more susceptible) from all others and then set the index to zero with Jonafree in the spring and KAZ 181 in the summer.

Cox's OP showed high resistance in spring and summer, though most genotypes showed high resistance in summer relative to KAZ 181 (Fig. 8). For developmental parameters we multiplied average development rate by average pupal weight then subtracted the genotype with the highest value (more susceptible) from all others. We then set the index for developmental parameters to zero for Northern Spy in spring and KAZ 181 in summer. In spring Viking was the most resistant genotype followed by Cox's OP, Keepsake, etc. In summer Florina and Cox's OP were the most resistant genotypes. All genotypes were more resistant than KAZ 181 in summer (Fig. 8).

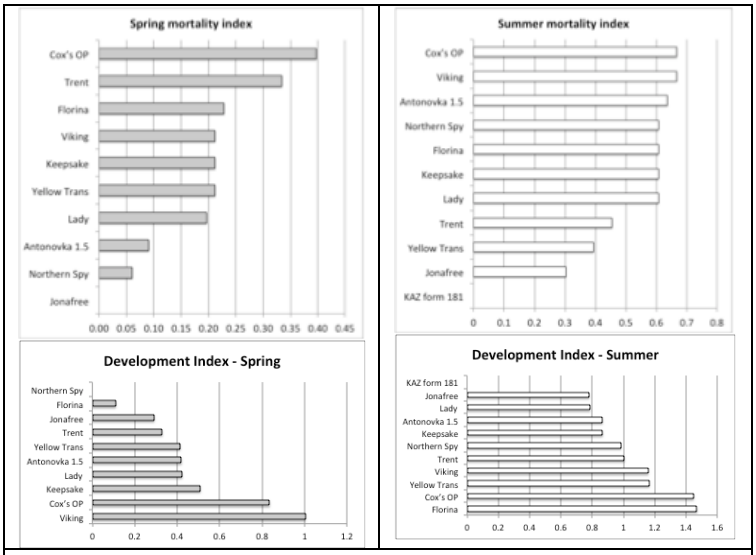

Figure 8. Mortality and development indices for OBLR reared on *Malus* genotypes in spring and summer.

Effect of oil on OBLR – As we were determining the cause of high OBLR larval mortality in bioassays established from the *Malus* parent block we identified an impact of oil on OBLR larvae that we had not anticipated. When oil was applied by an airblast sprayer at 1% concentration to apple trees, and leaves were then collected and ran them through our bioassay (exposing second-instar larvae to these treated leaves), we found that 100% of larvae died within the first 7 days of exposure. These data certainly point to an area that requires future investigation outside the scope of this project.

Plans for 2013 - Leafrollers. The leafroller bioassay has provided a good screening method for examining different expressions of resistance across *Malus* genotypes. However, this bioassay is labor intensive and requires careful choices of genotypes to evaluate. We will consult with the breeding team for direction based on 2012 results. We are hopeful that we will be able to screen parent varieties to search for a source of resistance that might occur

We will be screening several *Malus* genotypes for resistance to codling moth. We have a sound bioassay method and it does not take as much labor to conduct as the leafroller bioassay. The main limitation for this bioassay will be availability of fruit and access to codling moth larvae. We will focus most efforts on the early season fruit, which would be attached by first generation codling moth. Additional bioassays will depend on labor and available fruit.

Localize genes for resistance. We intended to be at a point where we might be able to search the apple genome for expressions of resistance to leafroller or codling moth by year three. We will discuss our data with genetics and breeding team that are collaborators on the project in order to determine next steps associated with this objective.

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

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

Project Title: Fire blight management in organic and conventional apple

PI: Ken Johnson
Organization: Oregon State University
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Address 2: 2082 Cordley Hall
City/ State/Zip: Corvallis, OR 97331-2902

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

Total Project Request: Year 1: $22,000 **Year 2: $22,660**

Other funding sources

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

WTFRC Collaborative expenses: None

Budget 1
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Kelvin Koong
Telephone: (541) 737-4066 **Email address:** .j.koong@oregonstate.edu

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

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

OBJECTIVES

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

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

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

SIGNIFICANT FINDINGS

- Oversprays of the bloom thinning agent, lime sulfur, suppressed populations of the fire blight pathogen after its establishment on apple flowers.
- Treatment with *Aureobasidium pullulans* (Blossom Protect) after lime sulfur and fish oil reduced fire blight infections by 92% compared with water only; for a 3rd year, this control level was similar to treatment with streptomycin against a streptomycin-sensitive pathogen strain.
- In parallel trials at Corvallis, OR, Wenatchee, WA, and Lakeport, CA, *Aureobasidium pullulans* was detected nearly 100% of flowers on trees treated with Blossom Protect once at early to mid-bloom.
- *Aureobasidium pullulans* colonized stigmas and hypanthial surfaces of nearly 100% of flowers sampled from trees treated with Blossom Protect.
- For a second season, the addition the systemic acquired resistance material, acibenzolar-S-methyl (Actigard) to antibiotic treatments significantly enhanced fire blight control.
- A non-crop destruct experimental use permit for Actigard has been obtained from EPA for the 2013-2014 seasons, which will allow for its continued evaluation in commercial orchards.

METHODS

All objectives were addressed with experiments in orchards located at the Oregon State University Botany and Plant Pathology Field Laboratory near Corvallis, OR. In addition, orchards in Wenatchee, WA and Lakeport, CA were used to address Objective 2. Experiments were arranged in a randomized complete block designs with 3-5 replications. Treatments were applied to trees during early morning (dates and bloom stages provided in results). Treatment suspensions and pathogen inoculum were sprayed to near runoff with backpack sprayers or with a motorized 25-gallon tank sprayer equipped with hand wands.

Microbial populations were measured by washing flowers sampled from the experimental trees. Generally, flowers were washed in 1 ml of phosphate buffer; the wash and two 1:100 dilutions of this wash were then plated onto a semi-selective culture medium appropriate for enumeration of each microorganism. Fire blight was measured by counting the number of blighted flower clusters (i.e. strikes) on each tree during weekly inspections from 29 May through 15 June. Blighted flower clusters were removed from the tree as they were observed. Microbial populations on flowers (log-transformed), total number of blighted flower clusters per tree, and disease incidence (diseased clusters divided by total clusters (based on prebloom counts)) were subjected to analysis of variance.

RESULTS & DISCUSSION

Obj. 1. Bloom thinning effects on microbial populations. Compared to the untreated control (UTC), lime sulfur oversprays onto pre-established epiphytic microbe populations on Golden Delicious flowers reduced significantly ($P < 0.05$) the population sizes of *Erwinia amylovora*, *Pantoea agglomerans* (Bloomtime Biological) and *A. pullulans* (Blossom Protect) regardless of sampling date or the rate of lime sulfur applied. Similarly, for the first sampling date (7 May) from Rome Beauty apple, epiphytic populations of *E. amylovora* and *A. pullulans* were significantly ($P < 0.05$) reduced by all rates of lime sulfur, but the effects of lime sulfur on the population size of *P. agglomerans* were inconsistent (Fig. 2). On 10 May, only the *A. pullulans* populations on the Rome Beauty flowers were suppressed significantly ($P < 0.05$) by the lime sulfur treatments on 3 May.

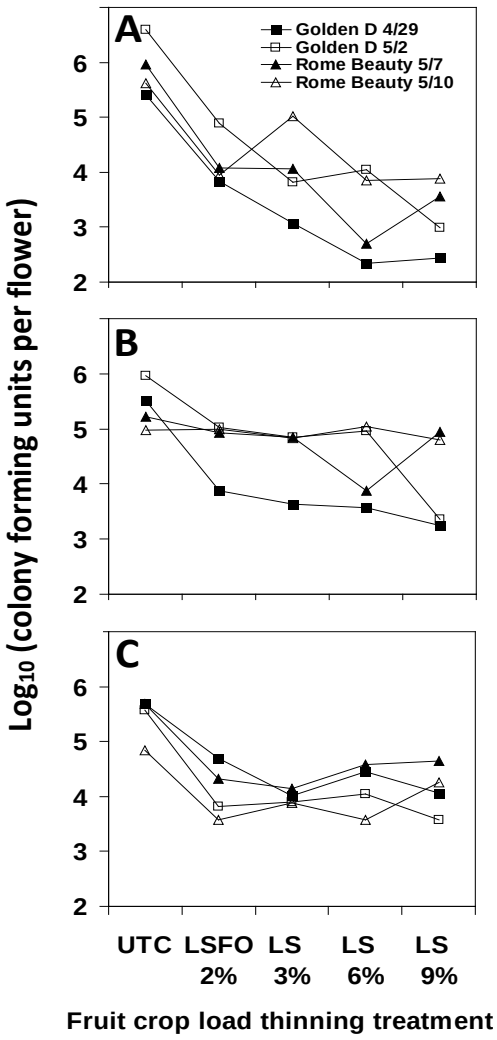


Fig. 1. Log_{10} (population size) of A) *Erwinia amylovora*, B) *Pantoea agglomerans* and C) *Aureobasidium pullulans* on apple flowers sprayed to runoff with the fire blight pathogen (1 x 10^6 CFU/ml) or with maximum labels rates Bloomtime Biological or Blossom Protect at 60 to 70% bloom (27 April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

2012 non-antibiotic fire blight control trials. Several programs of biological products were evaluated for non-antibiotic fire blight control in Gala apple. Treatment with lime sulfur and fish oil followed by Blossom Protect and Buffer A provided a level of control similar to that observed with Firewall (streptomycin sulfate). After three years of orchard trials, Blossom Protect (*A. pullulans*) after bloom thinning continues to be the most effective and consistent non-antibiotic program for fire blight control.

Table 1. Non-antibiotic fire blight suppression in Gala apple, 2012.

		Date treatment applied*								
Treatment	Rate per 100 gallons water	25 April 30% bloom	27 April 60% bloom	29 April 90% bloom	1 May Full bloom	5 May Before Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	------	---	**X** [§]	---	**X**	---	**215**	**a** [#]	**41.1**	**a**
Bloomtime then	5 oz.	X	X	---	---	---	125	ab	27.3	b
Optiva plus	16 oz.	---	---	---	X	X				
Biolink	4 fl. oz.	---	---	---	X	X				
Bloomtime then	5 oz.	X	X	---	---	---	120	b	27.1	b
Serenade Max plus	32 oz.	---	---	---	X	X				
Biolink	4 fl. oz.	---	---	---	X	X				
Bloomtime alone then	5 oz.	X	X	---	---	---	96	b	21.8	bc
with Regalia then	32 fl. oz.	---	X	---	X	X				
Regalia with										
Serenade Max plus	32 oz.	---	---	---	X	X				
Biolink	4 fl. oz.									
Fireline 100ppm	8 oz.	---	X	---	X	---	72	bc	16.9	cd
Rex Lime Sulfur &	2 gal.	X	X	---	---	---	70	bc	16.7	cd
Crocker's Fish oil	2 gal.	X	X	---	---	---				
Rex Lime Sulfur &	2 gal.	X	X	---	---	---	68	bc	13.0	de
Crocker's Fish oil	2 gal.	X	X	---	---	---				
then Bloomtime	5 oz.	---	---	X[§§]	---	---				
Bloomtime then	5 oz.	---	X	---	---	---	44	c	9.3	ef
Fireline 200ppm	16 oz.	---	---	---	X	---				
Firewall 100 ppm	8 oz.	---	---	---	X	---	25	d	5.1	fg
Rex Lime Sulfur &	2 gal.	X	X	---	---	---	18	d	4.5	g
Crocker's Fish oil	2 gal.	X	X	---	---	---				
then Blossom	21.4 oz.	---	---	X	---	X				
Protect plus buffer	150 oz.	---	---	X	---	X				
Rex Lime Sulfur &	2 gal.	X	X	---	---	---	18	d	4.0	g
Crocker's Fish oil	2 gal.	X	X	---	---	---				
then Bloomtime	5 oz.	---	---	X[§§]	---	---				
then Blossom	21.4 oz.	---	---	X	---	X				
Protect plus buffer	150 oz.	---	---	X	---	X				

*** Trees inoculated on evening of 28 April with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive); total inoculum concentration was 1 x 10^6 CFU/ml. * * 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. [§§] Applied morning of 28 April**

Discussion. The reason lime sulfur suppresses fire blight is likely twofold: 1) lime sulfur is directly toxic to epiphytic microbes on plants, and 2) the treatment causes flower abscission, which reduces the number of flower clusters that become diseased. Recent surveys that we made on the detectability of epiphytic *E. amylovora* in pear and apple flowers sampled from commercial orchards found that likelihood of positive pathogen detection is relatively small (<5%) from early to mid-bloom when thinning agents are applied, but increases five- to twenty-fold by petal fall. Consequently, because of its antibacterial properties, lime sulfur is likely sufficient in most orchards to delay/suppress the epiphytic increase of *E. amylovora* in early bloom, and that the biological materials specifically registered for fire blight control can be implemented after the bloom thinning protocol is completed. The deleterious effects of lime sulfur oversprays onto biological antagonists (*P. agglomerans* and *A. pullulans* also indicates that antagonist treatments should be delayed until after the bloom thinning protocol is complete.

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

Floral colonization. In 2011 and 2012, overspray of a 80% bloom timing of Bloomtime Biological (*P. agglomerans*) with Blossom Protect (*A. pullulans*) at full bloom and petal fall revealed differences in the colonization of the flowers by these microbes. On stigmas, the incidence of both microbes was high (2011: 63 and 98% for *P. agglomerans* and *A. pullulans*, respectively; 2012: 71 and 94% for *P. agglomerans* and *A. pullulans*, respectively) (Fig. 2A, B). In contrast, for floral cups, the yeast was again recovered from 98% (2011) to 100% (2012) of sampled flowers whereas *P. agglomerans* was detectable on 9% and 18% of washed hypanthia in 2011 and 2012, respectively (Fig. 2C, D)

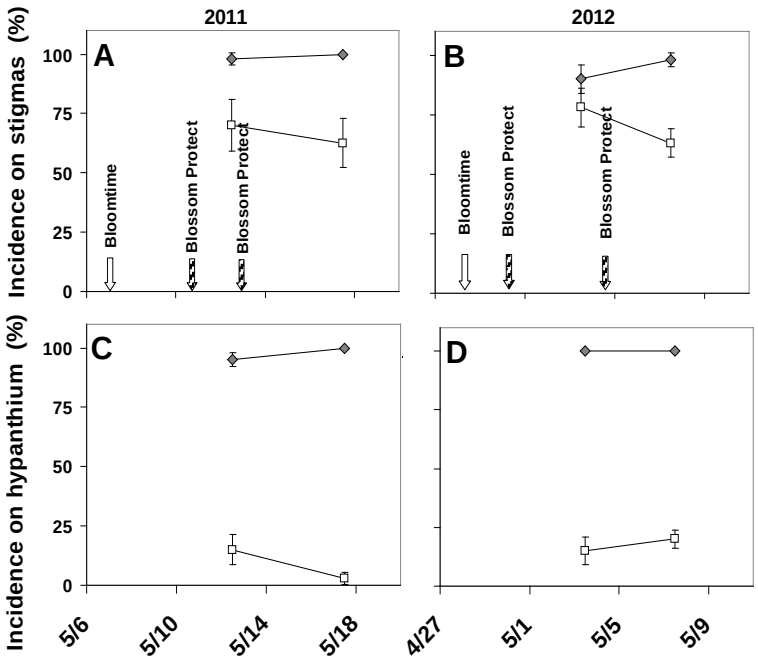


Fig. 2. Incidence of detection of *Pantoea agglomerans* (open squares) and of *Aureobasidium pullulans* (gray diamonds) on floral stigmas (A, B) and on hypanthia (C, D) by date of sampling from Gala apple trees treated with Bloomtime Biological at 80% bloom (open arrow) and with Blossom Protect at full bloom and prior to petal fall (hatched arrows) in an experimental orchard located near Corvallis, OR in 2011 and 2012. On each sampling date, incidence was determined by dilution plating dissected stigma and hypanthium subsamples from 10 flowers from each of four replicate trees. Error bars associated with each point represent plus/minus one standard error of the mean.

Environmental influences on floral establishment. In 2012, at Corvallis and Wenatchee, treatment with Blossom Protect at 20, 80 or at both 20 and 80% bloom resulted in recovery of *A. pullulans* from nearly every flower sampled between full bloom and petal fall (Fig. 3A,C,D). Flowers sampled from the untreated control trees also had a measureable incidence of *A. pullulans* on flowers that ranged from 26 (apple Corvallis) to 80% (pear Corvallis). At Lakeport, CA, pear trees treated

with Blossom Protect in early bloom were sampled in mid-June when fruit were thumb-sized. Calyx ends of these fruit were washed and subjected to dilution plating. Nearly every calyx-end of sampled pear fruit had a recoverable population of *A. pullulans* (Fig. 3B). The incidence of *A. pullulans* on fruit sampled from the untreated control was 98%.

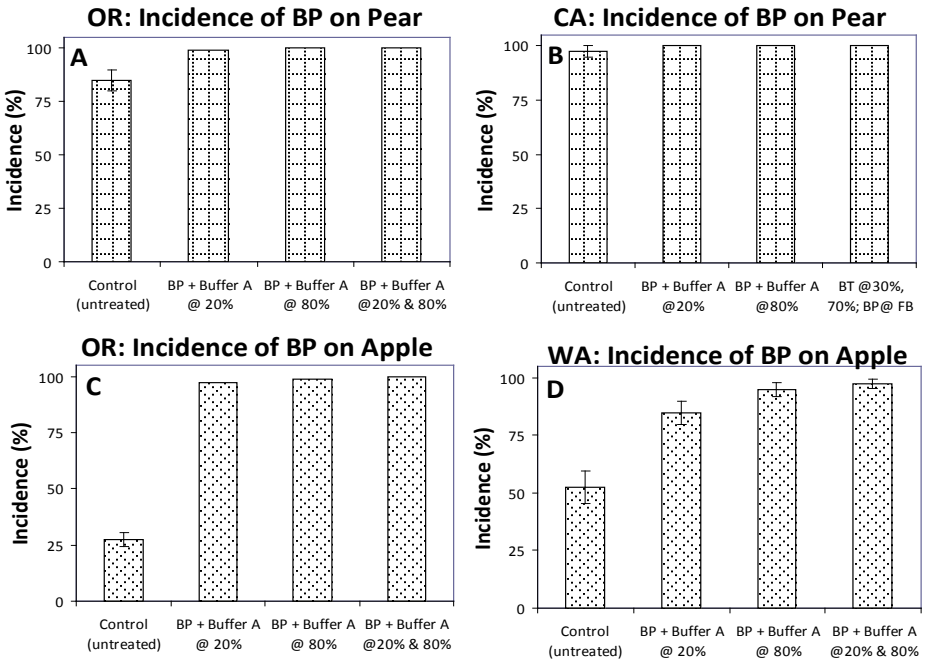


Fig. 3. Incidence of detection of *Aureobasidium pullulans* on pear (A, B) and apple (C, D) flowers after treatment with Blossom Protect at 20, 80 or both 20 and 80% bloom in orchards located near Corvallis, OR (A,C), Lakeport, CA (B), and Wenatchee, WA (D) in 2012. In Corvallis and Wenatchee, flowers were sampled twice between full bloom and petal fall. On each sampling date, incidence was determined by dilution plating 10 flowers from each of four replicate trees, the average incidence for the two sampling dates is presented in the figure. In Lakeport, samples were taken once when pear fruit were thumb-sized; The calyx ends of ten pear fruit from each of four replicate trees fruit were washed and dilution plated. Error bars associated with each point represent plus/minus one standard error of the mean.

Discussion. A. *pullulans* is an excellent colonist of both the stigma and the hypanthium, whereas *P. agglomerans* is only a good colonist of stigmas. The ability of *A. pullulans* to colonize the hypanthium may be a primary mechanism by which this organism provides outstanding fire blight suppression. Certainly, for yeasts used as biocontrol agents of postharvest fruit rots, the effectiveness of these antagonists is commonly attributed to an ability to rapidly utilize nutrients available at the site of infection (the hypanthial surface is the infection site for *E. amylovora*). As a biological product, Blossom Protect is produced to a very high quality standard, which results in a high level of viable colony forming units (spores) in the spray tank. For three environments (Corvallis, Wenatchee and Lakeport), *A. pullulans* became established in nearly all pear and apple flowers to which Blossom Protect was applied. Moreover, these strains apparently spread flower-to-flower after initial establishment as evidenced by the high recovery of *A. pullulans* from flowers treated at 20% bloom, and from flowers sampled from the untreated controls. In wetter climates, a negative aspect of *A. pullulans* colonization of flowers is a causal association with skin russet of developing fruit, which we observed in our 2011 Gala apple trial when frequent rains occurred in Corvallis after primary bloom. Russeting of fruit surfaces can greatly reduce crop value, and thus, treatments (e.g., sulfur,

new coppers and a new antimicrobial material from Canada) to suppress *A. pullulans* populations (and also the fire blight pathogen) at petal fall will be a research focus in 2013.

Obj. 3. Systemic acquired resistance for protection of apple. Relative to the water-treated control, each of the antibiotic and Actigard treatments significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree. In general, Actigard treatments in combination with Fireline (oxytetracycline) improved the control of fire blight compared to Fireline alone or Fireline in combination Bloomtime Biological (*Pantoea agglomerans*): the 8 trees treated with Fireline only or in combination with Bloomtime averaged 58 ± (s.e.) 7 blighted flower clusters whereas the 20 trees treated with a combination of Fireline and Actigard averaged 30 ± 5 blighted clusters. The Firewall (streptomycin) treatment averaged 25 ± 5 blighted clusters per tree.

Table 2. Evaluation of Actigard for suppression of fire blight of Gala apple, 2012

		Date treatment applied*						
		27 Apr	1 May	5 May				
Treatment	Rate per 100 gallons water	60% bloom	Full bloom	Before petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water control		**X[§]**	**X**	---	**215**		**41.1**	
Firewall 100 ppm	8 oz.	---	X	---	25	a[#]	5.1	a
Bloomtime then	5 oz.	X	---	---	44	ab	9.3	b
Fireline 200ppm	16 oz.	---	X	---				
Fireline 100 ppm	8 oz.	X	X	---	72	b	16.9	c
Actigard then	2 oz.	X	---	---	27	a	5.7	ab
Fireline 200 ppm	16 oz.	---	X	---				
Actigard alone then mixed	2 oz.	X	X	---	41	ab	8.0	ab
with Fireline 200 ppm	16 oz.	---	X	---				
Actigard mixed with	2 oz.	X	X	---	37	ab	8.8	ab
Fireline 100 ppm	8 oz.	X	X	---				
Actigard then	2 oz.	X	---	---	20	a	4.3	a
Fireline 200 ppm	8 oz.	---	X	---				
then Actigard	2 oz.	---	---	X				
Actigard then	3.2 oz.	X	---	---	27	a	5.5	ab
Fireline 200 ppm	8 oz.	---	X	---				
then Actigard	3.2 oz.	---	---	X				

Footnotes: *, **, *,** §, and # (**see bottom of Table 1).**

Failed SAR experiments in 2012: Experiments also were conducted in 2102 to evaluate protection of apple rootstocks with Actigard (against rootstock blight) and to evaluate Actigard as an aid to cutting of blight in scions of young trees. For a 2nd year, the rootstock field experiment failed because of inconsistent fire blight inoculations (frustrating!). The cutting experiment also failed because of a miscommunication about maintenance fungicide treatments, which resulted in a severe apple scab epidemic that completely defoliated the young trees. An Actigard aid-to-blight-cutting experiment in pear was successful (see pear report).

Discussion. We have made significant progress in understanding effective rates of Actigard for the various methods of application. Induction of systemic acquired resistance appears to have its greatest effect/value when blight symptoms are minimal (near time of infection or after cutting). Actigard shows value as mixture partner with antibiotics during bloom, and perhaps more significant, it may be effective as long residual protectant for rattail and shoot infection phases of fire blight; a 2013-14 non-crop destruct experimental use permit will allow for the evaluation of late Actigard treatments in commercial orchards. Use of Actigard paints as aid to pruning continues to show promise; these investigations will be continued.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP–12-100

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

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

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

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

Other funding sources

Financial support is expected from companies supplying products to be tested for effect on fire blight or orchard replant during this project.

I am Co-PI on the project "Development of Non-Antibiotic Programs for Fire Blight Control in Apple and Pear," from the USDA Organic Agriculture Research and Extension Initiative. My sub-award will be $29,887 in 2013.

Budget
Organization Name: Washington State University **Contract Administrator:** Carrie Johnston
Telephone: 509-335-4564 **Email address:** carriej@wsu.edu

	2012	**2013**	**2014**
Salaries	$10,125	$10,660	$11,086
Benefits	4,305	4,477	4,656
Wages			
Benefits			
Equipment			
Supplies			
Travel	600	600	600
Plot Fees	0	0	0
Miscellaneous			
Total	$15,155	**$15,737**	$16,343

Footnotes: **Salaries and benefits are in support of 0.25 FTE of a full time scientific assistant. Travel is to plot sites.**

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 "Blossom Protect" which 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.

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.

SIGNIFICANT FINDINGS

- Over five years and in eight separate apple and pear fire blight control material trials, a dried yeast product, *Auriobassidium pulullans*, registered for USA use in 2012 as "Blossom Protect," 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 has been 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.**)**
- Two proprietary copper compound formulations, Phyton27 AG and Previsto, often provided blossom protection equal to antibiotics when applied to open blossoms at full rates. These products are not available for use on pome fruits yet. 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 (especially Phyton27) must undergo much more fruit safety tests during the critical post bloom infection period.
- In 2010 and 2011, the 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/infection. Application of this product to the soil under the test tree reduced blight infection, but not significantly. This year's more extensive tests were promising, but not as conclusive. More work must be done to prove effect.
- Fruit production increased to about 40-50% of mature potential in the fourth 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 $12,700 to $19,900 per acre. This was a 27 to 1 economic return over four years on the cost of the fumigation.

Methods: This trial follows the European and Mediterranean Plant Protection Organization protocol on efficacy evaluation of bactericides, 2002, OEPP/EPPO, Bulletin 32, 341- 345.

The products were applied in 100 gallons water per acre (full wetting by spray, but no drip.) All treatments were applied with a backpack air-blast/mist sprayer at about 100 gallons per acre (full wetting, no drip). At about the time of "full bloom," the blossoms were inoculated with the bacterial Fire Blight pathogen, *Erwinia amylovora*. The pathogen was a research standard strain provided by Dr. Larry Pusey, Plant Pathologist, USDA-ARS, Tree Fruit Research Laboratory, 1104 N. Western Ave., Wenatchee, WA 98801. Description of pathogen: *Erwinia amylovora* (Burr.) Winsl. *et al*., strain Ea 153nal. This strain was is susceptible to streptomycin sulfate, unlike many wild strains currently in Washington. The bacteria were suspended in buffered water. The procedure duplicated lab standards that result in a concentration of about ten million colony forming units per ml of water and misted on about 100 flower clusters per replicate. Weather and flower condition were optimum during the inoculation**. Evaluation**: Trees were visually evaluated for flower cluster infections every week following treatment. Symptoms became visible about 14 days after inoculation, and continued to develop for about 28 days, after which data collection ceased. The foliage and fruit were inspected for damage a few days after spraying and again at harvest time.

Fire Blight Results & Discussion: Four non-antibiotic materials performed very well in the 2011 trials. Three copper compounds, one, "Previsto," (which I have referred to as "copper product TS - Trade Secret") from Gowan, "Phyton 27 AG" from Phyton, and "Cueva" from Certis, reduced fire blight infection as well as, or sometimes better than, standard and test antibiotics when applied to open blossoms and 80% open blossoms then again the day after infection. Copper compounds have rarely performed well in past trials, and have a history of causing fruit skin russet or marking. The "Previsto" copper was applied to both D'Anjou and Bartlett pears in a specific russet/phytotoxicity trial. There was no russet on the fruit skin observed at harvest, even on the usually russet-prone D'Anjou pears. The Phyton27 AG and Cueva coppers were applied at bloom to D'Anjou pears in more limited trials, with no marking. The Phyton27 induced browning of flower petals.
The biological product, which was 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 Protect"). This genus and species of yeast is commonly found in the Pacific Northwest as a natural colonizer of apple and pear flowers and apparently thrives and spreads 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. 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 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. The tests were greatly expanded again in 2012, using other effective antibiotics and copper compounds, with less clear-cut results. The Actigard has been 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. Sprayed as a curative after symptoms appeared, it had no apparent effect.

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

Products	Rate	Timing	% Infection	% Control
Agri-Strep 17%	100 ppm	100% bloom	0	100a
Actigard Agri-Strep	2 oz/A 100 ppm	50% bloom 100% bloom	0	100a
Actigard Kasumin	2 oz/A 100 ppm	50% bloom 100% bloom	3.35	94.4b
Actigard, Blossom Protect + Buffer Protect	2 oz/A, 1.34 lb/A 9.35 lb/A	50% bloom 50, 80 +100% bloom	3.85	93.5b
Blossom Protect + Buffer Protect	1.34 lb/A 9.35 lb/A	50%, 80% + 100% bloom	5.1	91.4b
Previsto (copper)	1 gallon /100/A	80% bloom and 1 day past infection	6.15	89.7bc
Kasumin	100 ppm	100% bloom	6.33	89.4 b c
Actigard Oxytet. Actigard	3.2 oz/A 200 ppm 3.2 oz/A	50% bloom 100% bloom 1-2 inch shoots	9.35	84.3 cd
Actigard Oxytet.	2 oz/A 200 ppm	50% bloom 100% bloom	9.38	84.2 cd
Oxytet.	200 ppm	100% bloom	9.63	83.8 cde
Actigard Oxytet. Actigard	2 oz/A 200 ppm 2 oz/A	50% bloom 100% bloom 100% bloom	12.48	79.0 de
Previsto	64 fl.oz./A	80% bloom and 1 day past infection	13.58	77.2 d
Actigard Oxytet. Actigard	2 oz/A 200 ppm 2 oz/A	50% bloom 100% bloom 1-2 inch shoots	14.63	75.4 eg
Actigard Oxytet.	2 oz/A 100 ppm	50, 100% Bloom 50, 100% Bloom	16.33	72.5 g
Serenade MAX	2 lb./A	50, 100% Bloom	31.2	47.5 h
Regalia	64 oz/A	50, 100% Bloom	38.93	34.5 i
Inoculated check	**na**	**na**	**59.48**	**0j**

Table 1. 2012 Fire Blight Control Product Efficacy on Pears: Values followed by the same letter should not be considered different**.**

ORCHARD REPLANT DISEASE PROJECT -

METHODS, *Orchard replant disease treatment trials:*

Establishment: In the fall of 2008, block of land south of Othello, Washington that had recently supported an apple orchard (with one fallow season) was selected as a site for the fumigant trial. The land was ripped thoroughly and smoothed prior to fumigation. On October 27, 2008 a replicated fumigation trial was established, with four treatments and untreated checks. Each replicate was approximately 0.8 to 1 acre, with a total of about three acres for each treatment. Fumigant application was by Trident Agricultural Products, Inc. The soil temperature and moisture were well within the optimum range. Treatments applied are as listed in table 2.

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

Table 2. Products used in treatments of orchard replant disease plots, Othello/Radar Hill site.

2012 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, with all treatments growing in fumigated replicates relatively equal in growth, and each significantly larger and more vigorous than in the untreated replicates. The trees produced a crop in 2011, one year prior to expectations, so tree vegetative growth was suppressed by fruit competition. In 2011 and 2012, and in all further evaluation seasons, fruit yields and size became the main evaluation criteria.

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.	35.5c	43.3b	42.1b	49.8a	23.8d
Weight lbs. Fruit / tree	16.6c	19.3b	19.0b	22.8a	10.3d
Weight lbs. per fruit	0.456a	0.438a	0.442a	0.452a	0.432a
Fruit Grams average	207a	199a	201a	205a	196a
Fruit box size average	87.7	91.3	90.3	88.6	92.7
% size 72 & +	9	6	10	7	7
80 & 88	41	37	47	39	35
100 & -	51	63	43	54	58
2012 Yield per Acre, lbs.	28,333	32,900	32,437	38,920	17.585
Total 2011-12	41,141	45,726	48,372	54,512	23,871

Table 3 . 2012 Fruit production in fourth season Cripp's Pink apples planted in 2009 as a "sleeping eye" on M9, planted after 2008 fall 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 lb.	Wt by size group	90% pack wt	Packed boxes	Price*	$ by size group
72+	9	28333	2550	2295	57	35	2008
80/88	41	28333	11617	10455	261	37	9671
100-	51	28333	14167	12750	319	30	9562
			Cull Value	$340		Total	21,581
		**Minus costs, adjustments of:			$5,059	**Adjusted:**	**$16,522**
	Total for 2011 & 2012 : $21,741						
Treatment B	***PicClor 60 (20 GPA: 144 lbs./A chloropicrin, 94 lb/A 1,3-DCP)***						
Box size	% in box size	Acre yield lb.	Wt by size group	90% pack wt	Packed boxes	Price*	$ by size group
72+	6	32900	1974	1777	44	35	1555
80/88	37	32900	12173	10956	274	37	10134
100-	63	32900	20727	18654	466	30	13991
			Cull Value	$395		Total	26074
		**Minus costs, adjustments of:			$6,185	**Adjusted:**	**$19,889**
	Total for 2011 & 2012: $25,307						
Treatment C	***Telone C-35 (25 GPA: 98 lb/A chloropicrin, 178 lb/A DCP)***						
Box size	% in box size	Acre yield lb.	Wt by size group	90% pack wt	Packed boxes	Price*	$ by size group
72+	10	32437	3244	2919	73	35	2554
80/88	47	32437	15245	13721	343	37	12692
100-	43	32437	13948	12553	314	30	9415
			Cull value	$389		Total	25050
		**Minus costs, adjustments of:			$5,792	**Adjusted:**	**$19,259**
	Total for 2011 & 2012: $25,898						
Treatment D	***Telone C-17 (30 GPA, 51 lb/A chloropicrin 260 lb/A DCP)***						
	% in box size	Acre yield lb.	Wt by size group	90% pack wt	Packed boxes	Price*	$ by size group
72+	7	38920	2724	2452	61	35	2145
80/88	39	38920	15179	13661	342	37	12636
100-	54	38920	21017	18915	473	30	14186
			Cull value	$467		Total	29435
		**Minus costs, adjustments of:			$6,949	**Adjusted:**	**$22,486**
	Total for 2011 & 2012: $32,542						

Treatment E	***Untreated***						
Box size	% in box size	Acre yield lb.	Wt by size group	90% pack wt	Packed boxes	Price*	$ by size group
72+	7	17585	1231	1108	28	35	969
80/88	35	17585	6155	5539	138	37	5124
100-	58	17585	10199	9179	229	30	6885
			Cull value	$211		Total:	13189
		**Minus costs, adjustments of:			$3,140	**Adjusted**	**$10,049**
		Total for 2011 & 2012: $12,683					

Table 4. Rough estimate of fruit gross economic value per acre. *Approximate FOB average on 11/07/2012. ****Costs, adjustments***: picking @ $20/bin, packing @ $7 per 40 lb. box, and fumigation @ $650-750/Acre. Credit of 12 cents/lb. (2012), and 7 cents/lb. (2011) for cull fruit is in the adjustments. Fumigation costs were included in 2011 costs, so are already taken into account.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: CP-12-104A/B

Project Title: Incorporating fire blight resistance into Washington apple cultivars

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

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

Total Project Request: **Year 1:** $3,200 **Year 2: $19,679** **Year 3:** $63,077

Other funding sources

Agency Name: State Horticultural Association of Pennsylvania
Amt.: $22,690 (2012-2015)
Notes: Title **'**Identifying a QTL for Fire Blight Resistance in 'Splendour' Apple'. Does not duplicate research of this proposal but would support it in identifying markers for fire blight resistance coming from 'Splendour'.

WTFRC Collaborative expenses: None

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

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

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

Budget 2

Organization Name: WSU-TFREC **Contract Administrators:** Carrie Johnson & Kevin Larson

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

Item	2012	2013	2014
Salaries [3]		$5,990 [4] (1)[1]	$6,749 [4] (1)[1]
Benefits		$599[5] (1)	$5,218 [5] (1)
Wages			
Benefits			
Equipment			
Supplies		$1,000[6] (1)	$1,000[6] (1)
Travel Domestic [2]	$500[2] (1)[1]	$800[2] (1)	$1,000[2] (1)
Miscellaneous	$500[3] (2&3)		
Plot Fees: orchard maintenance	$1,000[7] (1)	$1,000[7] (1)	$1,000[7] (1)
Plot Fees: new orchard planting			$13,400[8] (2)
Total	**$2,000**	**$9,389**	$28,367

Footnotes: 1: (#) = Objective # associated with expense, **2:** travel to field, **3:** source and send budwood for tree propagation, **4:** summer student to assist with fire blight inoculation, recording data and plant maintenance, **5:** if same student is hired in 2013 and 2014, WSU benefit rate increases in year 2014, **6:** supplies to grow bacteria, inoculation process and plant labeling, **7:** maintenance of existing planting of *Malus sieversii* previously established with WTFRC support, **8:** planting of RosBREED Crop Reference Set for fire blight evaluation, new orchard planting $6,000/acre, fumigation $700/acre, 2 acre Obj. 2.

Budget 3

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

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

Item	2012	2013	2014
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous: Tree propagation			$23,080[2, 3] (2)[1]
Plot Fees:			
Total	0	0	$23,080

Footnotes: 1: (#) = Objective # associated with expense; **2:** $21,000 tree propagation ($7/tree * 5 trees/accession * 600 accessions/obj.), $1,680 sales tax (0.065 State, 0.015 Local), and $400 shipping; **3:** Tree propagation originally estimated at $18,160; increased cost resulted from change in nursery (see Results and Discussion for more detailed information).

Objectives:

1. Identify the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program. ($48,976)

2. Establish planting of RosBREED apple Crop Reference Set and Washington State Breeding Pedigree Set in Wenatchee, WA for future fire blight evaluation. ($36,980)

Significant Finds:

- Field plantings of 194 *Malus sieversii* accessions and 7 control cultivars were established in Kearneysville, WV (USDA-ARS-AFRS) and Wenatchee, WA (WSA-TFREC) in 2012 for the purpose of identifying the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program.

Methods:

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

Because fire blight is a sporadic disease from year to year and in its distribution within the orchard, reliable evaluation of fire blight resistance requires artificial challenge of test plants with the fire blight bacteria. Vigorously growing shoots will be challenged by dipping a pair of scissors in a suspension of the bacteria and then cutting the youngest leaves of the shoot tip. Resistance will be determined by measuring the percent of the current seasons shoot length that becomes infected. Because economic losses from fire blight are the result of the death of young trees and woody tissue, rating cultivar resistance based upon progression of disease in shoot tissue has proven a reliable method of accessing fire blight resistance.

Trees of the *M. sieversii* accessions were grafted onto M.7 rootstock and planted in both Wenatchee, WA (3 reps per accession) and Kearneysville, WV (4 reps per accession) in 2012 (See Results & Discussion section). Each planting in WA and WV includes the same 194 *M. sieversii* accessions and 7 control apple cultivars. Having plantings in both WA and WV will allow determination of fire blight resistance in diverse environmental conditions that will result in more reliable and precise resistance ratings. Standard cultivars are included in trials so that results obtained in different locations and years can be directly compared. Moderately resistant 'Delicious', intermediate 'Empire' and moderately susceptible 'Golden Delicious' are included to establish the lower limit for a "resistant" rating. Two highly susceptible cultivars ('Gala' and 'Jonathan') are included to establish the high end of the disease scale when comparing tests and to ensure that a minimum disease pressure threshold is achieved in every test. A highly resistant cultivar ('Robusta 5') is included to establish the low end of the disease scale when comparing tests. 'Goldrush' and 'Splendour', two cultivars reported to be resistant to fire blight, are included to directly compare their resistance with that observed in *M. sieversii* accessions.

In year 1 (2012), the field plantings were established for later fire blight challenge in years 2 and 3. In year 2 (2013), 3 shoots per plant will be challenged with the fire blight bacteria. In year 3 (2014), 5 to 10 shoots will be challenged. Norelli has many years of experience evaluating apple for fire blight resistance and will travel to Wenatchee, Washington to assist Kate Evans in both the inoculation and disease evaluation process.

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

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

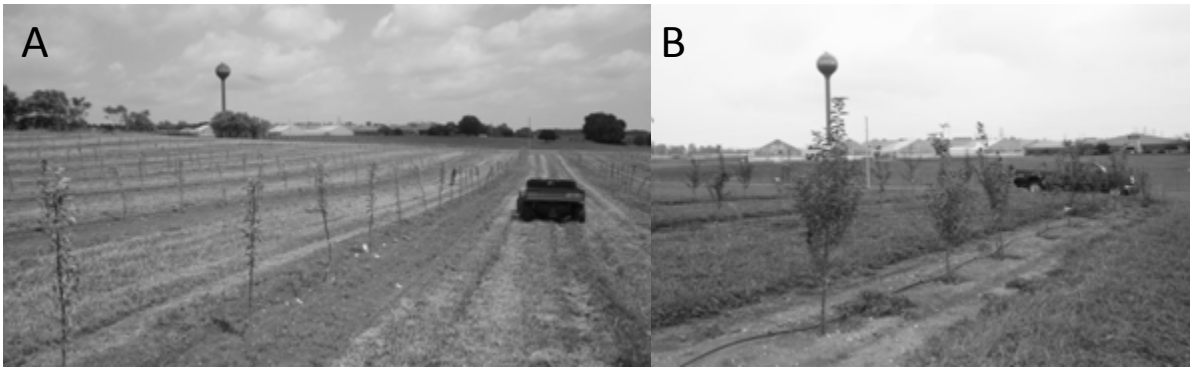
Because fire blight resistance will be determined on shoots, the tests for resistance can be conducted on young trees and the planting is expected to be of short term duration (3 to 4 years), allowing for planting at high density. Because fire blight challenge of the planting will be required for future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

Results & Discussion:

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

The primary goal of this project in 2012 was to establish field plantings of *M. sieversii* in order to determine the best *M. sieversii* accessions to be used as sources of fire blight resistance in the WSU apple breeding program. This goal was fully met. Field plantings of 194 *M. sieversii* accessions and 7 control apple cultivars were planted spring 2012 in Wenatchee, WA (3 reps per accession) and Kearneysville, WV (4 reps per accession, total number of trees in both plantings = 1,400) (Figure 1A). Irrigation was installed in both orchards. The trees grew well and are ready for inoculation with the fire blight pathogen in 2013 (Figure 1B). The Kearneysville planting was mildly impacted by Super Storm Sandy, with one tree lost (snapped off at graft union) and many trees (120) leaning after the storm. Leaning trees were staked upright and are expected to fully recover.

Figure 1. *M. sieversii* planting at USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV. A: Overview of planting shortly after planting (May 2012). B: Typical trees in planting at the end of growing season (October 2012).



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

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

We originally planned to propagate the trees on M.7 rootstocks however we were unable to locate a sufficient supply of M.7 for the project in 2012. We decided to propagate the trees on MM.111 when Cameron Nursery generously offered to donate MM.111 rootstocks to the project. MM.111 rootstock has sufficient tolerance to fire blight to prevent rootstock blight from being a major problem within the block.

The estimated cost of tree propagation has increased from $18,160 in the original project proposal to $23,080 (see Budget footnote for breakdown of costs). The increased cost resulted from a change in the nursery that will propagate the trees and the way each nursery charges for special orders (# of trees delivered versus # of trees propagated). Custom propagation orders for research purposes are difficult for nurseries to complete because they involve a small number of trees of many (600) different accessions and require accurate labeling of each individual tree. We were required to change nurseries when the nursery we originally planned to use for tree propagation decided they could not complete this special order.