2006 Apple Entomology Review
February 2-3
Confluence Technology Center
Wenatchee, WA

Thursday, February 2, 2006

Time	Page	PI	Proposal Title	Funding Period
			Final reports	
8:00	1	Unruh	Effects of new insecticides on natural enemies	**02-04**
8:15	11	Landolt	Field testing of multi-component host plant kairomones for the codling moth	**03-05**
8:30	16	Judd	Evaluation of a codling moth larval aggregation pheromone as an IPM tool	**03-05**
8:45	17	Lacey	Optimizing the use of the codling moth granulovirus	**04-05**
9:00	24	Beers	Biology and management of secondary pests of apples	**05**
9:15	35	Beers	Biology, migration, and management of Western flower thrips in apple orchards	**03-05**
9:30	45	Sheppard	Tien Shan mountain gray bee	**01-03**
			Poster Session - Continuing Reports - 3:00pm - 5:00pm	
1	51	Brunner	Codling moth management with pheromones: key unanswered questions	05-07
2	57	Jones	The importance of dispersal in biological control and IPM^2	04-06
2	63	Jones	Mechanisms underlying mating disruption	04-06
2	69	Knight	Developing ultra low volume microencapulated sex pheromones for CM control	05-06
2	75	Knight	Direct control of CM with formulations of the pear ester	05-06
2	81	Lacey	Codling moth granulovirus transmission and potential for autodissemination	05-07
3	87	Brunner	Sustainable management of leafrollers in apple orchards	05-07
3	93	Horton	Distribution of flower thrips eggs among vegetative, flowering & fruiting structures	05-06
3	96	Yee	Alternative hosts of apple maggot as threat to apple	04-06
3	102	Yee	Control of apple maggot using bait spray insecticides and traps	04-06
3	108	Unruh	Biological control of leafrollers through habitat modification	**03-05**

FINAL REPORT

Project Title: Effects of new insecticides on natural enemies

CO-PIs Tom Unruh, Dave Horton, USDA-ARS Yakima
Richard Hilton, Helmut Riedl, OSU, Medford & Hood River
Elizabeth Beers, WSU, Wenatchee
Nick Mills, U.C., Berkeley

OBJECTIVES (2002-2004):

1. Test acute toxicity of new insecticides to 7 arthropods using standard bioassay
2. Develop bioassay methods to measure sub-lethal effects on beneficial insects
3. Test sub-lethal effects in those cases where acute effects are modest.
4. Model acute and sub-lethal toxicity data to provide field testable predictions of pesticide effects and to better represent combined effects

SIGNIFICANT FINDINGS:

- Acute and sublethal bioassay methods were developed and used for 7 species including: the European earwig, the common green lacewing, a predatory mite, *Deraeocoris brevis, Anthocorus nemoralis, Colpoclypeus florus, Mastrus ridibundus*.
- Acute toxicity were tested for 7 to 13 insecticides and responses were often different among species; generally the neonicotinyl insecticides, Actara, Assail, Provado and Success were acutely toxic with the exceptions of earwig and predatory mite.
- The sublethal assay results also varied by species: negative effects were seen for Esteem, Intrepid and especially Novaluron, compounds which were not acutely toxic.

FUNDING: WTFRC funding by lab

Lab – scientist <arthropod tested> /year	2002	2003	2004
Wenatchee – Beers <predatory mite>	10,000	10,000	0
Hood River – Riedl <predatory mirid bug>	10,000	10,000	10,000
Medford – Hilton <European earwig>	10,000	10,000	10,000
Wapato – Unruh & Horton <leafroller parasitoid & predatory anthocorid bug	10,000	10,000	10,000
Berkeley – Mills <green lacewing, codling moth parasitoid>	10,000	10,000	20,000
TOTAL	50,000	50,000	50,000

USDA-IFAFS matched $220,000 over 3 years ($86,000, $86,000, $60,000)

INTRODUCTION

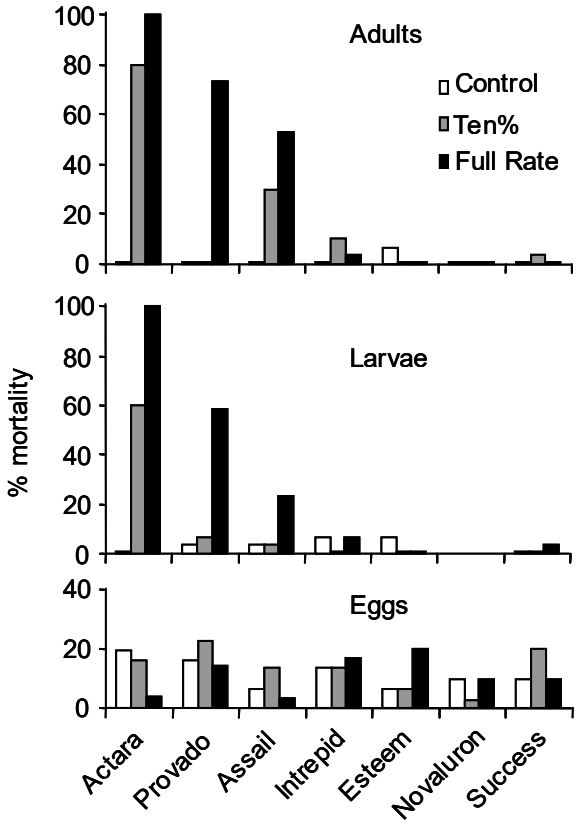
The purpose of this work was to discover the negative effects, if any, of the new, nominally more selective, insecticides being used for pest control in pome fruits in the Pacific Northwest. These studies represent 3-4 years of effort in 5 laboratories by six scientists working with 7 natural enemy species and testing effects of 7 or more new insecticides. Because each arthropod natural enemy has its own requirements, new methods were required. The uniqueness of the bioassay procedures used for each test organism lead to differences in responses and differences in the best ways to report this data for each species as is evident in pages that follow. The last page of the report provides a tubular summary of the effects and the page that precedes it outlines where we hope to go in the future to better summarize and understand sublethal effects..

Green lacewings, *Chrysoperla carnea*, predator of pear psylla, mealybugs, and moth eggs

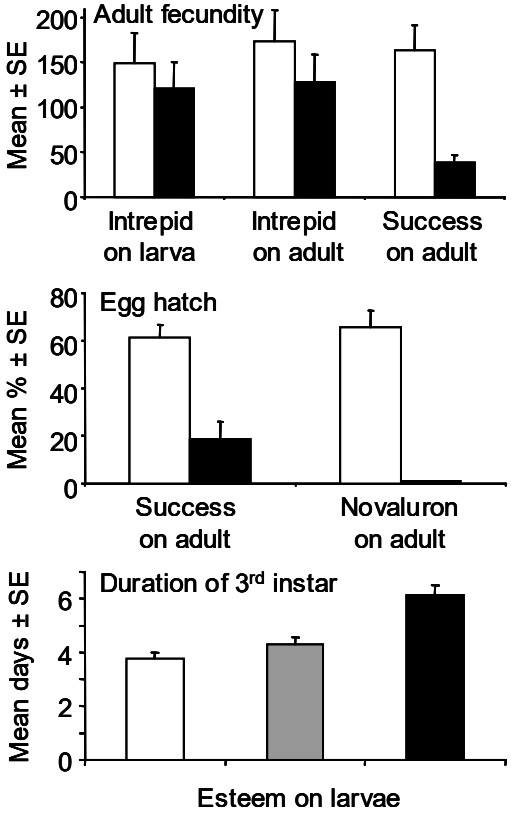
- Acute toxicity of neonycotinyls to lacewing adults and larvae, but not eggs
- Sublethal effect of larval or adult exposure to Intrepid, reducing adult fecundity
- Sublethal effect of adult exposure to Novaluron and Success, reducing egg hatch
- Sublethal effect of larval exposure to Esteem, increasing duration of the 3rd larval instar

We developed a 'worst case scenario' lab assay to determine both the acute (48 h) toxicity of a topical exposure, and the lethal and sublethal toxicities of a combined route of exposure (topical, residual, and oral) of each insecticide on eggs, larvae (2 day old) and adults (2 day old) of the lacewings. Following treatment, all lacewing stages were kept at 23°C, 70% R.H. and 16 hours of light. Measurements from assays included development time, survivorship, adult size (hind tibia length), fecundity (first 14 days) and success of egg hatch. Only those measurements that showed notable acute or sublethal effects are shown below for the influence of each insecticide on either larval or adult lacewings. Sublethal effects were followed into the F1 generation to check on the production of viable offspring. All acute topical assays used 100% and 10% field rates and distilled water controls. The sublethal bioassays were restricted to control plus the highest concentration that permitted greater than 25% survival from the acute assay.

Acute toxicity of neonicotinyls



Sublethal effects of IGRs and Success on adult fecundity, egg hatch and development time of 3rd instar larvae

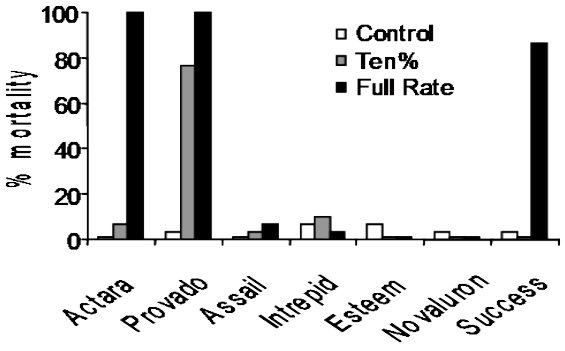


***Mastrus ridibundus*, introduced parasitoid of codling moth cocoons**

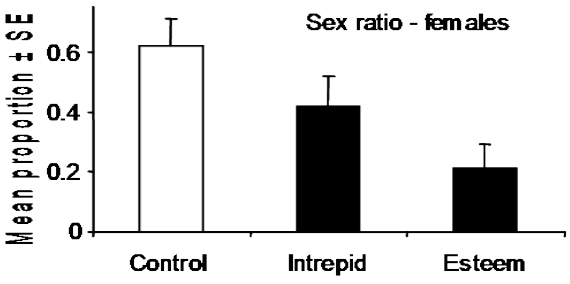
- Acute adult toxicity of Provado at 10% rate, and Actara and Success at full rate
- Sublethal effect of exposure to neonicotinyls, Novaluron and Success, reducing adult fecundity
- Sublethal effect of exposure to IGRs, biasing progeny sex ratio toward males
- Sublethal effect of exposure to neonicotinyls and Success, reducing adult survivorship

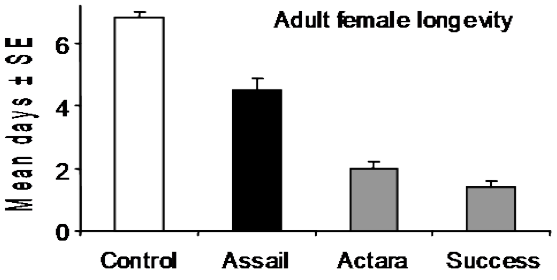
We developed a 'worst case scenario' lab assay to determine both the acute (48 h) toxicity of a topical exposure, and the lethal and sublethal toxicities of a combined route of exposure (topical, residual, and oral) of each product on adults (2-d old). Following treatment, all female parasitoids were kept at 23°C, 70% R.H. and 16 hours of light, and given 8 codling moth cocoons to parasitize each day. Measurements from assays included development time, survivorship, adult size (hind tibia length), and fecundity (first 6 days) and sublethal effects were followed through to F1 progeny to check on the production of viable offspring. Adult females (1.5-d old) were the only stage assayed in both sets of assays since the other stages are never exposed to insecticides in the field.

Acute toxicity and reduced survivorship of neonicotinyls and Success



Sublethal effects of neonicotinyls, IGRs, and Success on sex ratio and daily fecundity





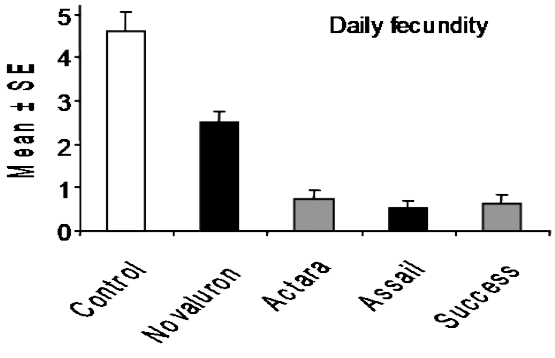


Table 1. Acute toxicity (48 hour) in topically treated females.

Product	High rate	Low rate
Provado	100	100
Agrimek	100*	30**
Assail	100	100
Pyramite	100	78
Actara	100	0
Success	0	0
Novaluron	0	0
Esteem	0	0
Intrepid	0	0

*Also killed untreated males added to Petri.
** 100% of females died before ovipositing.

Provado, Agri-Mek, Assail, and Pyramite caused high rates of mortality within 48 hours of treatment in female *A. nemoralis* (Table 1). Untreated males added to clean petri dishes containing females treated with a high rate of Agri-Mek also died within 48 hours, presumably due to contact with females during mating attempts. Females that survived 48 hours following treatment with a low rate of Agri-Mek nonetheless died before depositing eggs. No acute toxicity effects were noted for Success, Novaluron, Esteem, Intrepid, and low rate of Actara.

Newly eclosed adult females were sprayed as described, moved to a clean Petri dish and provide with untreated, psylla-infested pear leaves, and an untreated male was added to each dish; snow peas were provided as oviposition substrates. Acute toxicity was defined as death of the female within 48 hours of treatment. For females surviving longer than 48 hours, 15-day fecundity, 15-day female survival, hatch rates of eggs, development time of hatched offspring (hatch to adult eclosion), and survival of offspring (F1) was recorded. Offspring were reared on a diet of pear psylla eggs and nymphs.

Sublethal effects were monitored for Success, Novaluron, Esteem, and Intrepid, all compounds not causing acute toxicity (Table 2). For each effect, I express the treatment result as a percentage of the control result. Thus (for example), fecundity in the "Success - high" treatment was only 24% of fecundity shown by untreated controls. Values above 100% for development time indicate that development rate was slowed in offspring of treated females (relative to development in offspring of untreated females). The assays showed substantial effects of Success on fecundity and (for High rates) female longevity, with a suggestion also that offspring from Success-treated females showed reduced survival. Intrepid may have had modest negative effects on female longevity and fecundity. Novaluron had especially strong effects on hatch of eggs deposited by the treated females. All eggs deposited by Novaluron-treated females (high rate) failed to hatch; the low rate of Novaluron also resulted in strongly reduced egg hatch. Esteem did not have any detrimental effects on *A. nemoralis* for the life-history characteristics that were monitored.

Table 2. Effects of Success, Intrepid, Esteem, and Novaluron on fecundity, female longevity, egg hatch, and offspring development times. All values expressed as percentage of control.

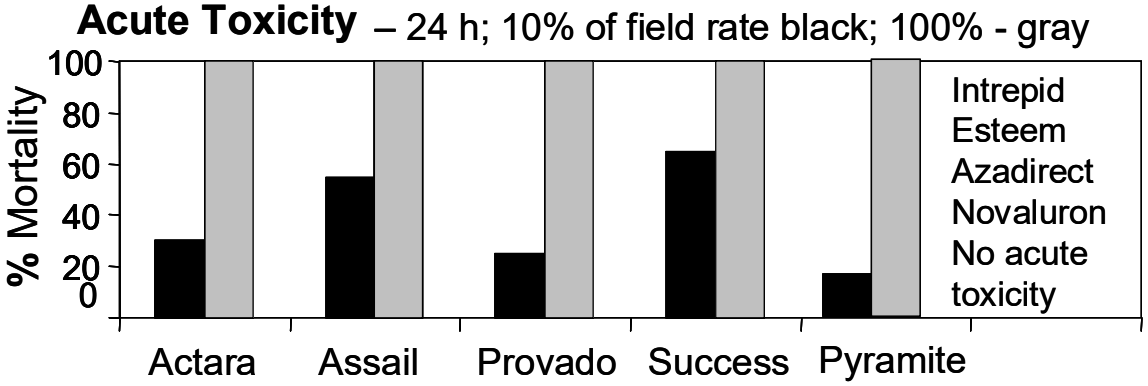
	Success		Intrepid		Esteem		Novaluron	
EFFECT MONITORED	High	Low	High	Low	High	Low	High	Low
15-day fecundity*	24	71	83	75	107	100	74	80
15-day female survival	67	100	89	78	100	107	100	90
Egg hatch	99	98	90	99	107	100	0	32
Development time	105	109	100	100	106	104	--	--
Offspring survival	76	95	106	109	116	116	--	--

* Calculated including eggs of females that died before reaching 15-day age

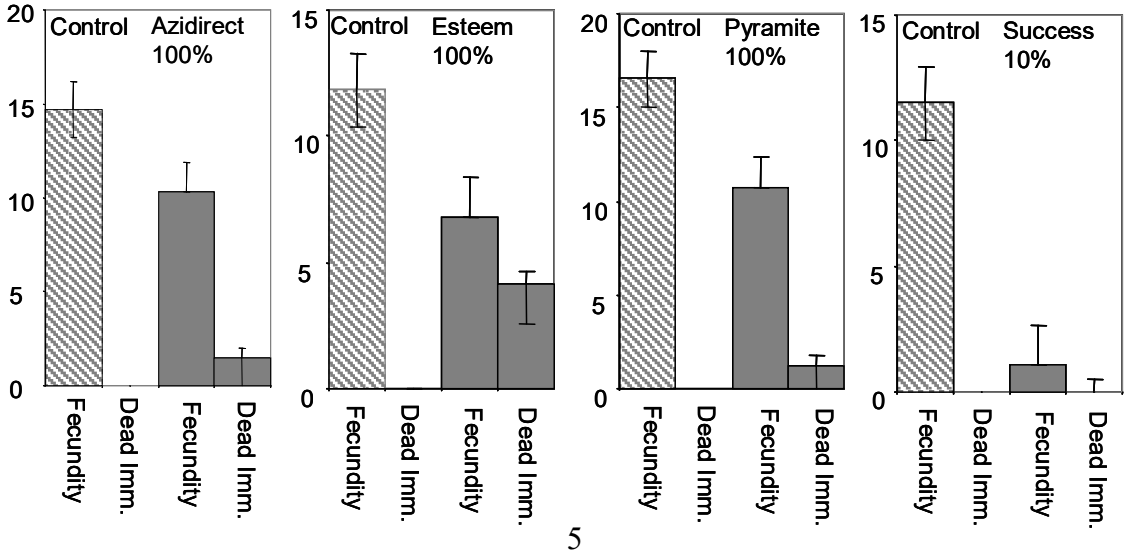
***Colpoclypeus florus*, imported parasitoid of leafrollers**

We evaluated acute toxicity for both adult female and males and recorded mortality after 24 h. The sublethal bioassays used the 3 routes of exposure combined. We used control plus concentrations that permitted greater than 25% survival from the acute assay for sublethal studies. Three- to five-d-old mated females were used and they were provided 2 hosts sequentially for 3 days each. With the first host the wasp, the host-leaf roll and honey droplets in the Petri dish were sprayed. The second host, the leaf and Petri substrate and the food were untreated.

- Acute adult toxicity to all the neonicotinyls and Pyramite at field rate and substantial mortality at 10% rates.
- Sublethal effects from field rates of Aza-Direct, Esteem, and Pyramite could be at least partially explained as death of eggs and larval wasps, mostly in the first clutch of eggs.
- Sublethal effects of 10% rate of Success are similar to that seen for the neonicotinyls (not shown) and are due to poor long-term survival and no reproduction of the adult.
- There were no sublethal effects of exposure to Intrepid at both rates(not shown)
- 100% mortality/sterility was cause by exposure to Novaluron (not shown)
- No significant effects on sex ratio were observed in any sublethal assay (not shown)
- Offspring of females exposed to Esteem also showed 46% reduction in fecundity; no other significant F-1 sublethal effects were observed.



Sublethal effects of selected compounds on *Colpoclypeus florus*

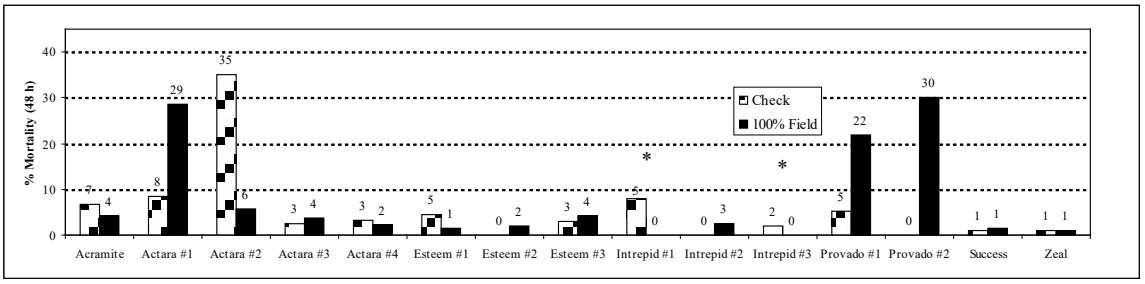


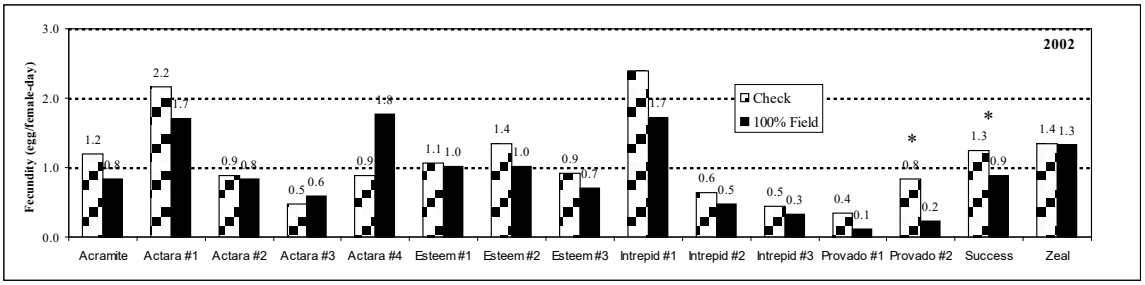
***Galendromus occidentalis* (western predatory mite), spider mite predator**

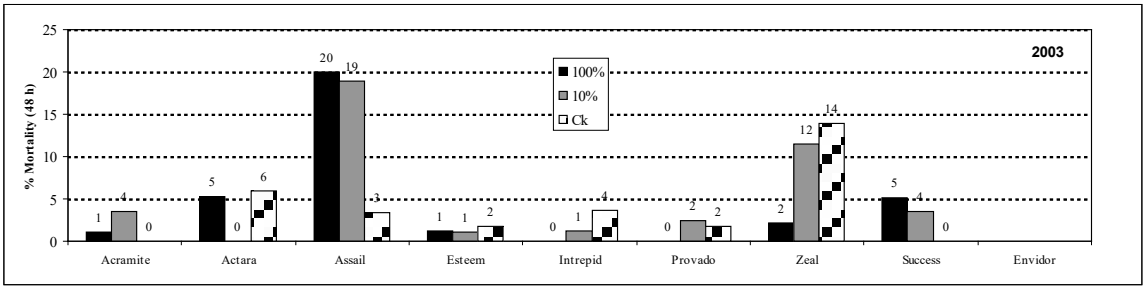
Acute mortality, fecundity and longevity of adult female *G. occidentalis* were tested in a topical-residual leaf disk bioassay. Twenty females/disk were treated in a Potter Spray Tower, and evaluated over a 7-d period. Acute toxicity was evaluated after 48 h. Tests done in 2002 used *G. occidentalis* females taken directly from commercial orchards; those in 2003 were from a cohort of eggs, and <24 h old.

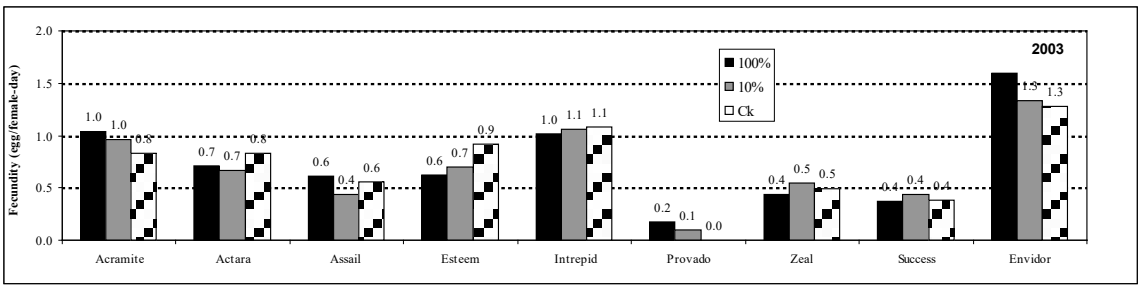
2002. Significant mortality caused by the test pesticide did not exceed 30% in 2002. Two neonicotinyls, Actara and Provado, caused the highest mortality, however, there was a signficant increase in mortality in two Intrepid assays. Significant reductions in fecundity occurred in only two assays, Provado and Success.

2003. None of the increases in mortality caused by pesticides were significant; however, the 10% and 100% field rate of Assail caused ≈20% mortality of adult females, and both rates of Zeal caused 12-14% mortality. Provado caused a slight increase in fecundity, and Esteem a slight decrease in fecundity.







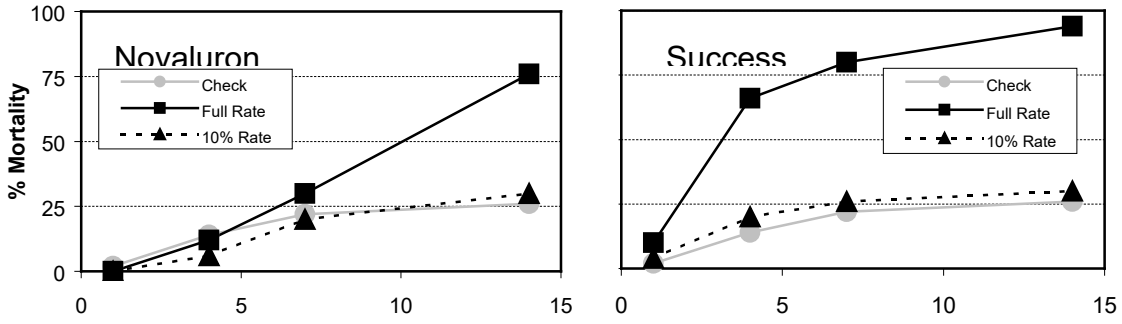


European earwig, *Forficula auricularia*, predator of aphids, pear psylla, and Lep. eggs

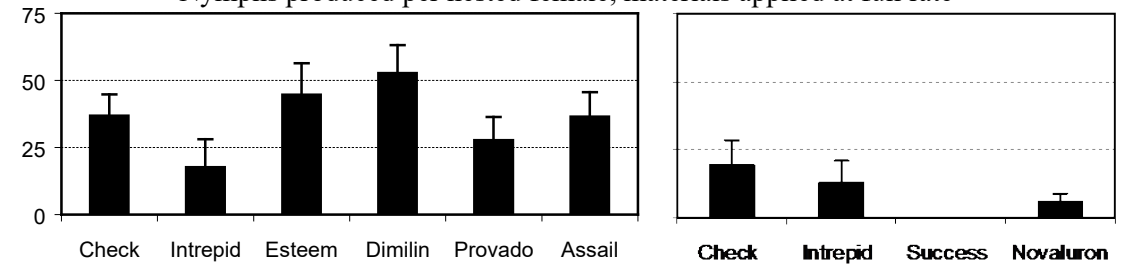
- High level of acute toxicity from Success to adults and nymphs
- Major sublethal effects from exposure to Success and Novaluron
- Moderate level of acute toxicity from neonicotinyls to adults and nymphs
- Moderate reduction in overall fecundity from exposure to Intrepid

Due to the European earwig's protracted life cycle, bioassays for sublethal effects required a minimum of seven months. Mortality often occurred in a delayed manner, this was particularly evident with the effect on nymphs from exposure to Success and novaluron where mortality continued to increase for up to two weeks following treatment. In the initial test on adults in 2002-3, the only significant sublethal effect observed was a 52% reduction in overall fecundity with Intrepid. This test was repeated unsuccessfully in 2003-4 and again in 2004-5 with novaluron and Success as additional treatments. In the 2004-5 test, overall fecundity was reduced by 32% with Intrepid, 68% with novaluron and 100% with Success. Low survivorship in the long term bioassays was a consistent problem and complicated measurement of possible carryover effects in the F1 generation. A total of thirteen materials were tested over the course of this study.

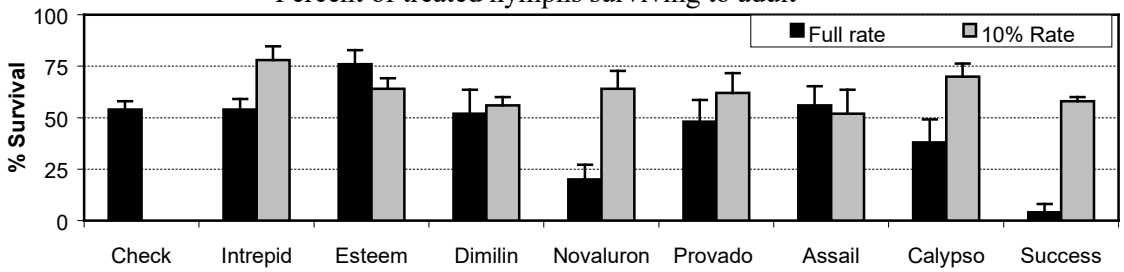
Percent mortality of nymphs up to 14 days after treatment



Nymphs produced per nested female, materials applied at full rate



Percent of treated nymphs surviving to adult

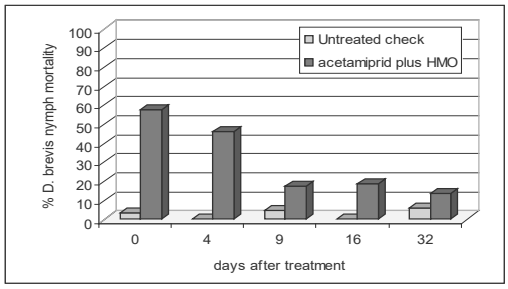


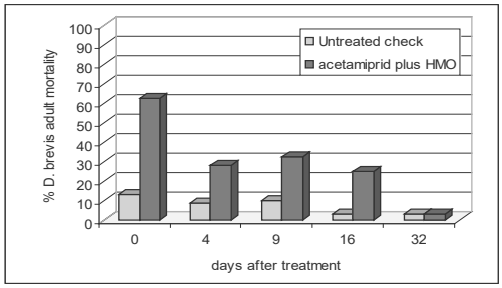
***Deraeocoris brevis*, predator of pear psylla and other insects and mites**

- Acute toxicity of Actara, Assail, Calypso, Clutch, and Provado; AgriMek; Danitol; and Imidan to *D. brevis* nymphs and adults
- Acute toxicity of Actara, Calypso, and Clutch, Provado; Danitol; Esteem, novaluron; and Imidan on *D. brevis* eggs
- Sublethal effect of adult exposure to novaluron; Success; AgriMek, reducing egg numbers and hatch
- Sublethal effect of adult exposure to AgriMek, increasing nymph development time in subsequent generation
- Sublethal effect of nymph exposure to Intrepid and AgriMek, increasing development time
- Moderate residual toxicity of Assail on apple foliage, causing mortality for 3-4 weeks
- High impact of neonicotinyls Assail, Actara, Calypso, Provado and AgriMek on *D. brevis* on pear in the field, with no or little recovery for at least 5 weeks

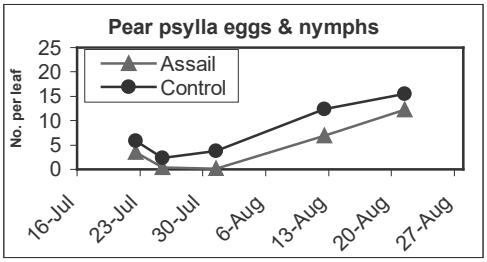
Laboratory tests: The acute toxicity of the following insecticides to *D. brevis* eggs, nymphs and adults was assessed: Actara, Assail, Calypso, Clutch and Provado (neonicotinyls); Esteem, Intrepid, and novaluron (IGRs); AgriMek and Success (fungal metabolites); Danitol (pyrethroid); Nexter (pyridazinone); and Imidan (organophosphate). Insecticides were tested at the full and 10% field rate with distilled water serving as untreated check. Insecticides with more than 25% survival in the acute bioassays were further evaluated to determine sublethal effects on development and reproduction. These included AgriMek, Assail, Intrepid, novaluron, and Success. Results have been published (see Kim et al. {2004} in Arthropod Management Tests, Vol. 29; and Kim et. al. {2006; in press} in BioControl, Vol. 51).

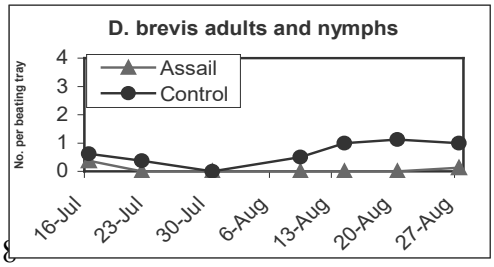
Semi-field test: The residual toxicity of Assail (acetamiprid) was evaluated by exposing *D. brevis* nymphs and adults to apple foliage treated in the field by air blast sprayer (3.4 oz/acre). Fresh deposits caused about 60% mortality in both stages. Impact declined to 15 to 20% mortality after 16 d. Exposure to sprayed apple foliage caused little or no mortality after 32 d.





Field tests: Spray trials using the full field rate were conducted to assess the impact of Actara, Assail, Calypso and Provado; AgriMek; and Mitac on *D. brevis* populations on pear. As an example, the impact of Assail on prey and predator are shown below. AgriMek and all neonicotinyl insecticides tested eliminated *D. brevis* from the orchard for at least 5 wks due to high initial toxicity, impact on prey, and possibly lack of a source population for recolonization.



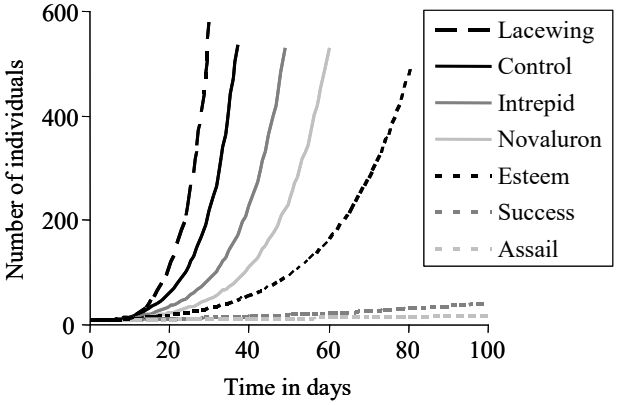


Demographic Approach to Risk of Pesticides to Natural Enemies

Traditionally the impact of pesticides on natural enemies has been based on acute mortality from topical exposure or accumulation from residues on the foliage, and expressed as an LC_{50} referring to the concentration of the product that causes 50% mortality of the natural enemy species. For the newer generation insecticides that have been developed to replace the organophosphates, impacts on natural enemies include more subtle effects such as reduced fecundity and male biased sex ratios, and thus LC_{50}s are no longer capture the overall effect on natural enemy populations. Sublethal effects are also more complex and thus it is less intuitive whether a 60% reduction in fecundity has a greater impact than 50% mortality. To resolve this problem, we have developed age-structured population models to incorporate the influence of any changes in life history parameters brought about by the impact of pesticides on the growth rate of natural enemy populations.

This demographic approach is based on an age-structured Leslie matrix model of life-history elements (survivorship, development rate and fecundity), allowing the incorporation of both acute and sublethal effects of exposure to pesticides. The model examines the impact of pesticides on natural enemies in terms of population growth or recovery time after exposure, providing a holistic population-level endpoint for the toxicology assays that can be used for comparisons among natural enemy species and products. The endpoints that have been considered are the intrinsic rate of population increase (r), the net reproductive rate (R_0), and the difference in time that it takes an exposed population to grow from 10 to 1000 individuals in comparison to a control population (D).

As an illustration, we present the results of this approach applied to the assay results from a predator, *Chrysoperla carnea*, and a parasitoid, *Mastrus ridibundus*. The graph compares the population growth of *M. ridibundus* populations in the absence (control) and presence of various insecticides, and in relation to the population growth of *C. carnea* (control). Note that *C. carnea* is in general a more rapid rate of population growth than *M. ridibundus* and so could recover more quickly after pesticide disturbance. Note also that the acute toxicity of Success to *M. ridibundus*, is matched by the reduced adult survivorship and fecundity caused by Assail, with the male bias in offspring sex ratio caused by Esteem not far behind.



The table shows the consistency of the three population endpoints and provides a comparison between natural enemy species and pesticide products.

	Chrysoperla carnea			*Mastrus ridibundus*		
	r	R_0	*D*	*r*	R_0	*D*
Control	0.175	60.52	0	0.128	12.40	0
Provado	0.011	1.32	393	-0.020	0.63	
Actara	-0.011	0.75		-0.020	0.63	
Assail	-0.004	0.91		0.007	1.18	630
Success	-0.006	0.85		0.016	1.41	251
Intrepid	0.158	44.76	4	0.094	7.20	14
Esteem	0.175	60.52	0	0.054	3.42	52
Novaluron	-0.124	0.00		0.079	5.37	27

Table 1. Acute toxicity summaries for 7 natural enemy species (2002-2004)
0=no effect, 1=up to 25% mortality, 2 = 25-50% mortality; 3= 50-75% mortality;
4 =75-100% mortality. Acute toxicities in the 3-4 range predict that few insects will survive a spray of this product in the field.

Acute Toxicity **0 = none** **1 = trivial** **2 = modest** **3 = strong** **4 = severe** **n = no data**	Earwig Nymph, adult	Lace-wing egg, larva, adul	Predatory mite (Adult)	*D. brevis*;	*A. nemoralis*; High, low rates	*C. florus* adult	*M. ridibundus* adult
Provado 1.6F	3,2	0, 3, 3	1	3, 4, 4	4,4	3	4
Actara 25WDG	n	0, 4,4	0	3, 4, 4	4,0	3	4
Assail 70WP	2,2	0, 1,3	1	0, 3, 4	4,4	3	0
Intrepid 2F	0,0	0, 0,0	1	0, 0, 0	0,0	0	0
Esteem 0.86EC	0,0	0, 0,0	0	2, 1, 0	0,0	0	0
Success 2SC	4,4	0, 0,0	1	1, 0, 0	0,0	4	3
Novaluron	3,0	0, 0,0	n	3, 1, 0	0,0	0	0
Pyramite 60W	n	n	n	0, 4, 3	4,4	3	n
Agri-Mek 0.15EC	3,1	n	n	0, 4, 4	4,2	n	n

Table 2. Sublethal effects of various insecticides on 7 insects tested. A value of 2 suggest that an insect exposed to this product will dye off in the orchard after this spray, more or less rapidly depending on the species tested.

Sublethal effects **0 = trivial** **1 = modest** **2 = strong** **n = no data**	Earwig Nymph, SR, fecundity	Lace-wing larva, adult, SR, fecundity	Predatory mite (fecundity)	*D. brevis*	*A. nemoralis; Fecundity, longevity, egg hatch*	*C. ;florus Female fecundity*	*M. ridibundus* Adult, sex ratio, fecundity
Provado 1.6F	0,0,0	2,1,0,1	1	n	n	n	n
Actara 25WDG	n	2,n,0,n	0	n	n	n	2,0,2
Assail 70WP	0,0,0	1,1,0,1	0	0,0,0	n	n	1,0,2
Intrepid 2F	0,01	0,1,0,1	0	0,1,0	1,0,0	1	0,1,0
Esteem 0.86EC	0,0,0	1,0,0,0	1	n	0,0,0	0	0,2,0
Success 2SC	2,0,2	0,2,0,2	1	0,0,2	2,1,0	2	2,0,2
Novaluron	2,0,2	0,2,0,2	n	0,0,2	1,0,2	2	0,1,2
Pyramite 60W	n	2,1,0,1	n	n	n	1	n
Agri-Mek 0.15EC	1,0,0	2,n,0,n	n	0,0,2	n	n	n

FINAL PROJECT REPORT

Project Title: Field testing of multi-component host plant kairomones
PI: Peter J. Landolt, Research Entomologist and Research Leader.
Organization: USDA, ARS, Yakima Agricultural Research Laboratory, Wapato, WA
Cooperator(s): Jay Brunner, WSU, Wenatchee

Contract Administrator : Carolyn Yager, cyager@yarl.ars.usda.gov/509-454-6575

Objectives:

Project objectives:

1. Determine 2-component blends that are attractive to codling moth females in apple orchards.
2. Determine if reported multi-component blends are due to responses to 2-component blends.
3. Compare doses and ratios of a select kairomonal blend, to provide researchers with an improved lure for study.

2005 Objectives/goals.

1. Optimize beta farnesene as a codling moth lure.
2. Optimize an attractive ester blend as a codling moth lure.
3. Compare kairomones for efficacy in attracting male and female codling moth and the seasonal pattern of codling moth response to these kairomones.

Significant Findings:

1. A new GC-EAD study of wild codling moth antennal responses to diluted apple volatiles revealed significant antennal responses to a small number of compounds.
2. Statistically significant attraction of codling moth was demonstrated to □-farnesene, E,E-□-farnesene, and the combination of ethyl benzoate and ethyl caproate.
3. Extensive and comparative field testing of the farnesenes, ethyl benzoate, ethyl caproate, and other apple volatiles indicated only weak attractiveness compared to the response of codling moth to pear ester.
4. Direct comparison of pheromone, pear ester, and the combination of both lures on back yard trees showed a consistent enhancement of male response with the combination of pear ester and pheromone (Figure).

Methods used:

Study 1.

Evaluation of GC-EAD active apple volatiles. In 2004 we used a GC-EAD set up to assess codling moth female antennal responses to volatiles of infested apple fruit, with the strategy of analyzing a serial dilution of samples of those volatiles. Looking at the most dilute sample that provided antennal responses,we saw a small set of consistent EAD-active compounds from these field collected apple fruit (nonanal, ethyl caproate, ethyl benzoate, bergamotene, and methylbutyl acetate). These compounds were tested in 2004 and in 2005 as partial and complex blends, to determine their attractiveness to codling moth in the field. One test evaluated a 5-component combination and blends with individual components dropped out. Other trapping tests evaluated single EAD-active chemicals (including compounds indicated by other laboratories to be EAD active), or multi-component blends. Compounds evaluated included beta farnesene, alpha farnesene, bergamotene, ocimene, linalool, pear ester, nonanal, methylbutyl acetate, ethyl caproate, and ethyl benzoate. Chemicals generally were formulated in rubber septa at one mg

loads, and were replaced every week or 2 weeks, depending on volatility. Pherocon 1C wing traps were used. Replicates of these tests were split between commercial orchards near Yakima, WA, and the Tukey Experimental Farm, WSU Pullman.

Study 2.
Beta farnesene optimization. Field tests were conducted in apple orchards to determine effects of changing the release rate, to compare trap designs, and to look for co-attractants with beta farnesene. The chemical was dispensed from vials for a high release rate range and rubber septa for a low release rate range. The trap designs tested were the Delta, wing, UniTrap or bucket, Sterling Smart, and pane traps. Trapping tests were conducted in both commercial and the WSU Tukey Experimental orchards.

Study 3.
Seasonal pattern of response and comparison of lures. The pear ester, beta farnesene, alpha farnesene, and ethyl benzoate with ethyl caproate were compared from April to late September. Traps were placed in commercial apple orchards and lures and traps were maintained through the Spring and Summer.

A season-long comparison was also made of the sex pheromone, the pear ester as a kairomone, and the combination of both lures placed in the same trap. Pheromone lures were Trece 1x lures, and kairomone lures were one mg pear ester on pre-extracted red rubber septa. Sterling Smart Traps were used for this experiment, and traps were placed in backyard and escaped or volunteer apple trees.

Results and Discussion:.

Study 1.

Testing of EAD active compounds. We showed in trapping experiments that beta farnesene, E,E-alpha farnesene, ethyl caproate with ethyl benzoate, as well as pear ester, are attractive to codling moth. However, the first four compounds have been only very weakly attractive, in comparison to pear ester.

Males responded significantly to the 5-component blend of nonanal, bergamotene, ethyl caproate, methyl benzoate, and methylbutyl acetate, as well as to the 4-component blends missing either ethyl caproate or methylbutyl acetate. Responses of females to these lures were not statistically significant, but greater numbers were in traps baited with the 5-component blend and the 4-component blend missing bergamotene.

Study 2.

Beta farnesene tests. The pane trap baited with beta farnesene captured the greatest number of codling moths, followed by the wing trap. Nearly no codling moths were captured in Multipher, red sphere, or Universal moth traps baited with beta farnesene. Numbers of male codling moths generally increased with the load (milligrams) of beta farnesene on the septum, up to the 10 mg maximum tested. With beta farnesene dispensed from vials, there was a negative correlation between numbers of males captured and vial hole size, indicating decreasing attractiveness with increased release rate. Numbers of males in traps with beta farnesene were increased with the presence of ethyl caproate and ethyl benzoate, but not synergistically.

Study 3.

Season-long response to lures. In the comparison of kairomones, by far the greatest number of codling moths were captured in traps baited with the pear ester, throughout both flights (Figure 2). In the comparison of pheromone and kairomone; throughout both flights the greatest numbers of codling moth males were in traps baited with the combination of pheromone and pear ester. In the first flight, numbers of males in pheromone traps were about 4X higher than in kairomone traps, but in the second flight these catches were comparable. Numbers of males in

traps baited with both lures were 2 to 3 X higher than in traps baited with either pheromone or kairomone (Figure 1). Numbers of females captured in traps baited with kairomone were similar to catches of females in traps baited with pheromone and kairomone together (Figure 1).

This work demonstrated codling moth attraction to several chemicals that are present in apple odor, including responses by females and by males. Although these responses were fairly consistent, they were also very weak in comparison either to codling moth attraction to pear ester, or attraction to the sex pheromone. Testing of combinations of chemicals did not show any significant positive interactions among compounds. These results are similar to that obtained earlier in the evaluation of a number of apple odor compounds tested in combination with the pear ester; none improved trap catch over that obtained with pear ester alone. It is possible that combinations or blends of compounds may be more attractive when released in a particular ratio.

The testing of doses of beta farnesene indicated improvement with 10 mg rather than the original one mg dose on a rubber septum, but also showed a decrease in attractiveness with the higher release rate range obtained with vial dispensers. The results of the trap design comparison, although not exhaustive, indicated that perhaps better results might be obtained using a pane or panel trap design for evaluation of kairomones. As with the pear ester, results with beta farnesene indicate a consistently stronger response by males than females, particularly in the first flight.

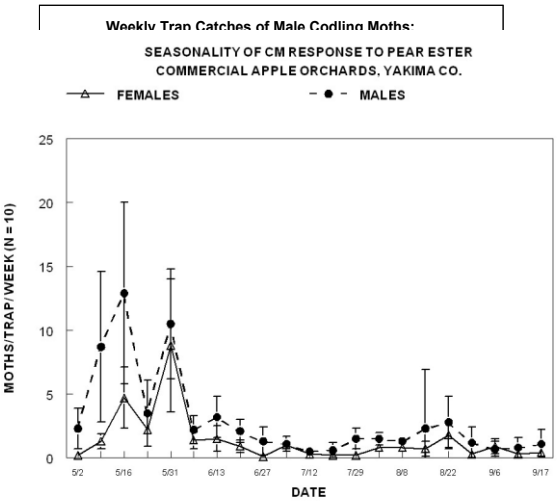
Other tests of blends, such as the combination of E,E-alpha farnesene, beta farnesene, linalool, ocimene, and hexyl hexanoate, did not produce significant results with blends. However, female codling moths were attracted by alpha farnesene, and males and females were sometimes attracted by beta farnesene. Other compounds were not attractive and did not enhance codling moth attraction to other compounds. Some were inhibitory at the levels tested (ocimene, linalool). Female response to alpha farnesene and female response to beta farnesene were not consistent. That is, results were statistically significant in some years or flights, and not in others. These are similar to problems experienced earlier with testing of pear ester. Possible confusing variables include competition from foliage and fruit odors that change with variety, pest levels, and season, as well as competition with other tested lures, and interaction with pheromone used in mating disruption.

Budget:
Project Title: Field testing of multi-component host plant kairomones
PI: Peter J. Landolt
Project Duration: 2003-2005
Current Year: 2005
Project Total (3 years): $53,200

Current Year Request: $18,100

Year	2003	2004	2005
Total	$17,500	$17,600	$18,100
Current year Breakdown	Year 1	Year 2	Year 3
Item			
Salaries	$14,300	$14,900	$15,400
Benefits			
Wages			
Benefits			
Equipment			
Supplies	$2,500	2,000	2,000
Travel	700	700	700
Miscellaneous			
Total	$17,500	$17,600	$18,100

Note: Funding from WTFRC Project "Codling moth Management with Pheromones: Key Unanswered Questions", permitted many more replicates as well as additional experiments to be conducted in tandem with this project. This included the costs of purchasing, purifying, and formulating much more of the kairomonal include replicates in Pullman, as well as Summerland, British Columbia, and Michigan.



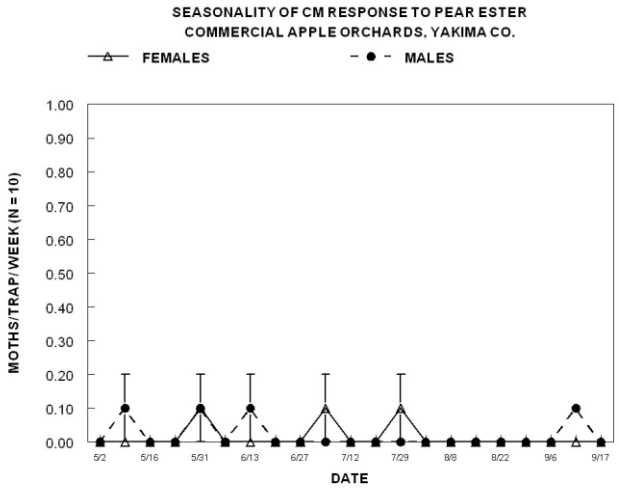


Figure 1 Mean numbers of male and female codling moth captured per trap, with traps baited with pear ester and codlemone. Back yard apple trees, Yakima County, 2005.

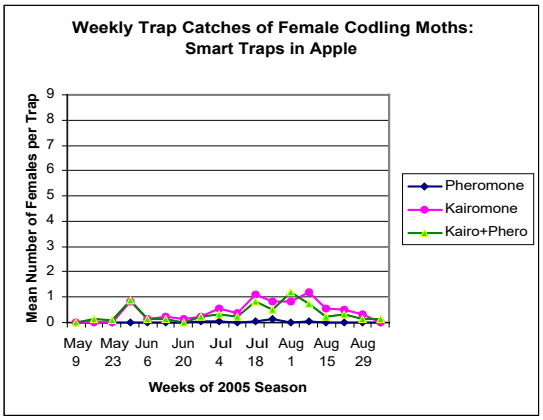


Figure 2. Mean numbers of male and female codling moths caught per trap, in traps baited with either pear ester or beta farnesene. Commercial apple orchards, Yakima, County, WA. 2005.

FINAL PROJECT REPORT

Project Title: Evaluation of a codling moth aggregation pheromone as an IPM tool
PI: Gary Judd, Pacific Agri-Food Research Center
Summerland, BC

No report submitted

FINAL PROJECT REPORT

Project Title: Optimizing the use of the codling moth (CM) granulovirus
PI: Lawrence A. Lacey, USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA, 509-454-4463, llacey@yarl.ars.usda.gov
Co-PI: Steven Arthurs, USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA, 509-454-4476, sarthurs@yarl.ars.usda.gov
Cooperators: Rob Fritts, Jr., Certis USA, Clovis, CA; Robert Behle, USDA-ARS, Peoria, IL
Contract Administrator: Chuck Myers, cwmyers@pw.ars.usda.gov, (510) 559-6108

OBJECTIVES:
1. Determine the lowest dosage of CpGV that will provide effective control of codling moth larvae.
2. Determine optimal intervals for spray application.
3. Continue to assess the shelf life of commercial formulations at various temperatures.
4. Investigate the potential of several adjuvants for protecting CpGV from solar degradation.

SIGNIFICANT FINDINGS:
- Season-long treatments of CpGV (Cyd-X) at 3 rates (1, 3 and 6 oz acre) and 3 application intervals (7, 10 and 14 days) resulted in significantly fewer deep entries and surviving larvae but did not reduce the proportion of fruit damaged by codling moth.
- There was a significant trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval.
- In replicated ½ acre plots, CpGV provided > 90% larval mortality at 1, 2 and 3 oz/acre, but was not as effective as Guthion in protecting fruit.
- The efficacy of 3 commercial CpGV formulations were significantly reduced (52-77%) by exposure to UV light (9.36×10^6 joules/m^2) in a solar simulator.
- Bioassay procedures to screen adjuvants providing possible UV protection of CpGV formulations were developed.
- The Cyd-X and Virosoft formulations of CpGV maintained larvicidal activity after storage at 2 and 25˚C for over 132 weeks, but activity was sharply reduced after storage at 35º for 16 and 40 weeks, respectively.
- Although lignin encapsulation provided significant protection of CpGV exposed to simulated sunlight in laboratory studies, under field conditions it did not.

METHODS
Optimal spray strategies (Objective 1 and 2)

Assessment of full-season virus programs adopting different application rates and spray intervals in an experimental orchard.
This study was conducted at the USDA experimental orchard near Moxee, WA. Virus applications were made to individual trees (Red Chief) using a Stihl SR420 backpack airblast sprayer with a large tarpaulin and a one-tree buffer used to confine treatments. Virus treatments (Cyd-X, Certis, USA) were applied in a factorial design with three levels for dose (1, 3 and 6 oz/acre) and application interval (7, 10 and 14 days). Dose rates covered the range labeled for use and intervals were based on persistence of treatments observed in 2003 (Arthurs and Lacey, 2004). For each treatment ten randomly selected trees were sprayed at a 100 gal./acre plus Nufilm17 at 8oz/acre. Control trees were sprayed with Nufilm17 plus water. Initial virus treatments were made at 5% egg hatch and continued until ≈ 95% (Beers et al. 1993). CM injury was assessed from 50 fruit per tree at the end of the first and second generations. Damaged fruit was removed to the laboratory to

assess both larval mortality and proportion of deep entries (> ¼ inch depth). Cardboard bands placed around trees captured surviving larvae.

Comparison of different rates of virus applied weekly to Guthion in a conventionally managed orchard heavily infested with CM.

This study was conducted within a 21 acre Delicious orchard near Zillah, WA. Virus applications were made using a 300 gal. tractor-mounted 'pull blast' sprayer. Individual ½ acre plots were marked out and treated in complete randomized block design with 3 rates of Cyd-X (1, 2 or 3 oz/acre). Five replicate blocks were sprayed at each dose @ 110 gal./acre plus NuFilm17 (8oz/acre) weekly throughout the season, with initial treatments made at 5% egg hatch. Three untreated areas served as controls. For the assessments, fruit injury was assessed from the central area of each plot and from adjacent areas treated with Guthion (azinphos-methyl). At the end of the first CM generation, 100 damaged fruit per plot were taken to the laboratory to assess larval mortality. Clear sticky 'interception traps' hung in the canopy at each plot's center were used to compared moth activity in the 2nd flight.

RESULTS AND DISCUSSION

Optimal spray strategy - study 1. Figure 1 shows fruit injury, proportion of deep entries and larval mortality for each virus treatment at harvest. Virus applications did not reduce fruit damaged by CM, but the majority of damage was in the form of shallow stings (< ¼") and larval mortality was high (>80% in all treatments). There was a statistical trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval. Rates of larval mortality were supported by the number of larvae captured in tree bands (data not shown).

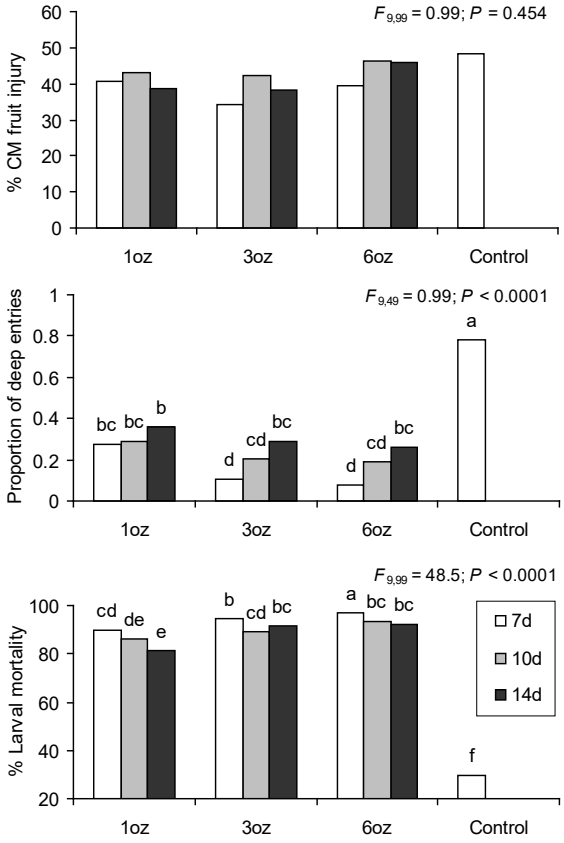
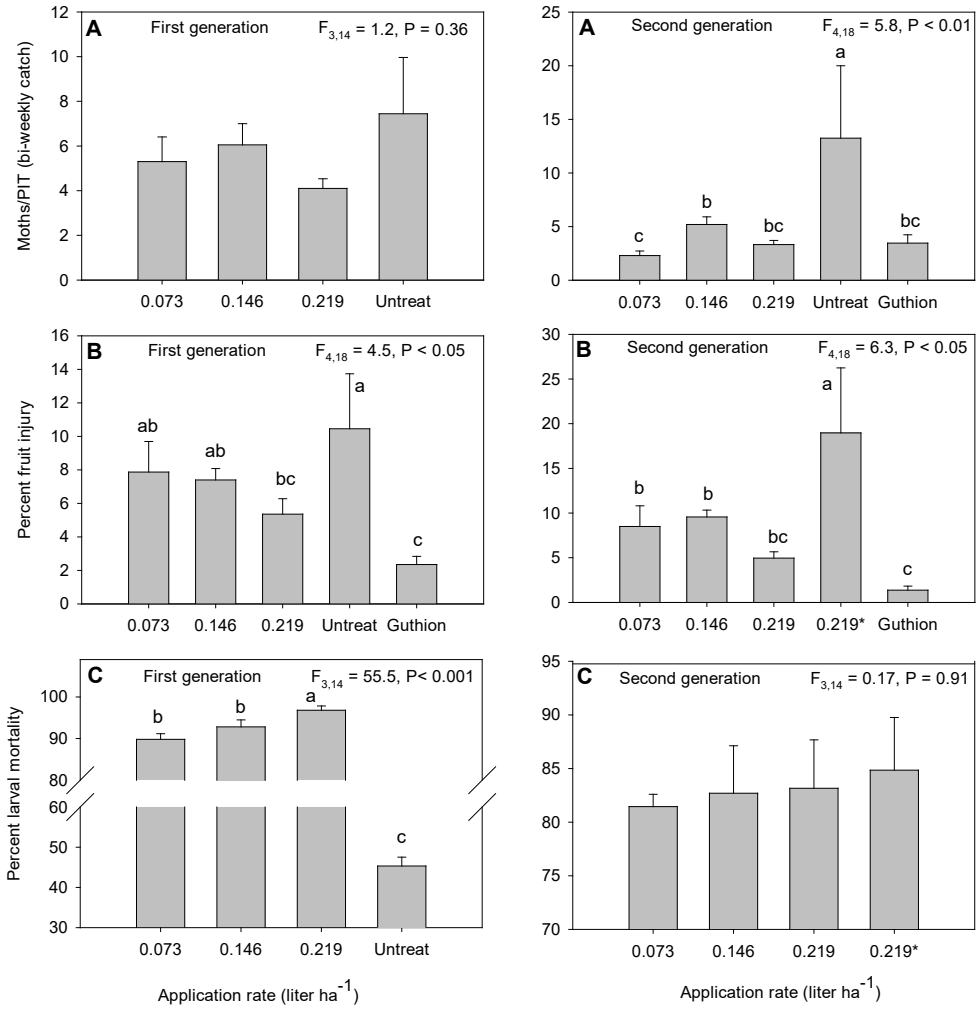


Figure.1. Fruit damage, deep entries and CM mortality following different treatments of Cyd-X in individual tree plots (data for 2nd generation). Letters indicate Fishers LSD at $P < 0.05$.

Optimal spray strategy. Figure 2 compares fruit injury, larval mortality and moth activity following 6 weekly applications of Cyd-X together with Guthion and untreated areas. There was less CM damage in virus plots compared with untreated areas, but more compared with Guthion-treated areas. Most damage was observed higher in the canopy (data not shown). Rates of CM mortality in virus-treated plots were similar to those observed in individual trees sprayed with equivalent rates of virus in the previous study. Data from interception traps showed fewer moths in virus-treated and Guthion-treated plots compared with untreated areas. Despite this, the virus study was terminated before harvest when fruit damage approached 10%. The heavy infestation (pheromone-baited traps averaged 70 moths/week) and untreated areas may have contributed to the high damage level.

Figure 2. Fruit damage, CM mortality and passive interception trap catches following different treatments of Cyd-X in ½ acre blocks in a 21A commercial orchard. Letters indicate Fishers LSD at $P < 0.05$.



Data from the experimental and commercial orchard provides information on the effectiveness of different virus programs against codling moth. The dosage and application frequency of virus that provides acceptable control (in many organic programs this will be a level at which a mating disruption program continues to be effective) will depend largely on the localized pressure of codling moth. Correlating moth counts from monitoring traps with the level of control required

will allow growers to make informed decisions about including codling moth virus into their spray programs.

Storage studies. 2003-2005. The Cyd-X and Virosoft formulations have maintained their larvicidal activity when bioassayed at 100,000 fold dilution after storage of product for 132 weeks at 2 and 25°C using bioassays techniques developed by Lacey et al. (2002). A significant decline in larvicidal activity was obsevered for Cyd-X and Virosoft after storage at 35ºC for 16 and 40 weeks, respectively. The larvicidal activity for the Carpovirusine formulation declined considerably at the highest temperature but maintained activity at 2˚C for 116 weeks (Table 1). Despite prolonged maintenance of larvicidal activity of the Cyd-X and Virosoft products after storage at 25°C, we recommend refrigeration of all products until they are used.

Table 1. Storage of codling moth granulovirus. Number of weeks stored at 2, 25, or 35°C with formulation producing ≥95% mortality in neonate codling moth larvae (10^{-5} dilution of product).

Temperature (°C)	number weeks stored with larvicidal activity maintained		
	Cyd-X	Virosoft	Carpovirusine
2	140+	132+	116
25	140+	132+	2
35	16	40	2

+ = end point not yet reached

UV protection studies. (Objective 4)

Because exposure to solar radiation limits the activity of CpGV in the Pacific Northwest, we established a bioassay system in the laboratory to assess various adjuvants for UV protection (see Lacey and Arthurs, 2005). In the procedure apples are sterilized, halved and the open end sealed using molten wax, aluminum foil and glue in preparation for virus treatments. Apples are treated in a DeVries spray cabinet which is calibrated to deliver specific quantities of experimental virus formulations (standard and high dilution) and then subsequently exposed to a controlled dose of UV and other wavelengths of light equivalent to 9.36×10^6 joules/m^2 in a solar simulator (Atlas) for 4 hours. Treated and control apples are then challenged with neonate codling moth larvae from a lab colony. Resulting fruit damage and larval mortality are measured in order to compare the activity of the virus treatments and hence quantify the most effective sunscreens for CpGV. The same approaches without the solar exposure can be used to test other adjuvants conferring possible enhancements to virus formulations, such as phagostimulants, rain-fasteners etc.

2004. In preliminary tests, we assessed virus degradation of 3 commercial formulations without additional adjuvants. The results showed a severe decline in activity resulting from the UV exposure (Table 2). These data suggest that efforts to identify sunscreens and other adjuvants that will be effective for conditions in the Pacific Northwest are worthwhile.

Table 2. Mean CM mortality on apples treated with standard rate of CpGV (1000-fold dilution) and exposed to 9.36×10^6 joules/m^2 simulated sunlight plus controls.

	Virosoft	Carpovirusine	Cyd-X
UV	29.7	20.4	46.8
No UV	95.1	90.2	98. 2
% reduction	*68.8*	*77.4*	*52.3*

2005. We evaluated lignin-encapsulated formulations of CpGV and use of various sun blocks for improved ultraviolet (UV) protection based on laboratory bioassays with a solar simulator and in field tests in an infested apple orchard. In laboratory tests spray-dried lignin-based formulations

with and without the additives titanium dioxide (TiO_2) and sugar provided extended UV-protection of virus when applied at a high dosage of 3×10^{10} OBs/L (i.e. 92-94% control compared with 66-67% from a commercial glycerin-stabilized or unformulated product) but not at a lower dosage containing 3×10^8 OB/L (Table 3). Equivalent dosage-dependent patterns in solar protection was observed in further tests with the lignin only formulation, when an intermediate dosage (3×10^9 OB/L) was also found to be ineffective. The dosage of a blank lignin formulation did not affect larval mortality, suggesting that the UV protection at the high dosage reflected the combined effect of lignin and virus (Table 4).

Table 3. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with unformulated, glycerin-stabilized and experimental spray-dried lignin formulations of CpGV and irradiated with a solar
simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with two rates of virus.

Formulation	High dose (3×10^{10} OB/L)		Low dose (3×10^8 OB/L)	
	% mortality	% deep entries	% mortality	% deep entries
Untreated	34.4c	97.6a	34.4c	97.6a
Unformulated	67.2b	67.3b	39.2bc	86.1ab
Cyd-X	66.8b	65.9b	55.0ab	78.8b
Lignin	93.6a	29.7c	52.8ab	75.8b
Lignin + sugar	92.8a	28.5c	58.0a	79.4b
Lignin + TiO_2	92.0a	41.1c	42.4bc	81.3b

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.

Table 4. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with glycerin-stabilized and experimental spray-dried lignin formulations of CpGV and irradiated with a solar simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with three rates of virus

Formulation	High dose (3×10^{10} OB/L)		Med. dose (3×10^9 OB/L)		Low dose (3×10^8 OB/L)	
	% mortality	% deep entries	% mortality	% deep entries	% mortality	% deep entries
Untreated	21.4c	83.2a	21.4b	83.2	21.4	83.2
Cyd-X	55.7b	62.0a	44.5a	73.2	37.8	61.9
Lignin	95.4a	25.6b	41.7a	73.5	37.3	70.6

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.

The use of several spray adjuvants, NuFilm-17 and Organic Biolink (sticker-spreaders at 0.06% v/v), Raynox (sunburn protectant at 5% v/v) and 'Trilogy' (neem oil at 1% v/v) did not protect a commercial CpGV preparation from solar inactivation in laboratory tests (Table 5). In season long

orchard tests (Golden Delicious), the lignin formulation of CpGV applied at 6.57×10^{12} OB/ha did not significantly improve control of codling moth or reduce fruit injury compared with a commercial preparation (Cyd-X) at equivalent rates (Table 6). Our studies show that lignin-encapsulated CpGV formulation provides solar protection but only at relatively high dosages. The testing of high concentrations of carrier containing reduced virus concentrations of virus would be worthwhile.

Table 5. Percentage mortality and deep entries (≥ 6 mm) of codling moth larvae recovered on half apples previously treated with CpGV (Cyd-X) with and without spray adjuvants and irradiated with a solar simulator. Data show average for five replicate tests (n = 25) for fruit sprayed with two rates of virus.

	High dose (3×10^{10} OB/L)		Low dose (3×10^{8} OB/L)	
Adjuvant	% mortality	% deep entries	% mortality	% deep entries
Untreated	29.6c	87.8a	29.6	87.8
None	58.8a	68.2b	38.9	89.3
NuFilm-17	58.4a	60.6b	44.8	86.2
Biolink	58.4a	75.3ab	37.3	87.9
Raynox	52.8ab	74.4ab	35.0	89.8
Trilogy	42.4bc	72.1b	39.2	85.2

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.

Table 6. Orchard tests with CpGV against codling moth. Assessments of spray-dried lignin formulations were compared to Cyd-X following 4 applications at 6.57×10^{12} OB/ha (3 oz/ac) against the 1st generation and 3 applications against 2nd generation (½ of trees were sprayed at a reduced rate of 2.2×10^{12} OB/ha= 1oz/ac).

Formulation	First generation			Se cond generation			
	% fruit damage	% mortality	% deep entries	% fruit damage	% mortality	% deep entries	Tree bands[1]
Untreated	6.1	38.5b	77.8a	33.8	27.4c	80.8a	84.1a
Blank Lignin	6.3	36.3b	75.4a	32.1	17.8c	86.1a	65.4a
Cyd-X	11.1	93.2a	30.7b	26.2	64.6ab	61.0b	17.4b
Lignin GV	9.1	87.8a	22.4b	27.9	71.4a	59.9b	22.8b
Cyd-X (½)	-	-	-	28.5	65.7ab	66.9b	23.6b
LigninGV(½)	-	-	-	23.2	58.6b	58.8b	18.6b

Column letters indicate mean significant differences using Fishers LSD at $P < 0.05$.
[1]Bands captured diapause-destined larvae, number includes any live larvae removed during fruit evaluations.

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FINAL PROJECT REPORT
WTFRC Project #AE-05-507 **WSU Project #13C-3643-6387**

Project title: Biology and Management of Secondary Pests of Apple
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Objectives:

1. Rosy apple aphid

1a. Identify the summer host or hosts of rosy apple aphid in Washington.

1b. Determine obligate status of the herbaceous host.

2. Woolly apple aphid

2a. Test chemical control tactics for aerial colonies.

2b. Test chemical control tactics for root colonies.

2c. Determine timing of peak migration root and aerial colonies.

2d. Conduct a world literature review of woolly apple aphid and its natural enemies.

3. Mites

3a. Examine the effects of newer codling moth control materials on integrated mite control.

Significant findings:

1. Rosy apple aphid (RAA)

1a. RAA colonies were found on both narrowleaf plantain, *Plantago lanceolata*, and broadleaf plantain, *P. major*, in orchards throughout central Washington. In a greenhouse experiment, aphids on these two weed species returned to apple trees in September and October. Rosy apple aphid was not found on other common weed species.

1b. Colonies on apple died out in July in a greenhouse, just as they did in the field; however, aphids kept under spring conditions (temperature and photoperiod) have continued to survive on apple alone.

2. Woolly apple aphid (WAA)

2a. Safe-T-Side oil had promising results for control of aerial colonies.

2b. Two systemic products applied as a soil drench, Admire 2F and Vydate 2L, and one applied as a foliar spray, NNP-316, showed activity against both root and aerial colonies on potted trees.

2c. First instars migrated from roots primarily from May through July, with a peak in early June (one site, Okanogan County). Very little migration was seen August through October.

3. Mites

3a. Of five commercial orchards, only one had seriously elevated tetranychid mite populations. The population was highest in the Rimon treatments, followed by Assail and Calypso,

with the OP treatment having the lowest mite population. In this orchard, the three non-OP treatments had lower overall levels of predatory mites.

Methods:

1. Rosy apple aphid

1a. Survey of weed species for RAA. A survey for rosy apple aphid on various weed species was conducted in the following Washington counties (no. sites): Okanogan (3), Chelan (4); Douglas (6), Grant (1), Yakima (11), Benton (7), Klickitat (1), Franklin (1), Skamania (1), and in Umatilla, Oregon (2). Sampling concentrated on plantain (*P. lanceolata* and *P. major*), but other species were also sampled periodically. These species included dandelion (*Taraxacum officinale* Weber in Wiggers), dock (*Rumex* spp.), lady's thumb (*Polygonum persicaria* L.), lamb's-quarter (*Chenopodium album* L.), common mallow (*Malva neglecta* Wallr.), white clover (*Trifolium repens*), and yarrow (*Achillea* spp.). Both plantain species were present at some sites, but most had one species predominant. The majority of sites were orchards, conventional or organic, with a current population or history of rosy apple aphid.

Samples of plantain and other common weeds were taken from inside the orchards or in areas directly adjacent. Samples were collected biweekly from late June through October. Plants were sampled destructively by cutting them off at ground level in sufficient quantity to fill a 1-gal plastic bag. Sample locations were identified by GPS coordinates. Aphids were extracted from plant material using Berlese funnels (2 h, ca. 100°F). A duplicate sample was taken at each site to rear aphid parasitoids; these plants were placed into plastic tubs and stored for a month at room temperature next to a window. In addition, confirmatory samples were taken from rosy apple aphid colonies in trees. All aphids were stored in 70% ethanol and sent to WSU-IAREC, Prosser, for identification.

1b. Life cycle of RAA. The life cycle was investigated with a series of greenhouse experiments. The following three questions were addressed by these experiments:

Will RAA on apple colonize plantain species? In April, bare root 'Delicious' and 'Golden Delicious' apple trees were planted in 5-gal pots, then infested with RAA from a TFREC orchard. Four herbaceous weed species, narrowleaf plantain, broadleaf plantain, woolly plantain, and common lamb's-quarter was collected from orchards and waste areas on 15 May. Narrowleaf and broadleaf plantain are perennials introduced from Europe, while woolly plantain is a native annual (Whitson et al. 1992). All three plantain species were of interest as possible summer hosts in central Washington. Lamb's-quarter, not known to be a host of RAA, was used as a check. Three replications of each apple-herbaceous plant combination were placed in cages on 23 May. All plants were maintained in a greenhouse without modified lighting.

Can RAA reproduce continuously on apple? In April, potted 'Delicious' and 'Golden Delicious' apple trees were infested with RAA from the same TFREC orchard. Trees were divided among three cages. When trees were severely damaged by RAA they were replaced (every 2-4 wk) with uninfested trees of the same cultivar. Aphids were transferred from the old to the new trees. Trees were kept in a greenhouse with ambient photoperiod.

A second group of apple trees was removed from cold storage in June, potted, and placed in a growth room in July. The growth room was kept at 62-68°F with a light:dark photoperiod of 14:10 h. Three times in July these trees were infested with aphids from wild colonies, without success. The final attempt, from a colony on 'Granny Smith' apple in Smith Tract Orchard, Orondo, was made on 22 July, and this colony became established. The colony was provided with new potted apple trees every month.

Will RAA return to apple from plantain species? The original apple trees were removed from the apple-plantain treatment cages on 11 July and colonies were left to develop on plantain. On 1 September, uninfested potted apple trees (seedling) were placed in the cages. These trees had not been previously exposed to RAA in the greenhouse.

Plants were inspected for aphids at least weekly, and specimens were collected for identification on 20 June and 4 and 29 September. Plantain and apple leaves containing aphid colonies were placed in 70% ethanol and shaken to dislodge specimens, while the entire *C. album* plants were placed in Berlese funnels for 2 h to extract aphids. All aphids were stored in 70% ethanol and sent to WSU-IAREC for identification.

2. Woolly apple aphid

2a. Chemical control of aerial colonies. This test was conducted in a commercial apple orchard in Bridgeport, Washington. Twenty-eight apple trees were selected along a road between two blocks. Three WAA shoot colonies per tree were tagged. On 18 July, live and mummified aphids were counted in the tagged colonies. Developing larvae of *Aphelinus mali* Haldemann, a parasitoid of WAA, cause later instars to turn black, instead of the usual pale purple-gray, before the adult emerges (Beers et al. 1993). Trees were assigned to one of four blocks based on the initial population density, then randomly assigned one of seven treatments within blocks. Treatments were applied with a handgun sprayer to the point of run-off on 19 July. Live aphids and aphid mummies were counted the following day, then at 3-4 d intervals throughout the rest of the test.

Data were analyzed using the Statistical Analysis System (SAS 1988). Data were tested prior to analysis for homogeneity of variance using Levene's (1960) test. Variances found to be non-homogeneous were transformed [ln(y+0.5)] before analysis. PROC GLM was used to conduct an analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test.

2b. Chemical control tactics for root colonies. This test was conducted on potted apple trees in a greenhouse. A bulk soil sample was collected adjacent to a commercial apple block near Orondo, Washington. The soil was primarily sand (Quincy series [Mixed, mesic Xeric Torripsamments]). Fifty ¾ inch 'Golden Delicious'/EMLA 7 rootstock were planted in 18 liters of soil in 14-in plastic pots on 4 April. On 4 May, after trees had grown shoots approximately 15 cm long, the trees were infested by placing WAA-infested leaves on the branches. By the middle of May, aphids were well established and had formed shoot colonies. A new generation of aphids began by early June. On 13 June, half the soil in the pots was removed and replaced with bark mulch to expose part of the roots to the new mobile aphids. Root colonies developed naturally by aphids moving from shoot colonies. Shoots were heavily infested by the middle of July, and trees were under extreme stress.

On 21 July, 24 trees with visible root colonies were selected. On each tree, three shoot colonies were randomly selected and all other aphids removed to reduce the pest pressure. After bark mulch was removed, all exposed WAA on the roots were counted, and the missing soil was replaced with additional field soil. Trees were distributed into four replicate blocks based on the population of WAA on the roots, and treatments were randomly assigned within each block. Insecticides were applied on 24 July and again two weeks later. One product, NNP-316, was applied to the foliage to run-off with a 1-gal sprayer. Water mixed with NNP-316 was first acidified to a pH of 6-7 with a few drops/liter of 1N HCl. All other products were applied as soil drenches. Trees were fully watered 3 d before application, and then 1 liter of insecticide solution was poured onto the soil. This volume completely saturated the soil in the pots without losing any solution. Starting 3 d after treatment, trees were watered regularly, but minimal water was lost through the drainage holes of the pots. Shoot colonies were assessed periodically for 6 wk after the

first application, and then all trees were lifted, the soil gently washed from the roots, and root colonies assessed.

Data were analyzed using the Statistical Analysis System (SAS 1988). Data were tested prior to analysis for homogeneity of variance using Levene's (1960) test. Variances found to be non-homogeneous were transformed [ln(y+0.5)] before analysis. PROC GLM was used to conduct an analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test.

2c. Timing of peak migration between root and aerial colonies. Three apple trees in a commercial orchard in Bridgeport were selected for monitoring. The trees were 8-yr-old Cameo scions grafted onto 15-yr-old rootstocks. Trees were selected for smooth bark that would accommodate sticky bands, history of a significant WAA population, and the soft insecticide program of the ranch. The trees had a circumference of 50-60 cm at 10 cm above the soil surface.

The sticky band trapping method was modified from that of Hoyt and Madsen (1960). Bands were made of a 3-cm wide strip of heavy-duty aluminum foil wrapped around the trunk and held in place with a thin layer of Tree Tanglefoot. A thin bead of Tree Tanglefoot was placed in the center of each band. One band was placed 15 cm above the soil surface to trap first instar nymphs moving up the trunk from the root colonies. A second band was placed 1 cm above the first to trap nymphs moving down from shoot colonies. Traps were set out 7 April and replaced weekly until frost, 27 October.

2d. Literature review: A collection of literature covering woolly apple aphid and its natural enemies was begun in the fall; an initial draft is written and will be finalized by 30 March, 2006.

3. Mites

3a. Effect of codling moth controls on mites: This test was conducted in five commercial orchards from Bridgeport to the Royal Slope. Cooperators had discretion over block size, with a minimum of one acre per treatment at each orchard. All treatments were applied by the orchard's personnel using their own equipment. Several growers elected to have 4-acre blocks so that an entire 400 gal sprayer tank could be used (at 100 gpa) to apply each treatment. Four of the orchards were primarily 'Delicious,' and the fifth one was 'Cameo.'

Treatments consisted of four different seasonal codling moth programs, applied at standard rates and timings for the given materials. Two applications of the products were made against first- and second-generation codling moth, with the key material paired with a "mite-neutral" material (Intrepid) in the second generation. The exception was Rimon, which was applied three times per generation. The treatments were Assail-Intrepid, Calypso-Intrepid, Rimon-Intrepid, and OP-OP.

Mites were sampled every 2-3 wk from late May through mid-September. One hundred leaves/plot were collected from the center portion of the plot and kept cool during transportation and storage. Mites were brushed from the leaves with a mite brushing machine and collected on a revolving sticky glass plate. The composite sample on the plate was counted using a stereoscopic microscope. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), *Tetranychus* urticae Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor [the eggs of TSM and MCD could not be distinguished and were recorded as a group]; western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); a stigmaeid predatory mite, *Zetzellia mali* Ewing; and motile stages of apple rust mite (ARM), *Aculus schlechtendali* (Nalepa).

Cumulative mite days (CMD) were calculated for tetranychid, predatory, and rust mites, giving an estimate of population densities integrated over the course of the test. CMD are the sums of the average density of mites on two dates multiplied by the number of intervening days.

Results and discussion:

1. Rosy apple aphid

1a. Survey of weed species for RAA. Both narrowleaf and broadleaf plantain support the summer generations of RAA in central Washington. Aphid identification is still in progress at the time of writing. In samples collected through September, 10 of 26 sites (38%) with broadleaf plantain had confirmed presence of RAA on that weed species. Sixteen of the 26 sites with narrowleaf plantain (62%) had confirmed presence of the aphid on that species. The presence of RAA on plantain appeared to be more related to the degree of RAA infestation in the orchard than the plantain species. The seven other weed species sampled were not a host for RAA, with the exception of one white clover sample. This was taken from an orchard with narrowleaf plantain, and two RAA were extracted from the foliage. These aphids may have crawled from a nearby narrowleaf plantain, and thus the clover was only a transient host on which the aphids do not reproduce or form colonies. Some parasitoid and hyperparasitoid specimens have been collected from apple and will be identified. A few parasitoid species have also been found on broadleaf and narrowleaf plantain.

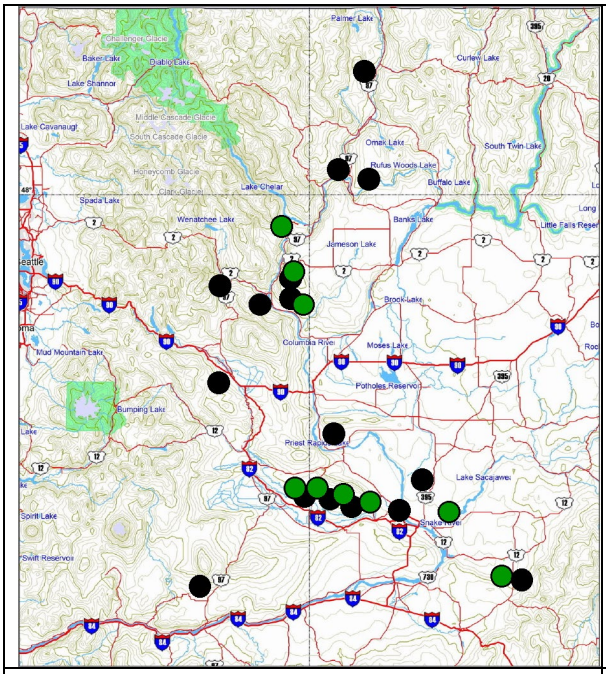

Fig 1a. Location of sites where rosy apple aphid was found (green circles) or not found (black circles) on broadleaf plantain, *Plantago major*.

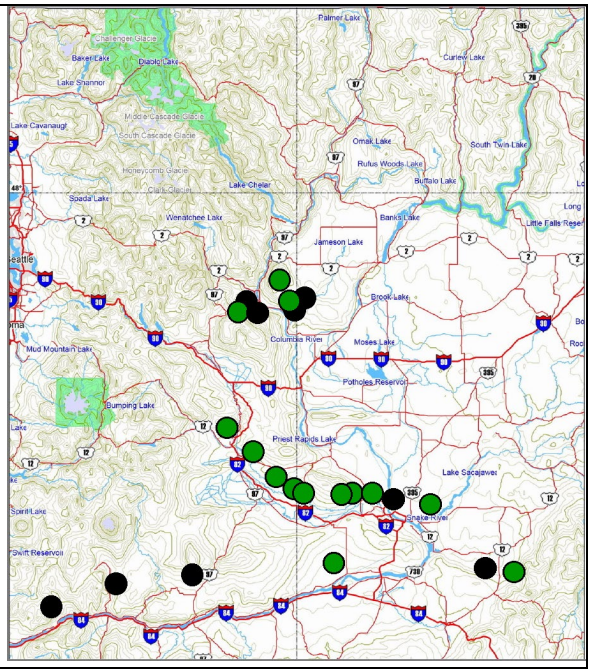

Fig. 1b. Location of sites where rosy apple aphid was found (green circles) or not found (black circles) on narrowleaf plantain, *Plantago lanceolata*.

1b. Life cycle of RAA

Will RAA colonize plantain species? On 15 May, winged aphids were present on the apple trees; then by 10 June, winged aphids were present on all of the plantain species. No winged aphids were observed on lamb's-quarter. Colonies of wingless aphids were observed on all plantain species by

20 June. On 12 July, these were confirmed to be RAA on all replications. Wingless aphids on plantain were pale yellow, and those on apple were pink or purple (Matheson 1919). No RAA specimens were ever identified on lamb's-quarter.

Can RAA reproduce continuously on apple? Although new apple trees were made available to RAA colonies grown exclusively on apple, the colonies produced more and more winged forms and had disappeared by 11 July. By this time, greenhouse temperatures reached 95-105°F each day. A detailed account of field observations in New York determined that there were only four generations on apple in the spring and summer, with most colonies producing winged forms in the second and third generation (Matheson 1919). An aphid colony in the growth chamber collected from the field on 22 July was still composed of wingless forms by the first week in December. What remains to be determined is if by chance an unusual strain was collected that is capable of continuous reproduction on apple or if all of the normally heteroecious RAA in central Washington are capable of this given the correct environmental conditions.

Will RAA return to apple from plantain species? Black, winged aphids were present on narrowleaf and broadleaf plantain by 22 August. Colonies of yellow RAA could still be located and positively identified on 2 of 3 replicates of narrowleaf plantain and 2 of 3 replicates of broadleaf plantain by 3 September. Woolly plantain plants flowered and then slowly dried during the summer, in spite of regular watering. All three plants, along with the aphids, died by 1 August. In cages with successful plantain colonies, black, winged aphids were seen landing on seedling apple trees in early September. Colonies of yellow, wingless aphids were observed on 4 of the 6 apple trees within a week. On 29 September, 2 of the 3 apple trees caged with narrowleaf plantain, and 2 of the 3 caged with broadleaf plantain were confirmed to be infested with wingless RAA, which were produced by the migrating winged females.

2. Woolly apple aphid

2a. Chemical control tactics for aerial colonies. Thiodan and Diazinon reduced WAA populations by one day after treatment (DAT - Table 1), and populations in these treatments remained low for the rest of the test. Raynox (10% v/v) provided some suppression, but this occurred more slowly than with the two standard insecticides. The lower rate (5%) and Aza-Direct treatment means were never different from the check. Although Safe-T-Side oil appeared to cause a noticeable reduction in populations due to the variability, differences were not significant. Variance in the data increased throughout the experiment, as some colonies decreased while others increased. The gradual decrease in populations on unsprayed trees, as well as initial decreases on sprayed trees, could be partly explained by an increase in parasitized aphids (Table 2).

Table 1. Woolly apple aphid densities on apple before (18 July) and after treatment with various insecticides on 19 July, 2005.

		Live woolly apple aphids/colony					
Treatment	Rate/100 gal or conc v/v	18-Jul	20-Jul[x]	23-Jul[x]	26-Jul	31-Jul[x]	2-Aug
Raynox[y]	10% v/v	21.25 a	11.75 a	5.42 ab	5.50 a	7.92 ab	7.33 a
Raynox[y]	5% v/v	23.17 a	23.50 a	22.50 a	23.00 a	19.17 ab	23.83 a
Thiodan 50W	4 lb	20.75 a	0.17 b	0.58 b	0.17 a	0.00 b	0.00 a
Diazinon 50W	4 lb	12.42 a	0.58 b	0.33 b	0.17 a	0.00 b	0.00 a
Aza-Direct 0.0987L	32 fl oz	16.08 a	14.42 a	9.50 a	9.75 a	17.92 a	14.17 a

Safe-T-Side oil	1.5% v/v	16.25 a	4.58 a	2.33 ab	0.83 a	0.83 ab	0.00 a
Check	-----	15.58 a	18.83 a	13.75 a	13.50 a	10.17 ab	7.17 a

Means within columns followed by the same letter are not significantly different, Waller-Duncan *k*-ratio *t*-test, *k*-ratio=100.

[x]Data transformed ln(y+0.5) prior to analysis due to non-homogeneity of variances.

[y]Water treated with 14 oz/100 gal EDTA.

Table 2. WAA mummies parasitized by *A. mali* on apple trees treated with various insecticides on 19 July 2005.

Treatment	Rate/100 gal or conc v/v	Parasitized (mummies) woolly apple aphids/colony					
		18-Jul	20-Jul[x]	23-Jul	26-Jul	31-Jul	2-Aug
Raynox[y]	10% v/v	5.00 a	9.67 a	11.08 a	10.08 a	10.83 a	11.50 a
Raynox[y]	5% v/v	5.42 a	6.25 ab	7.50 a	7.75 a	7.83 a	8.83 a
Thiodan 50W	4 lb	4.67 a	5.50 b	7.17 a	7.17 a	7.17 a	7.17 a
Diazinon 50W	4 lb	4.83 a	7.00 ab	7.17 a	7.17 a	7.17 a	7.17 a
Aza-Direct 0.0987L	32 fl oz	2.50 a	5.42 ab	7.08 a	6.33 a	7.67 a	9.25 a
Safe-T-Side oil	1.5% v/v	3.75 a	5.50 ab	7.58 a	7.67 a	7.92 a	8.17 a
Check	-----	6.58 a	10.50 a	10.83 a	11.92 a	11.08 a	12.00 a

Means within columns followed by the same letter are not significantly different, Waller-Duncan *k*-ratio *t*-test, *k*-ratio=100.
[x]Data transformed ln(y+0.5) prior to analysis due to non-homogeneity of variances.
[y]Water treated with 14 oz/100 gal EDTA.

2b. Chemical control tactics for shoot and root colonies: Aphid pressure was high in this test, with 138-191 WAA per shoot colony before treatments were applied (Table 3). Vydate provided the best knockdown of aphid colonies on shoots; however, both NNP-316 and Admire also significantly reduced populations by 7 DAT (31 July). Excessive heat in the greenhouse on 5-6 August, just before the second application, caused a substantial reduction in shoot populations on all trees. Although WAA began to recover on untreated shoots, populations remained extremely low on all treated trees. Heat, combined with heavy pest pressure, resulted in death of 5 of the 24 trees (treated as missing values in the analysis).

Table 3. WAA populations on shoots and roots of potted apple trees before (20 July) and after treatment with various insecticides

Treatment	Rate/vol	Shoot colonies/tree				Root colonies/tree	
		20-Jul	31-Jul	7-Aug[x]	27-Aug[x]	20-Jul	27-Aug
NNP-316[y]	150 g/liter	148.33 a	97.50 ab	0.00 b	0.00 b	27.75 a	9.25 b
Admire 2F	32 fl oz/100 gal	151.67 a	100.17 ab	0.00 b	0.00 b	28.25 a	0.00 b
Vydate 2L	2.5 ml/gal	137.50 a	1.67 d	0.00 b	0.00 b	23.25 a	0.00 b
Vydate 2L	5 ml/gal	239.17 a	31.67 bc	0.00 b	0.00 b	26.00 a	0.00 b
Vydate 2L	10 ml/gal	190.83 a	38.58 cd	0.17 b	0.00 b	42.50 a	0.00 b
Check	------	184.17 a	253.33 a	4.58 a	70.00 a	30.00 a	155.00 a

Means within columns not followed by the same letter are not significantly different, Waller-Duncan k-ratio t-test, k-ratio=100.
Treatments applied 24 July and 7 August.
[x]Data transformed ln(y+0.5) prior to analysis due to unequal variances.
[y]Tank mixed with 0.25% vol:vol Destiny methylated seed oil.

Root colonies on the trees were not extensive (Table 3), only becoming established on roots growing in the bark chips. However, large galls were produced around feeding sites. Five

weeks after the treatments began, all products had severely reduced root colonies. The low rate of Vydate, as well as Admire, provided excellent control. NNP-316 appeared to be transported throughout the apple tree, reaching the root colonies by a foliar application alone.

2c. Timing of peak migration between root and aerial colonies. Very few first instars were caught in April. Aphids moving from root colonies started increasing in May and peaked in early June (Fig. 2). Migration declined slowly until reaching near zero in mid-July. Aphids moving down from shoot colonies increased in May and stayed constant until mid-July, after which they, too, decreased to near zero. During peak activity, movement up from root colonies surpassed movement down. After July, very few first instars were trapped for the remainder of the year.

In West Virginia, aerial colonies increased in May, peaked in June, and declined in July and August. In some years aerial colonies increased again in September or stayed low (Brown and Schmitt 1994). Observations were different in California (Hoyt and Madsen 1960), where first instars moving on the trunk were trapped continuously throughout the summer and fall. Activity seemed to decrease during the rainy season, February through May; however, no repeat observations were made in different years.

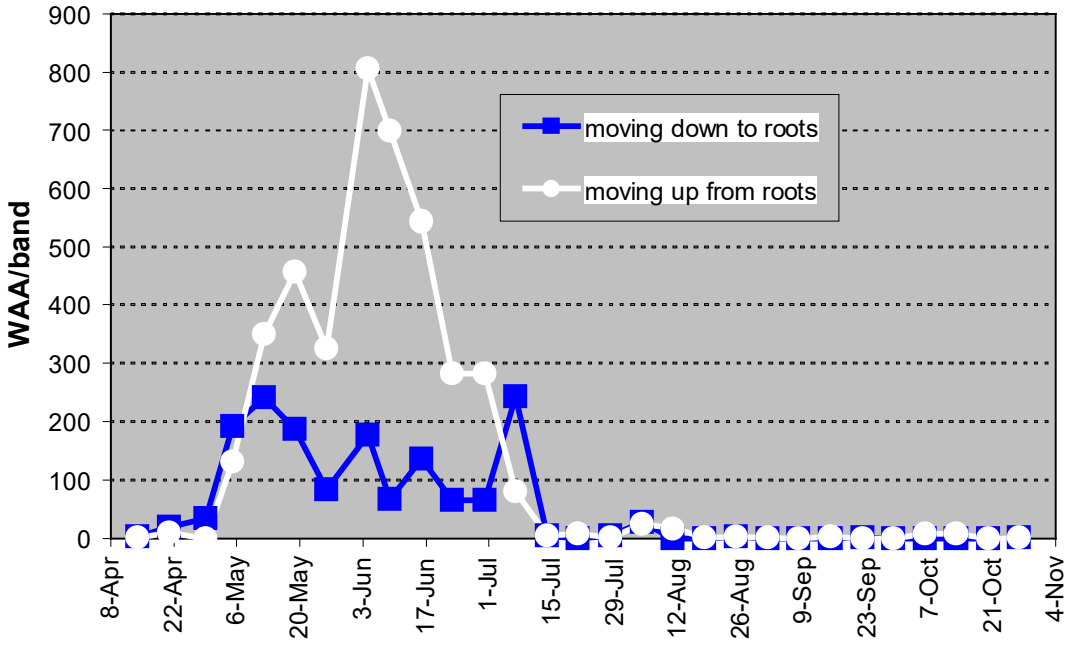

Fig. 2. Mean number of first instar woolly apple aphids trapped in sticky bands, Bridgeport, WA.

2d. Literature review. At the time of writing, the literature review is about half completed. The final version will be completed by February 2006.

3. Mites.

3. Effect of codling moth program on mites. Mites remained at relatively low densities (<2 mites/leaf) in four of the five orchards. One orchard, QLR, peaked at >50 mites/leaf in the Rimon treatment, although this orchard had slightly elevated levels in all the treatments (Fig. 3a). Because the treatments were not replicated within orchards, statistical analysis is not possible; however, at QLR the cumulative mite days were highest in the Rimon plot, followed by Assail and Calypso (very similar), with a lower level in the OP plot. Cumulative mite days did not differ greatly

among treatments (within orchards) in the orchards in which mite populations stayed low. Predatory mites peaked in midsummer in three of the five orchards, with ARR and SLH having the highest seasonal populations in late August-early September. There was little variation among the treatment CMDs for predatory mites in ARR and SLH, but some of the treatments had lower predatory mite seasonal densities in relation to the OP standard in QLR and BAN (Fig. 3b). The RYL orchard had lower predatory CMDs in the Rimon treatment but a high level in the Calypso treatment.

No codling moth damage was found in any orchard or treatment after either first or second generation (data not shown).

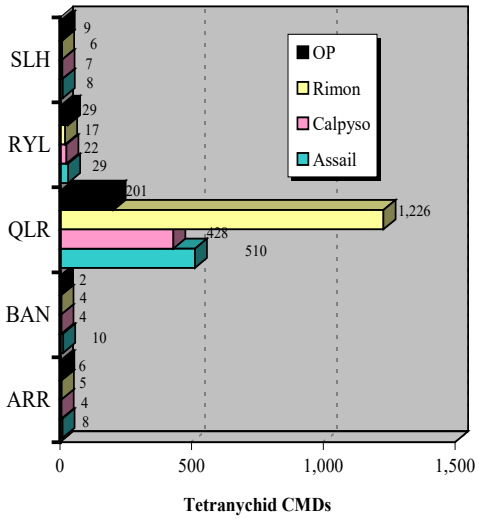

Fig. 3a. Tetranychid CMDs resulting from different codling moth programs.

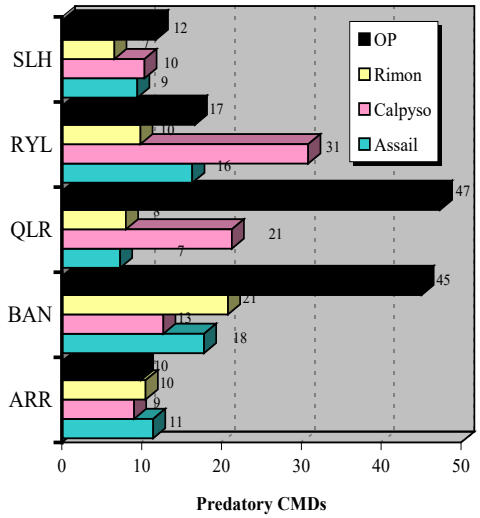

Fig. 3b. Predatory mite CMDs resulting from different codling moth programs.

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Budget:

Project title: Biology and Management of Secondary Pests of Apple
PI: Elizabeth H. Beers
Project duration: One year
Current year: 2005
Project total (1 year): $38,344

Item	Year 1 (2005)
Salaries[1]	$17,453
Benefits[2]	6,412
Wages[3]	9,360
Benefits (11%)	1,092
Equipment	-
Supplies[4]	1,000
Travel[5]	3,000
Miscellaneous	-
Total	**$38,344**

[1]Salaries: S.D. Cockfield (0.4 FTE); R. Talley (0.0625 FTE).
[2]Benefits: 36% for Cockfield, 41% for Talley.
[3]Time-slip wages (600 hours).
[4]Cell phone charges are allowed under this grant.
[5]Travel to research plots.

FINAL REPORT
WTFRC Project #AE-03-330 **WSU Project #13C-3643-3386**

Project title: Biology, migration, and management of western flower thrips in apple orchards
PI: Elizabeth H. Beers, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

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Objectives:

1. Determine the mobility of thrips in orchards and between orchards and near-orchard habitats.
2. Determine the efficacy of managing alternate hosts in the orchard groundcover during apple blossom for management of the resident WFT population.
3. Determine the period of susceptibility of apple fruit to oviposition injury.

Significant findings:

1. The source of thrips in apple flowers, as revealed with protein markers, depended on the characteristics of a particular block. A block bordered by sagebrush steppe on two sides was influenced by migration from that habitat. A block with a high population of dandelions in the groundcover had more thrips originating from the orchard floor.
2. Although rubidium (Rb) was detected in thrips exposed to the element, not enough was present to mark individual thrips. Rb was therefore not chosen as a marking technique.
3. Populations of thrips in apple flowers were highest on the orchard border next to sagebrush steppe, supporting the hypothesis of significant migration from dry, uncultivated areas into the orchard. There was typically a decrease in density within 30 feet of the border, although some orchards did not show this trend. The overall relationship in thrips density was reflected in thrips fruit damage.
4. A large reduction in dandelion densities led to a small reduction in thrips densities in apple flowers and shoots and no significant reduction in fruit injury.
5. Sampling thrips eggs revealed that very few eggs were laid in fruit during bloom. Most eggs were laid after petal fall. Excluding thrips with cages, an insecticide reduced fruit injury before peak egg lay, in that trial a week after petal fall, after which the treatments were ineffective at reducing damage.

Methods:

1. Contribution of orchard floor and near-orchard uncultivated habitats to WTF populations on apple blossoms.

1a. Distribution of thrips within orchards bordered by sagebrush steppe habitat.
This investigation was done jointly by personnel from WSU-TFREC, Wenatchee, and USDA-ARS, Yakima.[1] Seven orchards from Bridgeport to Moxee were selected for their history of thrips problems and direct proximity to sagebrush steppe habitat. In 2003, all orchards were 'Granny Smith' and had one or more edges bordered by uncultivated land. Broadleaf plants were plentiful in some blocks while others had few weeds. Transects were measured from the edge bordering uncultivated habitat into the center of the orchards. Plant samples were taken along the transects at the orchard border, 100, 200 and 300 ft at four phonological stages (pink, king bloom, full bloom and petal fall). Twenty-five open apple flower clusters and 25 dandelion flowers, if available, were collected at each location and time. Adult and immature thrips were extracted from the samples by washing with soapy water. Bloom phenology was visually estimated at each location. Five blue sticky cards were placed just above the level of ground vegetation and in the lower canopy of the trees. Sticky cards were left in place during bloom, and total adult thrips were counted at petal fall.

In 2004, the number of orchards was increased to eight and the sample locations were increased to six: orchard border, 30, 60, 100, 200, and 300 ft. Samples were taken at the same four phenological stages as above. Twenty-five flower clusters were collected at each sampling location and phenological time. Up to 25 trees were sampled in a broad band at each distance. Thrips were collected and processed as described previously. Ten sticky cards were placed in the native vegetation during bloom to measure the relative population densities of thrips available to migrate.

Data were analyzed as repeated measures using ANOVA with each distance being a repeated measure within orchards. If the distance effect was significant at $P < 0.1$, paired contrasts of adjacent distances (e.g., edge vs. 30 ft) were examined for significant differences. Contrasts were used to determine where the populations ceased to change significantly.

1b. Mark-recapture to determine inter-habitat migration.
Preliminary studies: In 2003, a number of greenhouse studies were conducted to find effective methods of marking thrips in their native habitat with broadcast sprays. The objective was to select methods that could be used in field trials in 2004 and 2005. Two different techniques were tried, common non-arthropod proteins detected with enzyme-linked immunosorbent assay (ELISA) and a rare element, rubidium (Rb), detected with chemical analysis.

Protein markers: Proteins were used as an inexpensive, external insect marker that can be detected by ELISA, an assay technique commonly used in medical diagnosis. In a series of initial studies, three non-toxic protein solutions were used: soy milk (10-20%), egg whites (10%) and cow's milk (15%). Blooming potted plants (sweet alyssum, dandelion, and marigold) in insect cages in the greenhouse were sprayed with protein solutions. Thrips were collected after one week by hanging sticky cards in the cages, and thrips were removed individually from the cards for ELISA. In addition to the potted plants, bouquets of flowers from plants in or near orchards were tested in the greenhouse. Plant species included dandelion, bitterbrush, and arrowleaf balsamroot.

[1] Dr. Dave Horton and Gene Miliczky, USDA-ARS, Yakima Research Lab, Wapato, WA.

The flower bouquets were artificially infested with thrips from the greenhouse colony, then sprayed with protein solutions. Thrips were collected as for the potted plants.
In a field trial, a sagebrush plant was sprayed with 10% egg whites in the fall to determine the success of the marker under field conditions. Thrips were again collected with sticky cards and tested for the presence of the protein marker with ELISA. Negative control samples of thrips were collected from naturally occurring field populations remote from the sprayed site.

Rubidium: In the second technique, rubidium chloride (RbCl) was applied to plants in a 500-ppm solution. Rb is incorporated into plant or insect tissues in place of potassium and becomes a permanent, internal marker that can be detected with atomic absorption spectrophotometry. Rb can also be acquired by a insect by feeding on an Rb-marked plant. RbCl was soil-applied to potted, greenhouse-grown dandelion and marigold plants that were infested with thrips. A composite sample of about 30 thrips was assayed at each sample date to determine the level of Rb marking. Thrips were sampled 1, 3, and 7 d, then weekly for 6 wk to determine the longevity of the mark.

Mark-recapture field trials with protein markers
Orondo site, 2004: This experiment was conducted in a 13-yr-old 'Granny Smith' planting of approximately 0.7 acres near Orondo, WA. The site is bordered by an extensive area of native vegetation on the east and north sides. The south side is bordered by a road and a cherry orchard, and to the west is a larger apple block. An area of 0.5 acres of native habitat was tagged adjacent to the orchard, extending approximately 50 m from the orchard border.

At pink (9 April), the tagged area in the native vegetation was sprayed with a high volume (400 gpa) of 17.5% milk solution with a handgun sprayer. The orchard drive row and weed-spray strip areas were sprayed with a 10% egg white solution at 286 gpa using a small boom sprayer. The volume used was sufficient to wet the soil, in addition to covering the vegetation. The protein spray applications were repeated at king bloom (13 April). Although the egg white concentration was not changed, the milk concentration was increased to 32.5%. Flower samples were taken after each spray as soon as the spray had dried. Flowers of four arrowleaf balsamroot plants in the native vegetation and four dandelion plants in the orchard were immediately frozen with dry ice to collect thrips. Samples of the solution in the tank were taken at each spray application timing.

A third sample of flowers of various plant species, including apple, was taken when the orchard was in full bloom (16 April). One hundred apple flowers were collected from each of six locations in the orchard. Dandelion flowers from the orchard floor were collected at the same locations as the apple flowers. Balsamroot flowers were collected from the native vegetation. Thrips samples were immediately frozen with dry ice. Negative control samples were collected from balsamroot flowers two miles from the test plot. Protective clothing was worn during spray applications and sample collections to prevent cross-contamination of the plots.

Orondo site, 2005: The experiment was repeated at the same site described above. Slight changes to the protocol included the addition of EDTA (7 fl oz/50 gal) to the spray water to improve longevity of the proteins and the substitution of 20% soy milk for cow's milk for the application to the native vegetation. Also, an additional 5 gal of the soy solution was applied with a small mist sprayer to individual flowers to increase the percentage of thrips marked. Egg white solution was applied to the orchard floor as above. Flower samples were taken at pink (11 April), king bloom (19 April) and full bloom (25 April) and processed as described above.

Bridgeport site, 2005: This site was located on a commercial orchard near Bridgeport which bordered sagebrush steppe habitat and had a very high dandelion population in the groundcover. A square area of 0.5 acres from the 4-acre block was selected for the experiment. An area in the

adjacent sagebrush steppe measuring 0.35 acres was marked. The native vegetation had arrowleaf balsamroot, lupine and phlox in addition to sagebrush.

The protocol for this site was similar to the Orondo 2005 site. Spray applications were made at pink (15 April) and king bloom (22 April). Flower samples were taken at these dates and also at full bloom (26 April).

In late May, the dandelion density was estimated at the Orondo and Bridgeport sites. A 1-m square frame was placed randomly in the row middles of the experimental blocks and the number of dandelion plants counted. Dandelions were measured in 2 locations (at least 15 m apart) in each of the 8 drive rows (total of 16 samples).

ELISA analysis of thrips: All flowers were examined under a microscope and thrips were collected on a toothpick with a tiny drop of adhesive (Stickum Special™, Seabright Industries, Emeryville, CA). Each insect was placed in the bottom of a 1.5 ml microcentrifuge vial. Thrips in vials were washed with a buffer solution which put the marker in solution. This solution was placed in a tray of wells lined with a polymer which binds the egg, soy, or milk proteins. After these proteins are bound, the remaining surface of the well was filled with a neutral protein (bovine albumen). Next, the primary antibody was added: rabbit anti-chicken albumen for egg white; rabbit anti-casein for cow's milk; and rabbit anti-soy for soy milk. Then the secondary antibody (donkey anti-rabbit) was added to bind with the free end of the protein chain. Color was formed by the addition of a substrate reacting with the secondary antibody. Color was detected with spectrophotometry. Color greater than a certain intensity was declared positive for the marker. The original buffer solution was tested for all proteins sprayed at a given site.

2. Management of resident WFT populations by reducing alternate hosts in the orchard: Four Central Washington orchards with a history of thrips problems were selected for inclusion in this experiment. Unlike the orchards used for the protein marker studies, the orchards in this study were not adjacent to native vegetation. The orchards consisted primarily of the cultivar 'Braeburn' in Quincy, Pateros and Brewster, and 'Granny Smith' in Bridgeport. Each orchard was approximately 5-10 acres. The orchard floor of one-half of each block received regular full-coverage broadleaf herbicide treatments, as well as spot treatments, to reduce the weed density. Both halves received full-spectrum herbicides (broadleaf and grasses) in the herbicide strips beneath the trees, as per the grower's normal management practices. The plots were managed and sampled for three years. At approximately 3-5 m intervals, ten 1-m square areas were randomly selected and marked in the center drive row of each treatment block. Flowering and non-flowering dandelion plants were counted in these areas to estimate dandelion density. Estimates were made monthly from April through November. In addition, dandelion plants (four flowering and four non-flowering) were collected monthly from random locations in the row middles to estimate thrips densities. In May 2003 and 2004, during full bloom but before insecticide applications were made, 25 individual open apple blossoms were selected from each of eight trees within the middle row. In 2005, due to low thrips populations, the number of flowers from each tree was increased to 300 at Bridgeport and 150 at all other sites. In June, July and August, 10 vegetative shoot tips were collected from each of eight trees. Plant tissue samples were washed with soapy water to dislodge adult and immature thrips. Specimens were slide-mounted for identification. Fruit injury was assessed in the summer by examining 500-1000 fruit *in situ* in the center row of each block. Data for thrips and dandelions were analyzed by date using ANOVA, followed by an LSD test for weedy and herbicide-treated plots. Data for proportion (p) of fruit with pansy spot injury were first transformed by arcsine(square root(p+0.001)), then analyzed using ANOVA.

3. Susceptibility of apple bloom stages to WFT damage:

Thrips exclusion, 2004*:* Starting at tight cluster, exclusion cages measuring 45 × 10 cm were placed on 100 branches of 'Delicious' trees in block 29S, TFREC. Each selected branch had 7-10 flower clusters. Cages were placed on the branches (but not closed) by 2 April. The experiment was a completely randomized design with 10 treatments (exclusion periods) and 10 replicates (cages). At each exclusion period, thrips were killed and cages closed. Thrips were killed by spraying the branches to drip with a solution of ¼ lb of Carzol 92SP/100 gal. Material was mixed in a 16-oz spray bottle and applied to run-off. The cages were left closed until the start of the next bloom stage, about 3-4 d, then after bloom cages were closed for 7-d periods. Carzol applications were made at tight cluster (5 April), pink (9 April), king bloom (13 April), full bloom (16 April), petal fall, 2.6 mm fruit diam (20 April), 2.9 mm fruit (23 April), 5.6 mm fruit (27 April), 10.9 mm fruit, (4 May), and 15.8 mm fruit (11 May). Fruit in the cages were harvested on 24-25 May. The proportion (p) of fruit injured by thrips was transformed by arcsine(square root(p)), then analyzed using analysis of variance for a CRD. Treatment means were separated with a Least Significant Difference test, α=0.05.

Thrips phenology, 2004: Phenology of egg, immature and adult thrips was assessed in the same block used for the thrips exclusion study. Blossom clusters or, after petal fall, king fruit (100/sample) were collected at intervals corresponding to the developmental stages of the apple bloom. One sample was taken per tree. Sample timings were early tight cluster (2 April), late tight cluster (6 April), pink (9 April), king bloom (13 April), full bloom (14 April), petal fall (2.6 mm fruit diameter) (19 April), 2.9 mm fruit (23 April), 5.6 mm fruit (27 April), 10.9 mm fruit (4 May), and 15.8 mm fruit (11 May). Plant tissue was washed in soapy water and the contents poured through sieves to collect insects.

After plant tissues were washed, the tissue was trimmed so that only the king bloom fruitlet remained, and this was stained with an acid fuschin solution. Next, the tissues were cleared in another solution of lactic acid and glycerin heated in a double boiler under a fume hood for one hour. After clearing, the skin (0.5 mm thick) was trimmed from each fruit. Skin was placed between two glass slides and examined under a microscope for the presence of eggs.

Thrips exclusion, 2005: The trial was conducted in a commercial orchard in Omak, WA planted with 'Braeburn' apples. Treatments consisted of different timings of Carzol 92SP at a rate of 0.5 lb/100 gal. Each plot consisted of five trees in a single row, and treatments were replicated four times. Sprays were applied to run-off with a 4-gal. capacity knapsack sprayer. Treatment times were tight cluster (17 April), pink (20 April), king bloom (24 April), full bloom (28 April), petal fall (2.9 mm fruit) (1 May), 3.7-mm fruit (4 May), 5.8-mm fruit (8 May), 6.0 mm fruit, (11 May), 11.6 mm fruit (16 May), and 18.3 mm fruit (23 May). Up to 100 fruit were sampled per plot on 16 and 18 June and 1 July and checked for pansy spot. The proportion (p) of fruit injured by thrips was transformed by arcsine(square root(p)), then analyzed using analysis of variance for a randomized complete block design. Treatment means were separated using the Waller-Duncan k-ratio t-test.

Thrips phenology, 2005: Thrips phenology was studied in the 'Braeburn' orchard used for the 2005 thrips exclusion study, immediately adjacent to the spray trial described above. From tight cluster through bloom, 100 flower clusters (or, after petal fall, 100 king fruit) were sampled at intervals corresponding to the developmental stages of the apple bloom. After bloom, ten king fruit were randomly selected out of those sampled to measure the mean fruit diameter. Plant tissues were collected into plastic bags, then stored under refrigeration and processed within a week. Samples were processed as described for 2004.

Results and discussion:

1a. Distribution of thrips within orchards bordered by shrub-steppe habitat.

2003. Thrips densities 100 ft from the orchard border were significantly lower than those at the border (king bloom, *P*=0.03, and in samples summed over time, *P*=0.05). There were no significant differences between the densities at 100 ft and 200 or 300 ft.

2004. Thrips densities 30 ft from the orchard border were significantly lower than those at the border (king bloom, *P*=0.035, summed sample *P*=0.013). The same decrease was evident for fruit injury (*P*=0.018).

The results above are for the aggregate of orchards; not every orchard (replication) showed these trends. In some, there was no relationship between population density and location. Overall, these results were consistent with the hypothesis that sagebrush steppe habitat is a significant source of thrips that infest the flowers and damage the fruit of apple in many orchards.

1b. Mark-recapture to determine inter-habitat migration.

Preliminary studies: Adult thrips incorporated some Rb within a day of application. The strongest mark resulted from larvae feeding on Rb-treated plants throughout their development. The element was detected in composite samples of thrips more than a month after it was applied to the food plant. However, when individual thrips were tested, the amount of Rb was not significantly greater than the check. Thus, the Rb technique could not be used to mark individual thrips because the detection equipment was not sensitive enough. This method was abandoned in favor of the protein markers, where marks of individual thrips can be detected.

Orondo site, 2004: After the first spray of milk solution to the sagebrush steppe, 35% of the thrips in arrowleaf balsamroot were marked with milk protein. The number increased to 45% after the second spray and decreased to 24% at full bloom (3 d after the last spray). Too few thrips were collected from dandelion in 2004 to estimate the percentages, but egg white protein was detected after both sprays to the orchard groundcover. Of the thrips collected from apple flowers at full bloom, only 16% carried one or more marks. Of the total, 14% were marked with milk protein (from native vegetation), while only 2% were marked with egg white (orchard groundcover). Less than 1% was marked with both (Fig 1). Also, <1% of thrips collected in arrowleaf balsamroot at apple full bloom was marked with egg white protein (from orchard groundcover), indicating little movement out of the orchard. Thus, of the thrips that could be traced to a specific origin, the majority had come from the native vegetation between pink and full bloom.

Orondo site, 2005: With the first spray, 29% of the thrips in arrowleaf balsamroot were marked with soy protein (native vegetation). The number increased to 40% after the second spray and decreased to 21% at full bloom, 6 d after the second spray. In dandelion flowers, 79% were marked with egg protein after the first spray, 66% after the second spray, and 9% at full bloom. Thrips marked with egg protein (orchard groundcover) were also detected in balsamroot after the first spray (21%), the second spray (8%), and at full bloom (6%), indicating significant migration out of the orchard. Of the thrips collected from apple flowers at full bloom, 13% carried one or more marks; 12% were marked with soy protein (native vegetation), while only 1% were marked with egg white (orchard groundcover) (Fig. 1). The majority of thrips that could be traced to a specific origin had come from native vegetation. In spite of the increased spray coverage of flowers in the native vegetation, and the different protein used, results of both years' trials in Orondo were nearly identical.

Bridgeport, 2005: After the first spray, 43% of the thrips in arrowleaf balsamroot were marked with soy protein. The number decreased to 25% after the second spray and decreased to 13% in the flowers sampled at full bloom. In dandelion flowers, 50% were marked with egg white after the first spray, 82% after the second spray, and 20% at full bloom. Thrips marked with egg white protein were also detected in balsamroot after the second spray (3%), and at full bloom (44%), indicating significant migration out of the orchard. This is counter to what is believed to happen, viz., that thrips migrate into apple orchards during bloom. Of the thrips collected from apple flowers at full bloom, only 4% were marked with soy protein (native vegetation), while 7% were marked with egg white (orchard groundcover) (Fig. 1). A slight majority of the thrips that could be traced to a specific origin had come from the orchard groundcover.

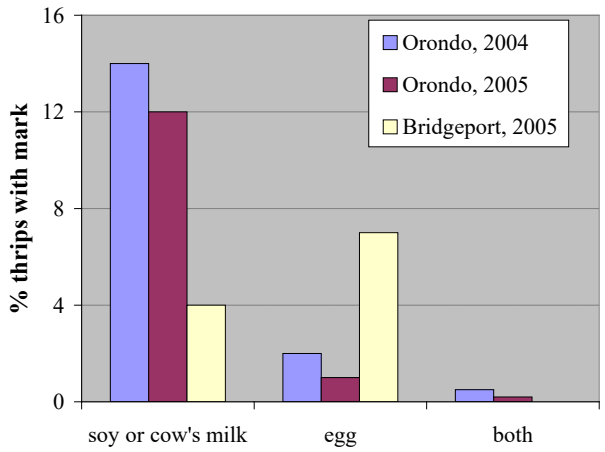

Fig. 1. Percentage of thrips in apple flowers marked with either soy or cow's milk (applied to sagebrush steppe) or egg white (applied to orchard groundcover) at two locations.

The Orondo site had fewer dandelions/m^2 (9.6) in 2005 compared with the Bridgeport site (32.6). The density of dandelions at the Orondo site in 2004 was similar to that of 2005, although no counts were made. This likely explains the higher percentage of thrips in apple flowers at the Bridgeport site originating from the orchard groundcover (Fig. 1). Thus, it appears that both the extra-orchard habitat and the groundcover may be a significant source of thrips in apple flowers, although more research is necessary to elucidate this under a broad range of conditions.

The highest number of thrips/sample collected in the three trials was at an edge of the orchard bordering native vegetation (data not shown). The highest percentage of thrips marked with soy or cow's milk (applied to native vegetation) was often distant from the edge. Apparently, thrips can move the distance of six rows or more within a short time, three to six days.

2. Management of resident WFT populations by reducing alternative hosts in the orchard. All the thrips specimens collected from apple flowers were western flower thrips, *Frankliniella occidentalis* (Pergande). Apple shoots contained a mixture of species, including *F. occidentalis*. The most common species in dandelion flowers during the late spring and summer was *Thrips trehernei* Priesner. Only *F. occidentalis* were counted in this experiment.

Dandelions slowly decreased in the low-weed plots during the first and part of the second year (Fig. 2). By the summer of 2003 dandelion population density was significantly reduced in these plots but remained moderate to very high (10 to >100/m^2) in high-weed blocks. Thrips were found almost exclusively in open flowers, rather than in non-flowering dandelions. No difference in thrips density was found in individual dandelion flowers from either low-weed or high-weed blocks. However, the very low dandelion population, especially in 2005, meant that this source of thrips was nearly eliminated in treated blocks.

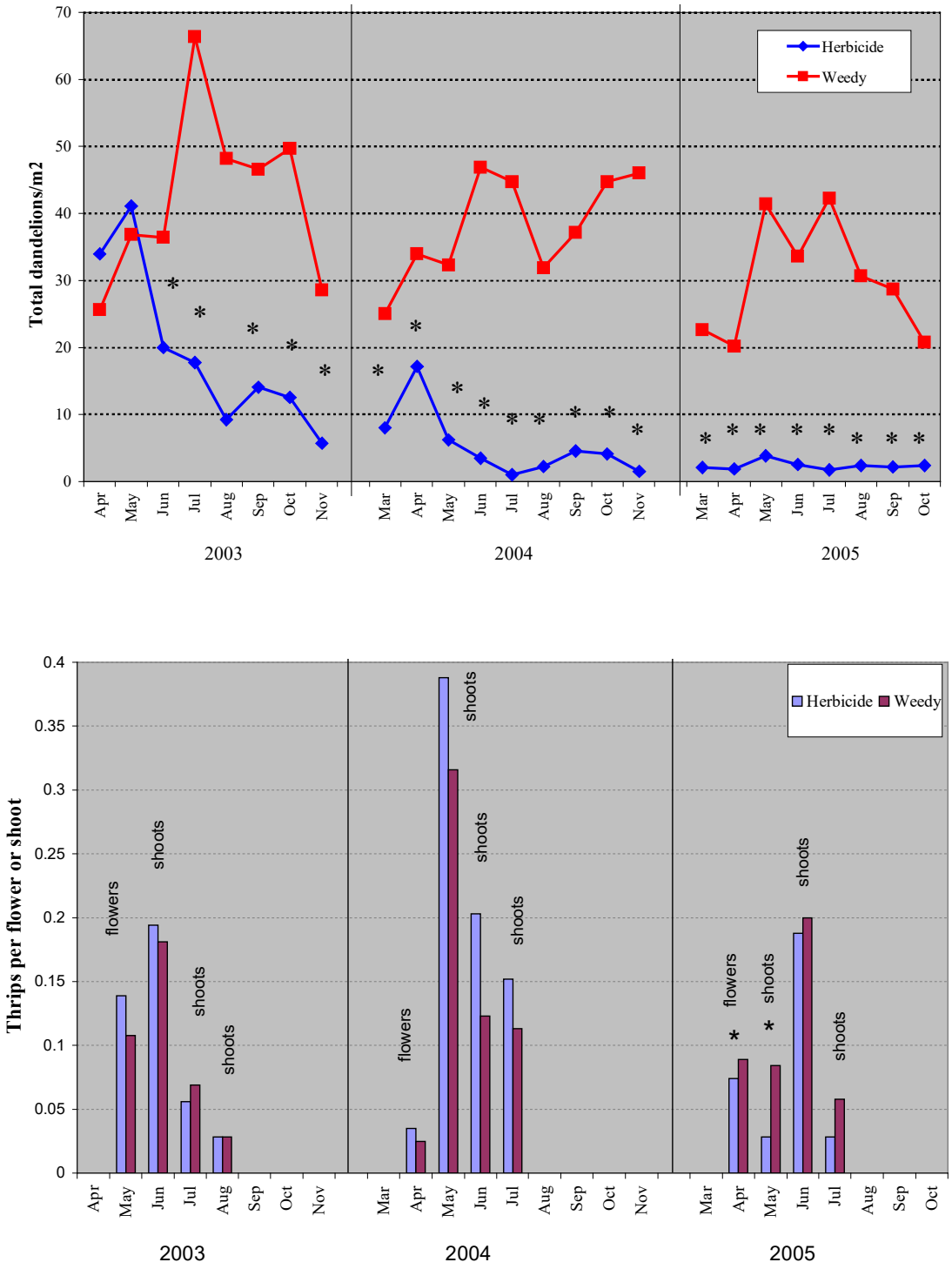

Fig 2. (Top) Mean population density of dandelions in herbicide-treated and weedy plots at four orchard sites. (Bottom) Thrips sampled from apple flowers or shoots in the herbicide-treated and weedy plots. Means marked with an asterisk (*) on a given date are significantly different, LSD test, α=0.05.

No significant differences in thrips populations were found in any of the apple flower or shoot samples in 2003 and 2004. However, in 2005, there was a significant difference in thrips populations in apple flowers between low-weed (0.074 thrips/flower) and high-weed blocks (0.089 thrips/flower). The significant reduction continued with thrips in the apple shoots after petal fall, with 0.028 thrips/shoot in low-weed blocks and 0.084 thrips/shoot in high-weed blocks. The effect was temporary, and no significant differences were found for the rest of the summer. The small decrease in thrips populations during bloom did not cause a significant reduction in fruit injury in any of the three years. The percentage of pansy spot was 1.8 (low-weed) v. 2.0 (high-weed) in 2003, 2.5 v 4.7 (2004), and 1.2 v. 0.8 (2005). Although dandelions can be a source of thrips in apple flowers (see Results, Section 1b), management of dandelions does not greatly improve management of thrips in apples or significantly reduce their damage. In spite of the loss of broadleaf weeds as alternate hosts and lack of access to native vegetation, thrips can still survive in the orchard during the summer (Fig. 2) and presumably find suitable hosts in the fall. The apple tree itself can serve as a host for months after bloom. Alternatively, in spite of the large blocks used in this trial, thrips could be moving from other habitats and colonizing the low-weed blocks.

3. Susceptibility of apple bloom stages to WFT damage.

Thrips exclusion, 2004: Exclusion of thrips after bloom was more effective in preventing fruit damage than those applied before or during bloom. Applications made when fruit was 10.9 mm or larger (2 wk or more after petal fall) were also less effective at preventing fruit damage. This timing of damage prevention corresponded to an increase in oviposition that occurred some time between 5.5 and 10.9 mm fruit size (1-2 wk after petal fall).

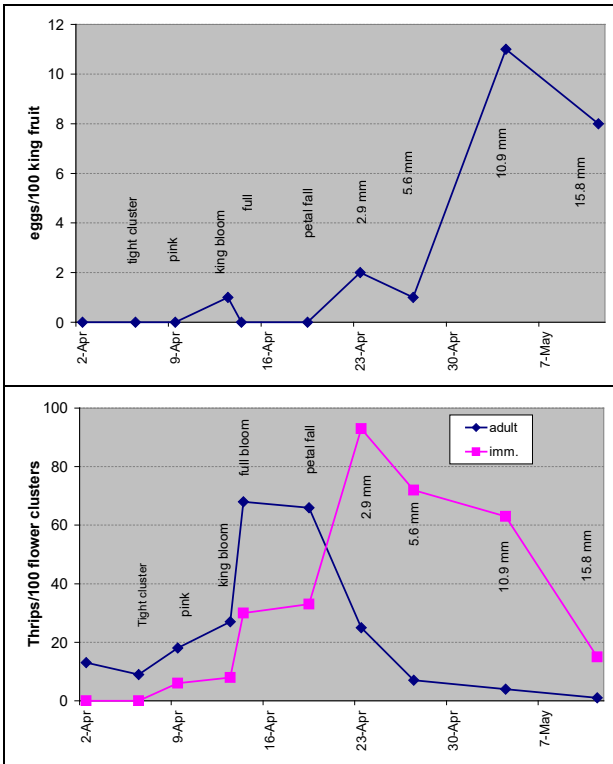

Fig. 3. (top) Eggs per 100 king fruit, TFREC, Wenatchee, WA, 2004. (bottom) Adult and immature thrips per 100 flower clusters.

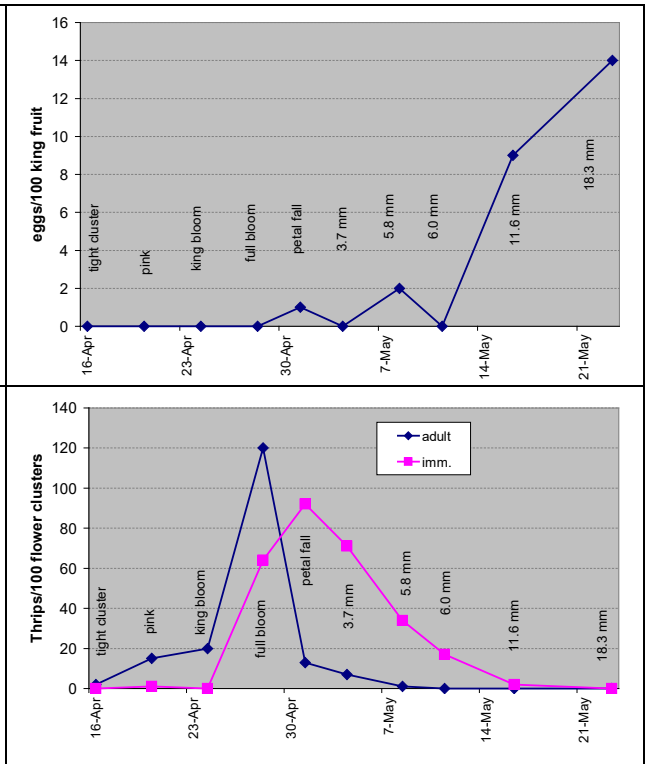

Fig. 4. (top) Eggs per 100 king fruit, Omak, WA, 2005. (bottom) Adult and immature thrips per 100 flower clusters.

Thrips phenology, 2004: Adult thrips were found in low numbers from tight cluster on; however, they increased in the blossom clusters as petals opened (Fig 3., bottom). There was a marked increase from king bloom to full bloom. Adults remained abundant until petal fall, then decreased rapidly. A low population remained on fruit clusters up to 15.8 mm, or 21 d after petal fall. Although eggs must have been laid on or near flower clusters well before bloom, judging from the presence of immatures at pink, very few eggs were laid on king fruit during bloom (Fig. 3, top). The majority were laid on king fruit shortly before 10.9 mm, or 14 d after petal fall. Immature thrips peaked just after petal fall, indicating substantial oviposition during bloom on vegetative tissue.

Thrips exclusion, 2005: Carzol applications did not significantly affect fruit damage on any date. Apparently either the knapsack sprayer used did not deliver sufficient insecticide into the interior of apple flowers to affect the population or the small block size allowed rapid reinfestation after a spray.

Thrips phenology, 2005: Adult thrips were found in low numbers at tight cluster and increased in the blossom clusters as petals opened (Fig. 4, bottom). There was a marked increase between king bloom and full bloom. Adults decreased rapidly after petal fall. The majority of eggs were laid on king fruit shortly before 11.6 mm fruit, or 15 d after petal fall. Immatures peaked at petal fall, before the peak of eggs found in the fruit, indicating the contribution of other (vegetative) tissues.

Impacts: Information gained by these studies has significantly enhanced our knowledge of thrips ecology and movement in near-orchard habitats and will influence management decisions and options in the future. The contribution of thrips hosts on the orchard floor can vary widely from site to site and may in some cases be as important as the contribution from surrounding native vegetation. However, there is little evidence that stringent weed control will significantly reduce thrips damage. Where immigration from native vegetation is the primary thrips source, border sprays may be a more appropriate management strategy. The evidence on timing of thrips oviposition is a significant indicator that pesticide applications should be timed much later than is currently recommended (pink-bloom), perhaps by several weeks; however, this needs further confirmation. Better timing provides more consistent control and optimal use of products with shorter residual activities.

Budget:

Project title: Biology, migration, and management of western flower thrips in apple orchards

PI: Elizabeth H. Beers

Project duration: 2003-2005

Project total (3 years): $107,084

Item	Year 1 (2003)	Year 2 (2004)	Year 3 (2005)
Total	36,953	35,131	35,000

FINAL REPORT

Project Title: A new pollinator from the homeland of apples
PI : W. Steve Sheppard, Entomologist, WSU, Pullman
Year initiated: 2001

Terminating year: 2005 (with no-cost extension)

OBJECTIVES:

1) to characterize the pollinating behavior of Tien Shan Mountain bees within regions of the wild apple forest and on cultivated apples.

2) to establish collaborators within this region and apiaries for queen rearing and importation

3) Initiate the importation, quarantine and field testing of stock.

4) Develop protocol for the dissemination of selected honey bee stocks to queen producers and beekeepers that serve the tree fruit growers of Washington State.

Explanation of deviation from original objectives or schedule (Relevant for objective 3 only). Importation and quarantine of *Apis mellifera pomonella* was delayed initially due to lack of availability of quarantine facility in Grande Terre Island, LA, in 2004 due to sabbatical leave of P.I. and in 2005 by a political coup in Kyrgyzstan that led to itravl warnings for US citizens by the state department.

SIGNIFICANT FINDINGS:

Obj. 1 - Data analysis from field and apiary studies indicated that the honey bees of the Tien Shan Mountains belong to a previously undescribed subspecies. The new subspecies was named scientifically *Apis mellifera pomonella*, in reference to its role as a pollinator of wild apples in their original range and area of endemism. Two articles about the new subspecies and its potential as an apple pollinator were published in The Goodfruit Grower and Bee Culture. The subspecies description was published in Apidologie, an international journal of bee research. Foraging data indicated that this subspecies forages for pollen at temperatures lower than reported for Italian honey bees in common use in the U.S.

Obj. 2 – Two areas within the homeland of the Tien Shan Honey Bee were evaluated as sources of honey bee germplasm for subsequent importation. **Kazahkstan:** Mr. Urazajev Zufar, a local beekeeper from the village of Jabagly agreed to provide honey bee stocks collected from a nearby genetic reserve. Mr. Zufar had assisted in the initial "discovery" of this subspecies during a WSU-USDA expedition (Unruh and Sheppard, 1999). Dr. Roman Jashenko of the Institute of Zoology in Almaty agreed to assist with all exportation permits. As President of the NGO Tethys, Dr. Jashenko is highly familiar with the regulations and procedures needed to export scientific material from Kazakhstan. **Kyrgyzstan:** Mr. Hugh Brown, Chief of Protocol of the ACDI-VOCA office facilitated travel in 2003 to 4 remote village areas in western Kyrgyzstan. The remoteness of the area surveyed, together with extensive interactions with local beekeepers and evaluation of *A. m. pomonella* from these locations, indicated that the honey bee stocks were unlikely to have any genetic introgression from European sources (unlike the Jabagly region). Collaborations were established for the importation of bees from the following specific areas:

Zerger Village – Malik Joldoshabaev (school principal with knowledge of local beekeepers); Kyzyl-Kol – Beishenbek Kenjebaev (school principal, beekeeper, with knowledge of local beekeepers); Kara-Suu – Arstanbai Shaidyldaev (beekeeper, leader of local bee association, knowledge of local beekeepers); Kara-Jygach - Mr. Bazabak (Deputy village administrator – limited knowledge of local beekeepers), Mr. Duishenbek (beekeeper)

Obj. 3 - Initiated importation protocols with USDA-APHIS and LA Department of Agriculture to bring queens into USDA quarantine on Grande Terre Island (LA). Actual importation of germplasm was delayed due to quarantine issues, Sheppard sabbatical leave in 2004 and presidential coup in Kyrgyzstan in 2005. Grande Terre will be returned to quarantine duty in 2006 (after clean-up completion from the 2005 hurricane) and WSU is negotiating for use of the facility in 2006. Importation funds will be provided through the Thurber endowment to complete this phase of the original project following the termination of WSTFRC support. The bees will be available for evaluation and inclusion in the WSU breeding program immediately after their release from USDA quarantine.

Obj. 4 – Selective breeding of mite and disease resistant stocks for use in tree fruit pollination. We have selected and maintained 8 genetic lines of honey bees for apicultural traits of interest to PNW beekeepers. These populations express high levels of hygienic behavior (a characteristic known to be linked to related to disease and mite resistance), overwintering and spring build-up ability under PNW conditions, honey production, gentleness and evidence of disease resistance. In 2005, mite levels measured in WSU apiaries were below our estimated IPM thresholds and we omitted any chemical control for *Varroa* mites. The ability to maintain colonies without miticides represents a significant jump in the selection pressure we can use to develop mite-tolerant honey bees. Collaborative arrangements with the WA State Beekeeping Association were made in 2005 to establish WSU-WSBA (Washington State Beekeepers Association) apiaries. Each apiary will contain queens of all 8 genetic lines from the WSU honey bee breeding program. This will serve as a model to distribute selected honey bee stocks to the industry in a rapid manner, as these apiaries will be used by Washington beekeepers to make daughter queens or to use as drone source for their own selection programs.

METHODS:

As part of a continuing WSU Honey Bee Breeding Program, the new subspecies will be assessed for overwintering ability, pollinating activity, disease resistance and other apicultural. The breeding program was initiated concurrently with the WSTFRC Project with the primary goal to develop mite and disease resistant lines for beekeeping under PNW conditions and to provide beekeepers with better adapted honey bee stocks for tree fruit pollination.

The original pool of germplasm for selective breeding was assembled by purchasing queens from all available commercial US honey bee stocks in 2001 and 2002. From this pool of several hundred colonies, we have selected and maintain 8 genetic lines through 2005. The eventual composition of the stocks maintained within the program will include 10 genetic lines, one of which will be the Tien Shan Mountain Bee.
The selective criteria and scoring system include:
1) overwintering ability under PNW conditions. Colonies that survive the winter and build up well in the Spring are given a numerical bonus

2) short term weight gain through the honey flow. This measure is known to be correlated with the trait of honey production and colonies are comparatively ranked.

3) gentleness and behavior on the combs. Colonies are given a numerical score during each inspection and a final average score for behavior at the time of selection.

4) hygienic behavior. Colonies are subjected to a freeze-killed brood assay that measures their propensity to clean out dead brood within a specified time. Hygienic behavior has been positively linked to disease resistance and possibly Varroa mite tolerance.

5) freedom from disease symptoms. Colonies that exhibit disease symptoms are removed from breeding consideration.

The breeding scheme uses family level selection to reduce the effects of inbreeding. Each of the 8 genetic lines are used to produce daughter queens for the next generation. Within the pool of daughters of each "family" or line, the highest scoring queen is used as the queen mother to produce virgin queens for the subsequent generation. All matings are done at our genetically isolated mating station on Smoot Hill, near Albion, WA. Up to 100 daughters of each line are produced and evaluated annually.

Beekeeping/grower input. Beginning in 2004, WSU selected lines were provided gratis to interested beekeeper collaborators to provide us with feedback on the progress of selection. In 2005 a USDA-SARE grant application was submitted that will continue the breeding program for 3 additional years and formalize the transition to higher levels of WA State Beekeeper involvement. In 2006, two collaborative WSU-WSBA apiaries will be established (run by WSBA members) to permit more widespread use of WSU selected stocks by WA beekeepers. A new project to test these stocks in large scale commercial beekeeping and pollinating operations began in Jan 2006. Two commercial collaborators (running 8,000 and 4,200 beehives, respectively) will take part in a large-scale experiment to compare WSU selected stocks and typical Italian commercial stocks under commercial pollinating practices in apples and alfalfa.

RESULTS AND DISCUSSION:

The Results have been largely addressed above in Significant Findings. Unquestionably, this WSTFRC-funded project has significantly improved our understanding of the native range and genetic potential of the honey bee. It led to the discovery and naming of a honey bee that was new to science (*A. m pomonella*) and native to the wild apple forests of central Asia. Just as this region is fundamentally important to the exploration for germplasm diversity within apples, the region holds the potential to unlock improved pollinating potential within the honey bee. Current honey bee populations in the US are primarily descendents of bees from the warm Mediterranean climate of Italy and, while these bees are well suited for commercial beekeeping and honey production when managed without significant overwintering, we can reasonably expect that honey bees from the native homeland of apples in the Tien Shan will be better-suited to climatological conditions that are ideal for apple production.

The biggest challenge to Washington beekeepers at present remains the parasitic mite *Varroa destructor* and the commitment of WSU to work to actively breed honey bees for tolerance to this mite has been substantial. In 2005, after 4-6 years of selection within the different genetic lines – progress was demonstrated by the overall low mite levels found in our apiaries in the Fall. In this

year, for the first time since the advent of *Varroa* treatment in the 1990,s, we DID NOT TREAT for *Varroa destructo*r. We plan to continue this selection and breeding program and, importantly, continue to also select honey bees for mite tolerance and other traits of importance to the industry (honey production, overwintering, build-up, behavior). The inclusion of *A. m. pomonella* within the breeding scheme is an important component of our plan to optimize honey bees for use in the PNW. With *pomonella* we will include an additional selective criterion, low temperature foraging activity, and combine it with our mite tolerant characteristics. I believe that this will result in a honey bee that is both highly desirable for WA State beekeepers and better suited to fulfill the pollination needs of the WA tree fruit industry.

The support of the WSTFRC has been a significant help in making this research possible. It is generally known that breeding programs involve a rather lengthy time horizon and bee breeding is no exception. However, I am committed to build on the progress made possible by WSTFRC funding and will use WSU Thurber endowment funds available to me to complete the germplasm importation in 2006 or 2007 (depending on USDA quarantine allotments). This importation will represent the only non-USDA importation of honey be germplasm for breeding purposes since 1922. A pending USDA-SARE grant will enable us to complete the transition from a research effort and breeding program involving a few hundred colonies to widespread availability of the genetic stocks to Washington beekeepers.. The exigencies of the market and industry forces remain an unkown, but the dire straits brought about by *Varroa* mite losses will clearly contribute to providing exposure and testing of the WSU-WSBA honey bee lines by beekeepers for use in tree fruit and other pollination activities.

ANTICIPATED BENEFITS AND INFORMATION TRANSFER:
The primary goal of this research is to facilitate the importation and distribution of a honey bee more appropriate to the pollination of tree fruit crops under the climatic conditions of Washington State. Results will be transferred via subsequent extension efforts to promote the use of these bees by queen producers and state beekeepers. Commercial honey bee populations will benefit through enhanced genetic diversity brought about by the inclusion of new bee germplasm. The goal is to incorporate desirable features into a stock better suited for the cool pollinating conditions of Washington State.

BUDGET: 2001-2005	28,145
ITEMS	
Beekeeping and analytical supplies	13,355
Equipment	5,512
Pending assignment ……	9,278……..

Zerger village Kyrgyzstan. Wild apple and walnut forests, apiary of A. m. pomonella

Previous page

A m pomonella

Apiary of A m pomonella in Russian style chest hives

CONTINUING PROPOSAL **YEAR 1/3**
WTFRC Project #AE-05-503 WSU Project #13C-3643-4190

Project title: Codling moth mgmt. with pheromones: key unanswered questions

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Project objectives:

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

Project highlights:

- **New blood** – Two new PhDs, Lukasz Stelinski and Matthew Grieshop joined the research team in 2005. Lukasz completed his PhD at MSU in 2005 working with Larry Gut and James Miller on pheromone mediated behavior of tortricid moths and moved seamlessly into the new project. Matt joined WSU in August 2005 after completing his PhD at Kansas State University, has an excellent background in entomology and brings a fresh, new and energetic member to the research team.
- **A team effort** – The research team met on several occasions via conference call and in person in Michigan and Wenatchee to plan and coordinate research. There was one meeting with industry representatives to go over plans and some preliminary results during the season.

Significant findings:

- CM males were able to find females (CA) or female mimics (WA) when released 10 or 20 m in one night. Males farther away (40 m) also found females or mimics but the discovery was delayed 2-3 days.
- When pheromone point sources (fibers) were close (30 cm) to a CM female, mating was suppressed. In addition, when the distance from the female was the same, mating was suppressed as the number of point sources surrounding a female increased.
- A new vacuum device holds promise as a tool in the investigations of where CM males and females reside in trees and possibly where females call.
- Combinations of codlemone and the pear ester were more effective in disrupting male orientation than codlemone alone applied as hand-applied dispensers (400/acre) or wax droplets (27,000/acre).

- Promising new technologies for applying fibers, flakes and wax drops were developed and tested.

Objective 1 – Active space.

Methods – The distance at which a male CM can detect and orient to a calling female or artificial pheromone was tested at three locations using different approaches. Release and recapture of marked CM males at different distances were used to determine relative attraction of wild and colony moths (Judd) and various artificial lures (flakes, fibers, lures) and virgin females (WSU). Another method using caged males was evaluated where males (5-10/cage) were placed downwind of a known pheromone source and observed. The distance from different pheromone sources where male behavior indicated they sensed pheromone was recorded.

Results – In the spring releases, total male recapture (35%) in all 10 female-baited traps was greater than in summer releases (10%). Given cooler temperatures in May, this difference was not anticipated. The percentage of wild males recaptured in spring in calling female baited traps was not different over distances of 2.5-20 m (Fig. 1). During the August releases at PARC, percent recapture of wild males as a function of downwind distance from a calling female declined exponentially (Fig. 1). This decline in response to "calling" females with increasing downwind distance was more rapid than that observed using synthetic pheromone sources at WSU.

Figure 1. CM Mark-Release Recapture
"Calling" females - Spring & Summer 2005

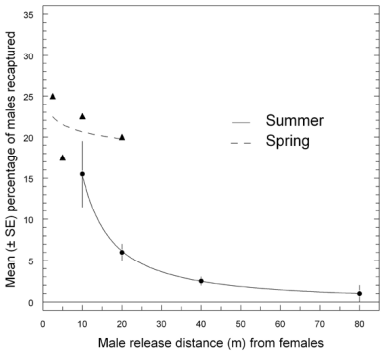
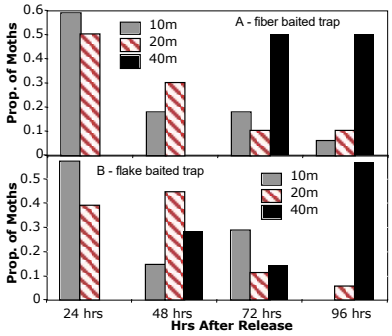


Figure 2 a&b. Proportion of marked male CM recaptured at three distances by 24h interval.



Marked moths were captured in both flake and fiber baited traps but not traps with virgin females. About equal proportions of marked males released at 10 and 20 f (11-18%) were recaptured; only at 40 m was there a significant decline (fiber only) in the percent moths recaptured (Fig. 2a,b). These data indicate that males move at least 20 m in one night to locate a pheromone source that mimics to some extent a female but when at greater distances their location of the source is delayed, suggesting that they move towards a source in incremental stages on successive nights.

Wing fanning as a means of assessing active space of a pheromone source was confounded by wind. The best activity was detected at speeds of 0.5 and 1.5 m/s. A significant difference was detected for the number of wing fanning incidences between the fiber and blank treatment.

Plans for 2006 – Preliminary results from these methodology development experiments suggest that mark-recapture techniques may be useful in helping us estimate female active spaces. Data generated from such experiments should allow us to develop a mathematical model of percent recapture as a function of distance, a proxy for the female active space. From this we should be able to demonstrate how the female active space is altered by presentation of synthetic sources of pheromone; for example, we might expect the active space to be truncated by competition from other attractive sources. We will continue to use the mark-release-recapture method to assess relative differences in active spaces of different pheromone sources in untreated environments. Similar experiments will then be conducted in pheromone treated orchards or small plot trials using different pheromone dispensing technologies (fibers, flakes, wax, hand-applied, etc.).

Objective 2 – Female calling.

Methods – One aspect of this objective was to determine what artificial pheromone release device might best mimic the calling CM female relative to attraction of males. We placed different pheromone devices in traps in both generations of CM and monitored male capture. These tests were conducted in WA and MI.

Results – In WA the 0.1 mg (0.1X) lure was highly attractive but for only a short period. The Trécé L2 lure was more attractive than a calling CM female but not as attractive as the 0.1X lure. A Scentry fiber consistently attracted males over the entire flight in both generations. The Hercon flake was probably the best mimic of the CM female when both were in a trap environment.
Plans for 2006 – The location of females emerging from the base of the tree will be followed to determine when they leave the emergence site and where they fly. Tethering of females at different locations will be used to determine if there are differences in mating preferences in spring versus summer. Mark-release-recapture method will be used to determine where males can find female mimics in pheromone and non-pheromone treated orchards. The effect of orchard micro-environment (wind, temperature) on mating behavior will be assessed.

Objective 3 – Aggregation of CM in orchards.

Methods – Much of 2005 was dedicated to methods development. In WA pyrethroid insecticides were evaluated for their ability to rapidly knock down CM adults as a means of assessing spatial distribution. Cardboard banding was applied to individual trees in an experimental plot assessing new products. The number of larvae per tree was compared to the level of fruit injury in the tree.

In MI several versions of a modified leaf blower vacuum system were evaluated and a final version adopted for field studies. The vacuum technology was used in plots with and without mating disruption to sample at three canopy heights (high, mid, and low). Larval distributions were assessed using cardboard banding. The number and distribution of larvae trapped in bands were compared to the level and distribution of fruit injury.
Results – Pyrethroid insecticides applied by handgun mist sprayer or fogger did not prove effective for sampling CM adults. In contrast, over 100 moths were collected using the MI vacuum. Female moths were evenly distributed throughout the tree in the non-pheromone treated plot but were more concentrated in the upper and middle portions of the tree in the pheromone treated plot. The number of CM larvae collected in bands was highly correlated with fruit injury ($r^2 = 0.9$), including trees providing very good control under high pressure.
Plans for 2006 – The ability of pyrethrum insecticides to knock down CM adults is being evaluated over the winter. If this product is promising, plots will be treated in 2006 to determine its efficiency as a sampling tool for CM adults. If results are positive, areas of commercial orchards will be sampled in the second generation. The vacuum method appears promising as a tool to assess locations of CM adults in orchards. Vacuums will be built for other locations and cooperative experiments designed to address the objective. Tree banding proved to be a good means of measuring population density and distribution, and studies will be continued in 2006.

Objective 4 – Pheromone purity, components and plant volatiles.

Methods – Most of the research was focused on plant volatiles. Electrophysiologically active volatiles identified from apple fruit were tested in 2005 as partial and complex blends for their attractiveness to CM. Field tests were conducted to determine effects of changing the release rate beta farnesene, to compare trap designs, and to look for co-attractants.

The relative response of male CM, wild and sterile moths from the SIR program, to two pheromone sources, female pheromone gland extract and >99% pure codlemone, was tested in the laboratory flight tunnel. The incidence of wing fanning, take off, upwind flight and source contact was recorded for each moth.
Results –We showed in trapping experiments that beta farnesene, E,E-alpha farnesene, ethyl caproate and ethyl benzoate, as well as pear ester (PE), are attractive to CM. However, the first four compounds have been only very weakly attractive in comparison to PE.

The pane trap baited with beta farnesene captured the greatest number of CM, followed by the wing trap. Few CM were captured in multipher, red sphere, or Universal moth traps baited with beta farnesene. Numbers of male CM generally increased with the load (milligrams) of beta farnesene on the septum, up to the 10 mg maximum tested. Numbers of males in traps with beta farnesene were increased with the presence of ethyl caproate and ethyl benzoate, but not synergistically.

In the comparison of kairomones, by far the greatest number of CM was captured in traps baited with the PE throughout both flights. In the comparison of pheromone and kairomone throughout both flights the greatest numbers of CM males were in traps baited with the combination of pheromone and PE.

This work demonstrated CM attraction to several chemicals that are present in apple odor, including responses by females and by males. Although these responses were fairly consistent, they were also very weak in comparison either to CM attraction to pear ester or attraction to the sex pheromone.

Flight-tunnel tests suggest that a female pheromone gland extract is more attractive and elicits more source contact from both wild and laboratory-reared SIR males than does a very pure form of codlemone. However, this difference was only statistically significant in wild males. These preliminary data suggest that pheromone gland components other than codlemone may be relatively more important to wild moths than laboratory-reared moths. Behavioral differences between the two populations tested here may be the result of many years of inbreeding. Similar tests need to be conducted on other wild (CA, MI and WA) and laboratory-reared (USDA) populations followed by other behavioral responses to such things as plant volatiles. Such tests will form the basis for characterizing and providing baseline behavioral differences on different populations (Objective 7).

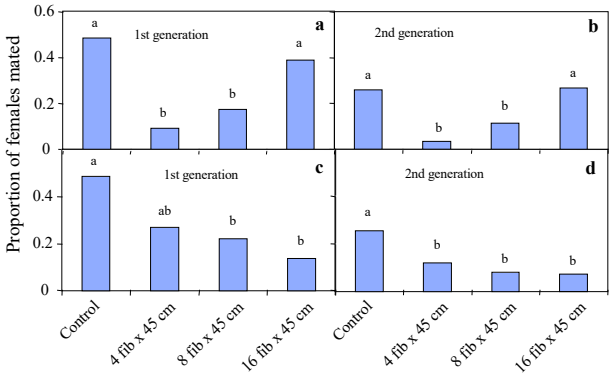
Plans for 2006 – Focus in 2006 will be on those plant volatiles that show promise as attractants and on issues of purity, minor pheromone components, and release rate in pheromone and non-pheromone treated orchards.

Objective 5 – Spatial arrangement of competing sources.

Methods – New pheromone delivery technology offers the opportunity to consider alternative explanations of how mating disruption works. WA and MI researchers selected Scentry NoMate fibers as the dispensing source to investigate 1) whether single-tree replicates could provide meaningful information for pheromone research, and 2) to determine the effect of point source density and distance on CM male orientation to females or pheromone baited traps. Experimental designs were coordinated between WA and MI. Fibers were placed manually on leaves around a tethered female or trap at different densities (4, 8 or 16) and distances (30, 45, or 60 cm). Mating success or trap capture was monitored at frequent intervals. In the first generation the test orchard was untreated; in the second generation a low rate (50 dispensers per acre) of Isomate C+ ropes was applied throughout the test orchard.

Results – In MI in the first and second generation there was a clear indication that the closer to the "calling" source (female or trap) the competing sources were placed the greater the impact on mating or capture (Fig. 3a, b). When distance from the "calling" source was constant, it appeared that as more competing sources were added the interference of males' ability to find (mate or be captured) declined (Fig. 3c, d). When treatments were evaluated using trap captures instead of tethered females similar trends were observed, but increased variation reduced statistical separation between treatments. Similar results were obtained from WA studies (not shown), but differences between treatments were not as clear. These data suggest that mini pheromone dispensers need to be close, within 30 cm (12 inches), and that the more per tree, well distributed, the better.

Figure 3 a-d: Proportion of females mated by the spatial arrangement of pheromone fibers (# of fibers x distance from female).



Plans for 2006 – In 2006 we will again coordinate research on this aspect of the project. We will most likely treat larger plots with new applicators using either the Hercon flake or MSU wax pheromone technology. New applicators offer the ability to apply different rates of mini-dispensers to

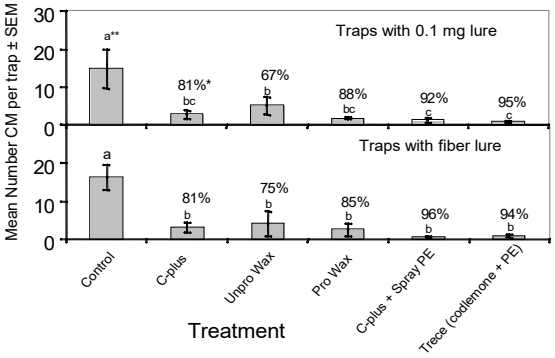
small plots. Evaluations will then use simultaneous assessment with tethered females and female-mimic baited traps. Mating and captures will be used to evaluate the impact of different treatments.

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

Methods – The objective of this study was to test the hypothesis that mating disruption of CM could be improved under high CM populations by deploying the CM pheromone (in some cases with other minor components) in combination with a plant volatile, either the PE or (E)-β-farnesene (Far), over that which could be achieved with pheromone dispensers alone. In the first experiment different volatiles were applied (MEC or wax) over the top of an Isomate C+ treated orchard. In the second experiment 6 treatments were directly compared under high CM pressure. The treatments evaluated were: 1) no pheromone control, 2) Trécé PVC dispensers releasing both codlemone and PE at 1000 dispensers/ha, 3) Isomate-C+ dispensers at 1000 dispensers/ha, 4) Isomate C+ plus MEC PE as described above, 5) protected wax, and 6) unprotected wax. Treatments were evaluated with traps baited with either an 0.1 mg codlemone lure or a Scentry fiber in each replicate plot.

Results – In the first experiment, disruption of CM to pheromone traps was increased by the addition of plant volatiles to plots treated with C+ dispensers; this effect was more pronounced with the pear ester (PE) treatment compared with (E)-β-farnesene (data not shown). In the second experiment, the highest level of disruption was recorded in plots treated with C+ dispensers with the addition of a PE-MEC application and with Trécé PVC dispensers releasing both codlemone and PE (Fig. 4). Releasing a combination of plant volatiles with pheromone holds promise as a tactic for improving mating disruption of CM under high population densities.

Figure 4. Average CM moths captured in traps placed in different pheromone or pheromone+PE treatments.



Plans for 2006 – The encouraging results in 2005 have stimulated more interest in Trécé working with the research team to evaluate the interaction between codlemone and PE. Trécé PVC dispensers releasing codlemone+PE will be compared with identical dispensers releasing codlemone alone. In addition, detailed laboratory flight tunnel studies will be conducted to elucidate the behavioral and electrophysiological mechanisms responsible for improving disruption by combining these two compounds. Small plot studies similar to those conducted in MI in 2005 will be conducted in MI and WA in 2006. A limited set of on-farm trials is planned as well.

Objective 7 – Baseline characterization of behavior and electrophysiology.

Methods – Different populations of CM (controls not exposed to pheromone) were collected from pome fruit and walnut growing regions in 2005. These are being kept under cool (diapausing) environmental conditions. Later this winter the larvae will be transported to laboratories where baseline behavioral and electro-physiological comparisons will be made between different populations, including from two laboratory populations (USDA-ARS, Wapato and SIT, Canada). Populations of CM will be compared to determine if there are differences in their behavior when pre-exposed to codlemone. Once methodology and baseline data are obtained, CM populations from orchards exposed for several years to mating disruption will be collected and subjected to the same behavioral and electrophysiological assays deemed appropriate based on previous studies.

Results – These data will not be available at the research review but will be presented at the next review.

Plans for 2006 – Baseline behavioral and electrophysiological comparisons will be made between different CM populations already collected. Those assays deemed appropriate will be used to characterize CM populations collected from crops not sampled in 2005 and from sites treated with pheromone for several years.

Objective 8 – Development and optimization of pheromone delivery technology.
Methods – Five pheromone delivery technologies (Hercon flakes, Scentry fibers, Suterra MEC, MSU wax, and Isomate CM/LR) were evaluated against CM in different studies. On-farm trials with fibers and wax drops were conducted in MI using newly developed, tractor-mounted applicators.
Results – Hercon flakes applied by air were retained (85%) for 42 days and for 20 days when applied by a new ground applicator. There was a rate response based on CM capture in traps from flake and fiber but not MEC applications. Isomate CM/LR Twin Tube demonstrated good efficacy in suppressing CM catch and reducing injury. A fiber applicator designed by MSU agricultural engineers attaches to a conventional tractor and uses articulating arms to deliver the product high in the canopy. Up to 18,000 fibers/acre adhered to the trees, and about 75% were retained through the targeted CM flight. Good suppression of moth captures in traps and reductions in fruit injury were recorded in fiber-treated plots, especially during the summer CM flights. A similar unit for applying pheromone wax drops showed promise in preliminary trials.
Plans for 2006 – Similar kinds of evaluations will be conducted in 2006 using improved technology and knowledge gained from experiments on point source density and effects of PE-codlemone combinations. We will continue to work with the pheromone industry to evaluate new technologies.

Budget:

Project duration:	Three years (2005-2007)	**Current year**: 2006
Project total (3 years):	$598,710	**Current year request**: $194,925

Year	**Year 1 (2005)**	**Year 2 (2006)**	**Year 3 (2007)**
Total	203198	**194925**	200587

Item	**Year 1 (2005)**	**Year 2 (2006)**	**Year 3 (2007)**
Salaries [1]	130205	**112417**	116635
Benefits [2]	38081	**38783**	40227
Wages [3]	15000	**19500**	19500
Benefits	1800	**2025**	2025
Equipment [4]	3000	**0**	0
Supplies [5]	7012	**11700**	11700
Travel [6]	8100	**10500**	10500
Total Request	203198	**194925**	200587
WSU total	83586	**80860**	83563
MSU total	48730	**53756**	54915
USDA total	35082	**39609**	40689
Ag. Canada total	35800	**20700**	21420

[1] Salary (4 mo.) for Senior Scientific Assistant ($19,269); Salary (9 mo.) for Research Assoc. ($30,900).
[2] Benefits for Senior Scientific Assistant ($6,551); 34% for Research Associate ($10,815).
[3] Hourly help to assist with setting up experimental apparatus, collection and analysis of data.
[4] Automated digital photo system equipment for recording insect behavior in field and laboratory studies.
[5] Supplies will include lures, traps, flagging materials, cell phone charges and fuel.
[6] Travel to experimental plots; pays for one car for 6 months.

CONTINUING PROPOSAL **YEAR 2/3**
WTFRC Project #AE-04-428 **WSU Project #3643-8366**

Project title:	The importance of dispersal in biological control and IPM
PI:	Vincent P. Jones, Associate Entomologist
Organization:	WSU Tree-Fruit Research and Extension Center
Address, phone, e-mail:	1100 N. Western Avenue, Wenatchee, WA 98801; (509) 663-8181 ext. 273; vpjones@wsu.edu
Co-PIs and affiliations:	Jay F. Brunner, WSU-TFREC Tom Unruh, USDA-ARS, Wapato Dave Horton, USDA-ARS, Wapato
Contract administrators:	Mary Lou Bricker (mdesros@wsu.edu), 509-335-7667; Sally Ray (saray@wsu.edu), 509-663-8181 x221

Objectives:

1. Determine the contribution of the orchard ground cover to natural enemy populations and biological control that occur in pear trees.
2. Examine the area of influence ("active space") of a rose/strawberry garden used to bolster parasitism of leafrollers.
3. Examine the movement of insect pests from areas of high population density to surrounding managed areas.

Significant findings:

- The diversity of predators found in the pear tree canopy and the ground cover was similar according to our mathematical diversity indices.
- A significant proportion of several natural enemies species that would be considered "tree species" had visited the ground cover. Even *Anthocoris* and *Deraeocoris* nymphs were found to move between the tree and ground cover, although at ≈1/3 the rate of adult movement patterns.
- We developed a new marking technique using powdered formulations of our markers and traps that allowed us to measure the movement of *Colpoclypeus florus*, a leafroller parasitoid, from the gardens into the orchard.
- Our *C. florus* movement experiment was performed on too small a scale, but even so, a 21□15-foot (315 ft^2) portion of the rose garden affected >2 acres of the adjacent orchard.
- Our movement studies of OBLR between apple and cherry showed that little movement occurred between the two areas.

Objective 1. Determine the contribution of the orchard ground cover to natural enemy populations and biological control that occur in pear trees.

Both last year and this year we applied the egg marker to the orchard ground cover with a weed sprayer mounted on an ATV. Last year we applied the marker as a 20% solution and had 97% marking for insects collected from the ground cover. This year we reduced the rate applied to 10% egg whites, and marking remained nearly the same. Insect samples were collected from both the ground cover and the canopy over the course of the experiment. Ground insects were tested for presence of the mark to determine marking success, and tree-collected insects were tested for the marker to determine movement between the two areas.

This year we concentrated on expanding our identification of the specimens collected. In particular, we identified the adult ladybird beetles and lacewings to species so that we could determine habitat specificity and the tendency of each species to move between the ground cover and the canopy. We also collected and tested immatures of ladybird beetles, green lacewings, *Anthocoris*, and

Deraeocoris. For the immature ladybird beetles and lacewings, identification to species was not possible.

Analysis: We wanted to determine the relative difference in species diversity of predators collected from the tree versus the ground cover. We used two different indices of diversity (Simpson's index and the Shannon-Weiner Function); the two have a slightly different basis and theoretical background. Simpson's index varies from 0 (low diversity) to almost 1 (high diversity). The Shannon-Weiner Function starts at 0 (no diversity) to 2.9 (in our samples) with larger numbers showing higher diversity.

We also classified each predator species collected in the tree canopy as to its habitat preference. To do this, for each species we calculated the percentage of the total captures (ground + tree) that occurred in the tree. If greater than 70% were collected in the tree, we tentatively considered this a species that preferred the tree; if between 30 and 70% were collected in the tree, the species was considered a "generalist"; and if less than 30% were collected in the tree it would be considered a species that preferred the ground cover. This classification should be viewed as a very rough guide, in part because the sampling methods were different between the tree and ground collections so we may have some differences in efficiencies between the two sampling methods that would distort the percentages.

Table 1. Species collected from the ground cover (GC) or canopy collected during summer 2005.

Species	N Ground	N Tree	% Tree Collected	% in Canopy Visiting GC	Preferred Habitat
Harmonia axyridis	0	35	100.0	8.6	Tree
Anthocoris tomentosus	10	100	90.9	31	Tree
Chrysoperla plorabunda	2	17	89.5	23.5	Tree
Deraeocoris brevis	49	266	84.4	15	Tree
Coccinella septempunctata	9	16	64.0	12.5	Generalist
Unknown ladybird beetle	9	10	52.6	10	Generalist
Spiders (various spp.)	256	259	50.3	16.2	Generalist
Hyperaspis lateralis	10	7	41.2	0	Generalist
Coccinella transversoguttata	13	6	31.6	16.7	GC
Hippodamia convergens	27	5	15.6	20	GC
Nabis sp.	17	2	10.5	0	GC
Lygus hesperus	264	9	3.3	66.7	GC
Geocoris sp.	145	3	2.0	33.3	GC
Orius tristicolor	411	6	1.4	33.3	GC
Immatures					
Anthocoris tomentosus	2	56	96.6	8.9	Tree
Deraeocoris brevis	5	74	93.7	5.4	Tree
Green Lacewings	5	26	83.9	11.5	Tree
Ladybird Beetles	49	4	7.5	75	GC
Total	**1283**	**866**			

The proportion of individuals in each species collected in the tree canopies that were positive for the ground cover mark was calculated. This value, along with the habitat preferences classification, was used to determine how important the ground cover was in population dynamics occurring in the canopy.

Results: The values for the two diversity indices were very similar between the predator samples collected from the tree and the ground cover (Simpson's: 0.784 (ground) vs. 0.753 (tree); Shannon-Weiner 0.290 (ground) vs. 0.238 (tree). There was a slight, but not statistically significant, increase in both indices for species collected from the ground, indicating that a slightly greater diversity occurred in the ground cover.

Using the classification system described above, four of the 14 species collected in the tree canopy could be described as "tree species" (Table 1). Of the four tree species, two were commonly collected (*Anthocoris tomentosus* and *Deraeocoris brevis*), with 31% of the *Anthocoris* and 15% of the *Deraeocoris* collected in the tree canopy testing positive for the ground cover marker. Immature *Anthocoris* and *Deraeocoris* were also found to move between the ground cover and the canopy but at roughly 1/3 the rates of the adults. A species of lacewing, *Chrysoperla plorabunda,* and a ladybird beetle, *C. septempunctata,* also moved between the ground and tree, with 23.5 and 12.5% of those collected in the trees testing positive for the ground cover marker, respectively. The ladybird beetle *Harmonia axyridis* was only found in the tree canopy, but 8.6% of that species also tested positive for the ground cover mark.

Spiders (a mix of species) were classified as habitat generalists and were found in nearly equal abundance in the ground and tree collections. In terms of overall abundance, spiders were the second most common predator found in the tree samples (first was *Deraeocoris*). About 16% of the spiders collected in the tree samples tested positive for the ground cover marker.

Conclusions: The ground cover is visited by a number of the different predators, even ones that abundance data would suggest are "tree" species. The *Anthocoris* found in the ground cover likely originated in the tree and only visit the ground cover for short periods (suggested by the high percentage collected in the canopy that tested positive for the ground cover mark and the low numbers found in the ground cover). Surprisingly, even the less mobile immatures move down to the ground cover and back. The importance of the ground cover cannot be further defined with the experiments we have performed to date because we did not collect prey abundance data (pea aphid in the cover crop and pear psylla in the canopy); therefore, the tendency of predators to move between the habitats and switch prey fed upon cannot be determined.

Plans for next year: We plan to use at least two markers so that we can mark insects in the canopy as well as those in the ground cover. This should help us to better define movement patterns. We will also attempt to manipulate the pea aphid population in the ground covers using sugar esters to determine if the reduction in pea aphid populations will force more of the ground resident predators into the canopy. On a small scale, we will use the powdered formulations of our markers to determine if we can define the importance of walking versus flying as the normal mode of movement between habitats for each of the different predator species. We will also collect prey density data in both the pear canopy and the ground cover to help determine the importance of prey density on movement patterns of the predators.

Objective 2. Examine the area of influence ("active space") of a rose/strawberry garden used to bolster parasitism of leafrollers.

Last year, we tried spraying markers on several rose/strawberry gardens but had virtually no luck at recovering marked or unmarked *C. florus* in the adjacent orchards. This year, we developed a new strategy for marking and we designed traps that allowed us to successfully capture adult parasitoids. Increased marking of the parasitoids was accomplished by placing a mesh netting (tulle) over a portion of the rose garden (Fig. 1). The holes in the netting were large enough that *C. florus* could easily pass through but not without coming into direct contact with the netting. We marked the netting by applying soy flour using a hand-held lawn fertilizer broadcaster. The result was that fine particles of soy flour stuck to the netting (see inset, Fig. 1), and the parasitoids contacted the soy flour on the foliage or when walking through the netting.

Fig. 1. Netting over the garden that has been dusted with soy flour. Inset shows a close-up of the soy flour on the netting.

Our research last year showed that *C. florus* was not attracted to yellow or white sticky cards. This year we devised a trap that consisted of an apple shoot infested with a late instar leafroller larva. We punched a hole in the center of a sticky card and inserted the leafroller infested shoot through the card and into a water vial (Fig. 2). These traps were placed at various locations in the orchard adjacent to the rose/strawberry garden. At 3-4 day intervals we would return to the orchard, replace the sticky cards if *C. florus* were on them, and refill the vials with water. The shoots were replaced at weekly intervals. Rolled leaves on the collected shoots were dissected to look for adult *C. florus* (they tend to remain in the feeding roll for 1-2 days after parasitizing a caterpillar).

Fig. 2. Trap used to collect adult C. florus. Shoot is infested with late instar OBLR and the shoot tip is inserted into a floral vial to keep the shoot succulent.

We placed 34 traps in the orchard in late July through the first week of September (Fig 3). We also took a population assessment of the leafrollers in the orchard once during the sampling period.

Results:

The traps allowed us to capture *C. florus* with relatively little effort (at least compared to our previous methods). A total of 182 *C. florus* were captured either on the cards (177) or in the leaf rolls (5). Our traps were concentrated near the rose garden, with the maximum distance being roughly 171 feet, through several tree rows of the orchard. The percentage marked was 13.2%, which was slightly higher than the percentage of the rose garden we actually marked. Four individuals were caught at the furthest trap, suggesting that our trap layout needed to be expanded. When we plotted the distance moved versus the percentage of

Fig. 3. Plot layout for C. florus movement experiment. Open circles represent trap locations, filled circles are where parasitoids were collected that were positive for the marker.

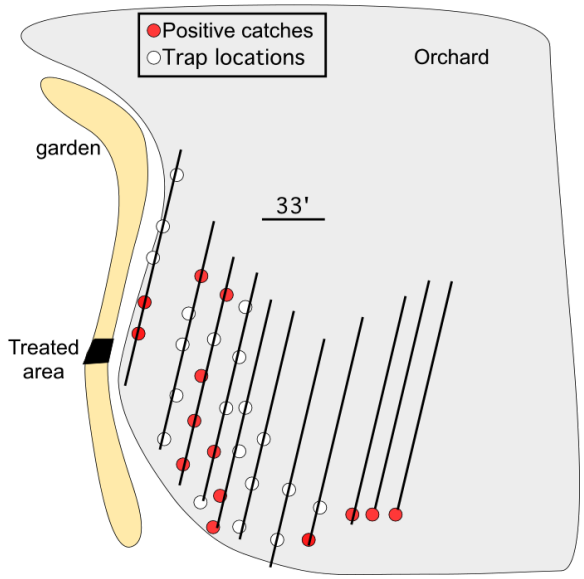
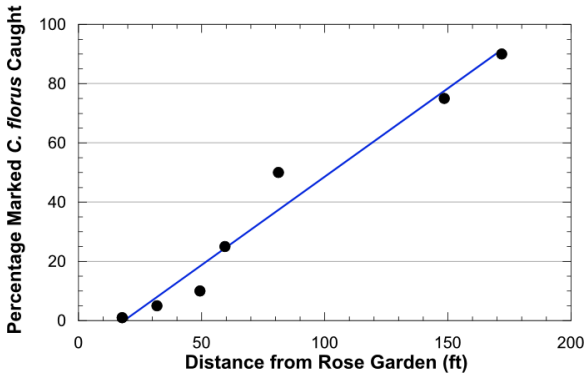


Fig. 4. Percentage of *C. florus* caught at different distances from the edge of the rose/strawberry garden.



marked individuals captured, 50% of the catch occurred at distances greater than 80 feet from the garden (Fig. 4). However, because we captured multiple individuals at the furthest trap, and because our trapping was concentrated closer to the garden, this figure is too low to be a realistic estimate of the influence of the rose garden. Even so, the 15x21-foot section of the garden influenced >2 acres of the adjacent orchard.

Plan for next year: We plan to increase the distance of our traps from the source gardens and increase the number of gardens we monitor. We will also set up the experiments early in the spring and monitor them throughout the season.

Objective 3. Examine the movement of insect pests from areas of high population density to surrounding managed areas.

We set up an experiment to measure movement of adult leafrollers between apple and cherry crops in late August (after the cherry harvest). The plots were roughly 200 feet deep and 380 feet long (≈1.6 acres each), with the long edges adjacent and parallel to each other. The apple block was sprayed with milk and the cherry block with egg whites. Each plot had 15 traps spaced uniformly throughout the area.

We also evaluated the movement of codling moth between apple orchards under different management schemes. This is reported more fully in our other progress report (AE-04-429), but the emphasis for this report is the movement between the primary (marked) orchard and an adjacent (unmarked) orchard. We marked 3 acres of the primary orchard and trapped throughout this orchard and a portion of an adjacent orchard. The adjacent orchard was between 100-220 feet away from marked area (the two orchards were not parallel to each other) and across an irrigation ditch. We used the combo pheromone/DA lure in all the traps.

Results leafroller movement: We captured 113 moths, 75 in the apples and 38 in the cherries. Overall, 22 (13%) of the captured moths were marked. Of these marked moths, only five of them moved from the area where they acquired the marks. Four of the five marked moths moved from apple to the cherry block, but obviously the number of moths that moved was relatively low

compared to the numbers of moths captured. Due to these low numbers of moths trapped out of the area where they were marked, we cannot draw any generalities on movement patterns.

Results codling moth movement: During the first generation, we captured 67 moths in the adjacent orchard, of which 12% originated in the marked plots of the primary orchard. During the second generation, trap catches in general declined, but 28% of the moths caught in the adjacent orchard originated in the marked areas. If we had marked a larger proportion of the primary orchard, it is likely the percentages of moths originating in the primary orchard would at least double for the traps in the adjacent orchard.

Plan for next year: We need to conduct the cherry/apple leafroller movement studies for a longer period and in an area with higher leafroller population levels. The cherry trees we marked were quite large, and we probably had lower spray coverage than in the apple block that consisted of much smaller trees. In addition, the cherry block was treated for leafrollers early in the season, which likely reduced OBLR populations there. We need to find orchards with cherry and apple trees of similar sizes and expand the acreage marked. For the movement of CM, our studies need to be done on a larger scale because our results (as reported in AE-04-429) suggest that movement can be much greater than 800 feet.

Budget:

Project title: The importance of dispersal in biological control and IPM

PI: Vincent P. Jones
Project duration: 2004-2006 (3 years)
Current year: 2006
Project total (3 years): **$**142,334
Current year request: **$**49,683

Year	**Year 1 (2004)**	**Year 2 (2005)**	**Year 3 (2006)**
Total	45,793	46,858	49,683

Item	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries[1]	20,487	21,306	**24,017**
Benefits (34%)[2]	6,146	6,392	**8,166**
Wages[3]	11,000	11,000	**10,000**
Benefits (11%)[2]	1,760	1,760	**1,100**
Supplies[4]	3,200	3,200	**3,200**
Travel[5]	3,200	3,200	**3,200**
Total	45,793	46,858	**49,683**

[1]Callie Baker, Associate in Research (changed from 0.50 FTE to 0.55 FTE).
[2]Benefits = 30% years 1 and 2, 34% year 3. Time-slip benefits decreased to 11% in year 3.
[3]Time-slip employees.
[4]Lab supplies. Cell phone charges are allowed.
[5]Travel to research plots.

CONTINUING PROPOSAL **YEAR 2/3**
Project #AE-04-429 **WSU Project #13C-3643-7366**

Project title:	Mechanisms underlying mating disruption
PI:	Vincent P. Jones, Associate Entomologist
Organization:	WSU Tree Fruit Research and Extension Center
Address, phone, e-mail:	1100 N. Western Avenue, Wenatchee, WA; (509) 663-8181 ext. 273; vpjones@wsu.edu
Co-PI and affiliation:	Jay F. Brunner, Entomologist and Director Tree Fruit Research and Extension Center
Contract administrators:	Mary Lou Bricker (mdesros@wsu.edu, 509-335-7667; Sally Ray (saray@wsu.edu, 509-663-8181 x221)

Objectives:

1. Examine the role that female and male mate choice plays in the mating success of CM and OBLR.
2. Examine the effect of male age on female reproduction in CM.
3. Determine if the delay in mating should be calculated on a calendar or a degree-day basis and determine if we can predict severity of CM and OBLR problems based on the average delay in mating experienced during the spring related to weather patterns.
4. Investigate the importance of CM dispersal between MD and non-MD areas.

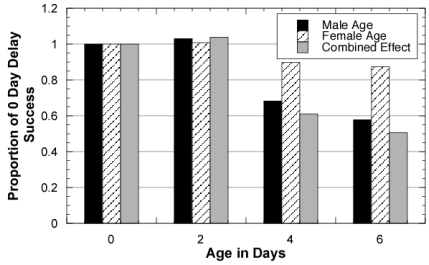
Significant findings:

- CM females appear to discriminate against older males. When paired with older males in no-choice studies, CM females showed a significant reduction in mating compared to when paired with younger males. Young CM males showed no tendency to discriminate against older females.
- OBLR males and females both discriminated against older individuals of the opposite sex. There were no apparent differences between the sexes in age discrimination.
- CM male age had an impact on population growth caused by both a reduction in mating (as above) and probably because they passed smaller sperm packets when they did mate.
- Laboratory studies proved conclusively that the delay in mating is based on a DD scale for both CM and OBLR. We also showed that mortality, the shape of the oviposition curve, and the height of the oviposition curve were predictable when the delay in mating was put on a DD scale.
- Our dispersal studies for CM showed that the scale of our experiments (7+ acres) needs to be expanded. Multiple marked moths were caught >800 feet away from the areas where they originated.
- Although a smaller number of CM females were caught in our dispersal studies compared to males, the dispersal range appears similar between the sexes. In one of our test orchards, 50% of the marked moths caught had moved >392 feet in both generations.

Methods: ***Objective 1.*** We used two different methods to test for age-based mating discrimination in both CM and OBLR. The first method compared the ability of 1-day-old and 4-day-old males to locate a female and pass a spermatophore. A young virgin female was tethered in a wind tunnel. One male of each age was marked with fluorescent dust (each a different color) and simultaneously released downwind of the female. The female was videotaped so that the mating could be observed. Also, the female was examined the following day to determine the dust color she was exhibiting (to see which male contacted her) and whether or not she was passed a spermatophore. This test measured the tendency of the different aged males to fly and their ability to locate the female and to successfully pass a spermatophore. This experiment was not a choice test but rather a measure of the overall fitness of males of different ages. It would only have been a choice test if both males arrived

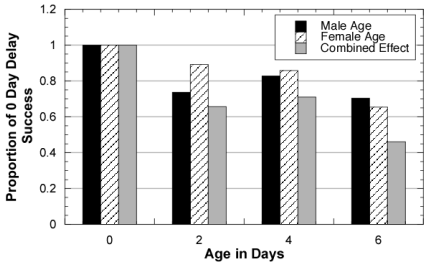
simultaneously and the female had to choose one versus the other. The second test was a no-choice test where we paired 0-, 2-, 4-, or 6-day-old virgin males with 0-day-old virgin females in mating cups (16 oz cups for OBLR, 10 oz for CM); in the reciprocal experiment, virgin females that were 0, 2, 4 or 6 days old were paired with 0-day-old virgin males.

Fig. 1. Proportion of CM pairs where a spermatophore was successfully passed. Values corrected relative to the success for 0 day old individuals.



Results: For both species, the 4-day-old males were significantly more likely to respond (by flying upwind) than 1-day-old males (90 vs. 71% for CM and 84 vs. 49% for OBLR). However, in both species, young males that flew were more likely to contact the female. Once contact was made there were no significant differences (using Fisher's exact test) in the ability to pass a spermatophore for CM (71.5 vs. 70% for 4- vs. 1-day-old males), or OBLR (33.3 vs. 14.3% for 4- vs. 1-day-old males). In both species, the tendency of older males to fly more frequently was cancelled by the younger males' ability to actually contact the female so that, overall, there were no differences in rates of mating between age categories.

Fig. 2. Proportion of OBLR pairs where spermatophores were successfully passed. Values corrected relative to the success of 0 day old individuals.

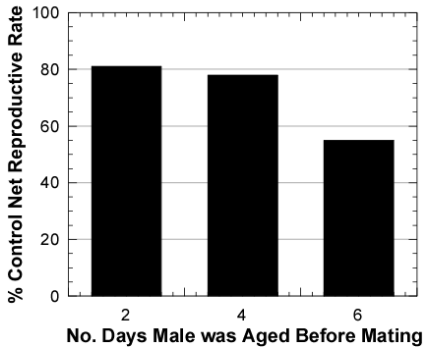


In the no-choice trial with CM, the largest effect on mating success was related to male age; that is, females were less likely to mate with males after they were >2 days old (Fig. 1). Six-day-old males were able to successfully pass a spermatophore only 50% as often as males aged 0 or 2 days. In contrast, males showed little age-based discrimination against females. Even 6-day-old females were able to mate at roughly 87% of the rate of newly emerged females. The combined effect is calculated by multiplying the probability of successful mating by males and females of the same age, but the combined effects are largely related to the females discriminating against older males.

In the no-choice trial with OBLR, mating success decreased with age in both sexes similarly compared to individuals paired on the day of emergence (Fig. 2). By day 6, males were only 70% as efficient at mating as males paired on the day of emergence and females were about 66% as efficient. ***Plan for next year:*** This objective has been completed.

Methods: ***Objective 2.*** We tested the effect of male age on female reproductive success by mating virgin males that had emerged 0, 2, 4, or 6 days before with 4-day-old virgin females. The resultant eggs were allowed to mature and the CM larvae that successfully emerged were counted. We found that the net reproductive rate (average number of daughters produced over the average female life span) dropped dramatically as the male age increased past 4 days (Fig. 3). The reduction in population growth associated with older males being mated with females of a fixed age is similar but of lower magnitude than our previous studies where females of different ages were mated with fixed-age (1-day-old) males. In this study, there were two components affecting female reproductive output: (1) as male age increased, the incidence of no eggs produced also increased and (2) there was a change in reproductive rate not associated with number of eggs produced (*i.e.* a decrease in percent egg hatch).

Fig. 3. Effect of male age on female reproductive output. All females were mated at 4 days old.



These data, in conjunction with our previous data on female age at time of mating, clearly show that the age of both sexes at time of mating is critical to population growth. Combined with our data in Objective 1, it appears that incidences of zero reproduction are likely to be caused at least in part by female

rejection of older males. While young males may reject some older females, it is relatively rare. ***Plan for next year:*** We have completed the laboratory portion of the experiments and just need to integrate the male age effects into our models that describe the effect of delayed mating.

Methods: ***Objective 3.*** Lab studies were set up so that females experienced a relatively constant number of degree-days in delay of mating by changing the temperature at which they were reared immediately after emergence. For codling moth, we reared newly eclosed females for 10 days at 15°C (=50 DDC delay) or for 2 days at 30°C (=40 DDC delay) before pairing with a male. With these DD delays, the expectation is that the reproductive output would be similar, although slightly higher for those that were reared at 30°C because the delay is slightly shorter. If the delay operates on a calendar date basis, then the females aged for 10 days at 15°C would have radically lower reproductive output than those reared for 2 days at 30°C.

The second part of this objective, whether we can predict severity of CM and OBLR problems based on the average delay in mating experienced in the field, required the answers to several additional questions:

1. What is the longevity of males and females subjected to field conditions on a degree-day basis?
2. Does the shape of the oviposition curve vary with a delay in mating on a degree-day basis?
3. Can we predict the magnitude of the oviposition curve with delays in mating on a degree-day basis?

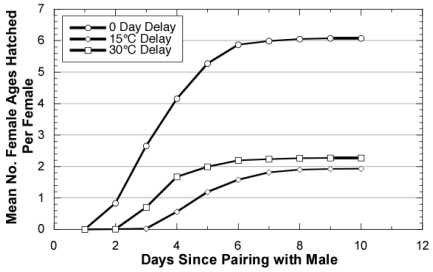
To determine the longevity of males and females under field conditions, we caged newly emerged CM and OBLR individuals in mating tubes, which were placed within large plastic delta traps. These were placed in the field primarily during CM and OBLR flight periods, respectively. This experiment was run last year in a small trial and in large trials this year. A total of 210 male and 476 female CM and 236 female and 368 male OBLR were tested in this manner. The time each moth was placed in the field and when that moth died were recorded, and the longevity on a degree-day scale was calculated. The data were then summarized as the cumulative mortality of moths versus DDF.

To determine if the shapes of the oviposition curves vary with mating delays on a DD basis for CM, we compared the cumulative percentage oviposition (from our previous laboratory studies) and our data from the degree-day delay studies. We standardized each curve and plotted them on a common DDF axis. We then used a cumulative Weibull function to predict the shape of the curve.

To determine the magnitude of the effect of delayed mating, we used the net reproductive rates computed in our laboratory studies on degree-days and calendar days mating. This was then plotted against the delay in mating on a DDF scale. A simple exponential curve was fit to the data.

To determine the likely effects of delayed mating that occur because of inclement weather conditions such as high wind or rain, we used data from TFREC during 2005. The temperature data were used to drive a simple model that accounted for: 1) the effect of mortality that occurred before females mated, 2) the amount of oviposition completed before death (*i.e.*, if the average female lived 200 DDF but experienced a delay of 100 DDF, then she only had 100 DDF of life left after mating and this reduces the total number of eggs she could lay); and 3) the effect of delayed mating on the magnitude of the oviposition curve. The data were summarized by comparing the net reproductive rate of the different delays over the course of the season to the net reproductive rate of the population that experienced no delays.

Fig. 4. Effect of aging CM females at different temperatures but constant degree-day accumulations on net reproductive rate.



Results: Our studies showed conclusively that the delay in mating affects codling moth on a degree-day scale. As predicted, the reproductive output was similar but slightly higher for those moths reared at 30°C for 2 days (40 DDC

delay) (Fig. 4) compared to those reared at 15°C for 10 days (50 DDC delay) before mating.

The information required to model the effect of delays in mating on population growth was obtained and highly accurate. The mortality rates of adult male and female codling moth were well predicted by the Weibull function with the regressions accounting for 99% of the variability in the data (Figs. 5 and 6). These data allow us to predict the longevity of moths depending on the degree-days accumulated since adult emergence. The cumulative percentage oviposition that occurred since pairing with a male was similar across the different delays experienced (Fig. 7) and allowed us to use the standard shape to determine how much of the reproductive potential could be used before death. The relationship between the DDF between female emergence and mating and the net reproductive rate was also highly accurate (Fig. 8), allowing us to determine the effect of delay on population growth. The OBLR results are similar and not shown because of space considerations.

Fig. 5. Mortality rate of CM females in the field at TFREC in 2004-2005.

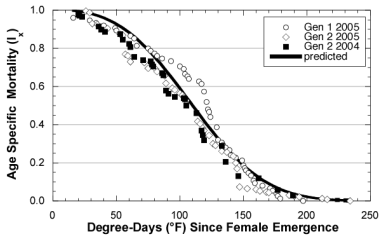


Fig. 6. Mortality rate of CM males in the field at TFREC in 2004-2005.

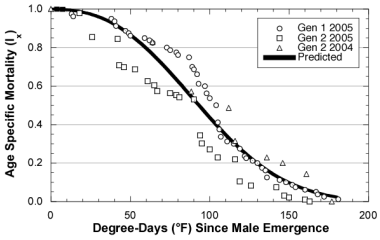


Fig. 7. Shape of the oviposition curve for CM females. Includes those with and without a delay in mating.

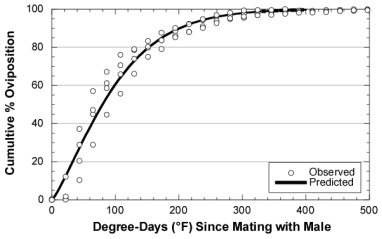
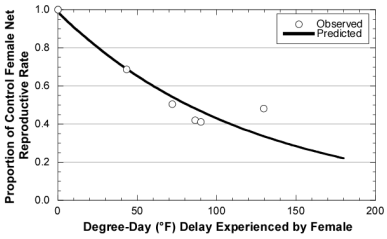


Fig. 8. Effect of delaying female CM mating on net reproductive rate. All rates scaled so undelayed females reproductive rate equals 1.



The model developed to date is based on females mating with a young male; therefore, it is very conservative in its predictions of the effect of the delay in mating. We have the data to incorporate male ages as well, but it requires a much more complex model than we will be working on this coming year. Our model projections this year show that the effect of a delay in mating of 2 days varies with the season, and during the first generation the average percent reduction in growth rate was ≈30% for a 2-day delay and 65 and 80% for a 4- or 6-day delay. During the second generation when temperatures are warmer, the effect is much more pronounced (roughly 50, 90 and 95% for 2, 4, and 6 days delay). We will be working on completing the CM model over the next year and starting a new one for the OBLR as well.

Plan for next year: The analysis of our laboratory data is nearly complete. However, we still need to evaluate several factors, particularly the role of wind velocity on mating. While there is some published data on this, it is poor and often contradictory. We want to evaluate mating at different wind speeds and include information on the effect of orchard design on wind patterns. To do this, we are requesting additional funds to purchase five data loggers so that we can map wind velocity in different orchard types. Once we get that data, we will do field studies where we release males downwind at different distances from virgin females placed at the orchard border and determine mating success. We will place a data logger at each distance and one at the orchard border. This will allow us to determine mating success at different wind velocities over the normal flight period. These data will then be used in a simple model that will use wind velocity, temperature at dusk, and our data on population growth rates of delayed moths to help predict the magnitude of the population growth compared to individuals that are not delayed.

Methods: ***Objective 4.*** We set up two experiments, each at a different orchard and each consisting of seven one-acre plots. At the first orchard, mating disruption was not used for the first generation, but before the second-generation flight Isomate C++ dispensers (400/acre) were applied to the center plot only. Also at the first orchard, we trapped in an adjacent orchard block that had MD present

throughout both CM generations. In the second orchard, mating disruption dispensers were uniformly present throughout the plots and during both generations. We used high density trapping to follow CM movement in both situations. In each of the orchards we used nine traps per acre (63 total) to trap moths. Combo pheromone/DA lures were used in both experiments, regardless of MD presence. The traps in each plot were numbered and their GPS coordinates were mapped. The GPS coordinates were used to determine the distance of each marked moth from the center of the plot of origin.

Analysis: We calculated the distance from the origin for each marked moth captured. To summarize the distance flown, we sorted the data from shortest to longest distance and calculated the distance flown by 1, 5, 10, 25, 50, 75, 90, 95, and 99% of the marked moths as well as the average distance flown. The different percentiles were then graphed on the y-axis, and the distance from the origin was graphed on the x-axis. This graph allowed us to compare the distances flown in the different MD situations and determine if differences existed.

Results:

First orchard, no MD present, first generation: The trap captures were highest at the south end of the plot and in the adjacent orchard (treated with MD). Marked moth captures were highest in these same areas. Fifty percent of the marked moths were captured within 392 feet of origin, but the converse of this is that 50% of the population traveled more than 392 feet (Fig. 9A). The cumulative percentage catch was linearly related to distance.

First orchard, MD present in the center plot only, second generation: As with the first generation, trap catches were still highest in the southern part of the plot and in the adjacent orchard. The distance moved and the shape of the cumulative percentage catch was virtually identical, indicating that the MD plot was unimportant in the degree of movement (Fig 9B).

Second orchard, MD present uniformly throughout the area, first generation: As with the other orchard, we found that trap catches were clustered, with most of the moths caught in three of the seven acres monitored. However, a plot of the cumulative percentage of moths caught versus distance was not a straight line, but a curve (Fig. 9C). In addition, in comparison to the first orchard, the distances flown were markedly shorter. For example, 50% of the moths were caught within 128 feet of origin versus 392 feet in the first orchard.

Second orchard, MD present uniformly throughout the area, second generation: The second generation showed a pattern of movement similar to the results in the first orchard; the cumulative percent moth catch was a linear function of distance (Fig. 9D). In this plot, 50% of the moths were caught within 308 feet of origin, compared to 128 feet in the first generation.

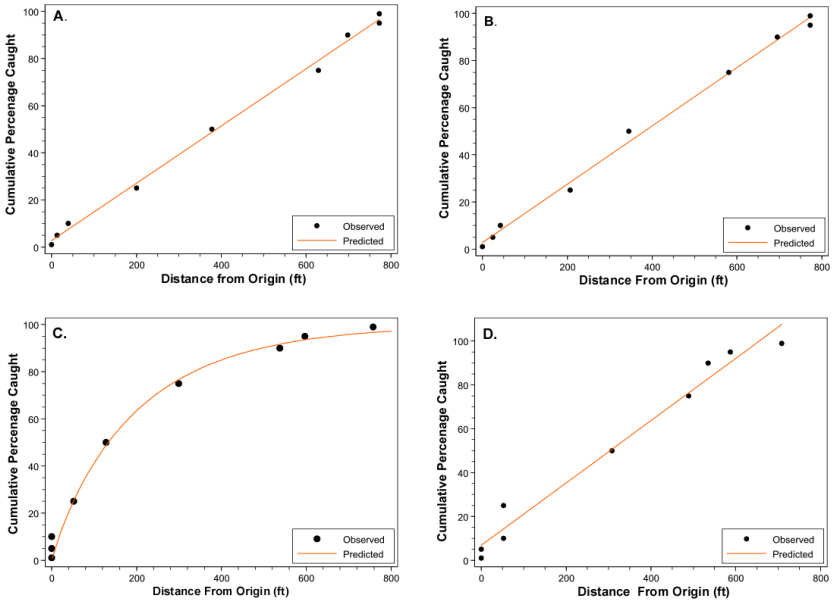
Difference in movement patterns between first and second generations in the second orchard (uniform MD): In reviewing the experimental procedures, we changed nothing in the protocols that would have affected the shape of the dispersal curve. However, a key difference is that the first orchard received Guthion® cover sprays for both generations. Guthion® was also used in the second generation in the second experimental orchard. For the first generation in the second experimental orchard, Assail® 70WP was applied for two cover sprays. Data from Jay Brunner's lab shows that Assail® causes high codling moth adult mortality and may have been the reason for our different shaped curves. A material with a high activity against adults would tend to kill them as they move throughout the orchard, resulting in an overall shorter distance flown.

The data obtained clearly show that codling moth is highly mobile. Although the combo pheromone/DA lures catch primarily males, we do have some data on the females and it tends to be similar (at least in the average distance flown) to the male data. Our data suggest that 50% of the individuals travel more than 400 feet, but it also means that the scale of our experiments needs to be increased. In all cases, we obtained multiple marked individuals at the traps farthest from their origins

(>800 feet). Our data do not indicate that MD plots are a source of moths or have the tendency to concentrate moths; however, more information on this point is required.

***Plan for next year*:** We will change our experimental design to increase the scale of the experiments so that we can get a better handle on the distances these moths are traveling. We would like to set up experiments in three separate orchards next year, two using Guthion® and one using Assail® only. This will allow us to determine if Assail® is causing the differently shaped dispersal curve. In one of the Guthion® orchards we will repeat the study where we add mating disruption to a portion of the plot before the second CM generation and compare the first and second generation movement patterns. It is likely that we need to expand the size of the MD plot as well.

Fig. 9. Cumulative percentage of marked moths caught at various distances from area where they were marked. **A**. First Generation in orchard one where no MD was present. **B.** Same orchard as figure A, but in the second generation with MD dispensers in the center 1 acre plot. **C.** Orchard 2 where MD was uniformly present in the first generation. **D.** Same orchard as figure C, but in the second generation.



Budget:

Project title: Mechanisms underlying mating disruption
PI: Vincent P. Jones
Project duration: 2004-2006 (3 years)
Current year: 2006
Current year request: $55,048

Item	Year 1 (2004)	Year 2 (2005)	Year 3 (2006)
Salaries[1]	22,724	23,634	**24,377**
Benefits (32%)[2]	7,272	7,563	**9,263**
Wages[3]	12,800	12,800	**12,800**
Benefits[2]	2,048	2,048	**1,408**
Supplies[4]	3,200	3,200	**5,700**
Travel[5]	3,200	3,200	**1,500**
Total	51,244	52,445	**55,048**

[1] Tawnee Wilburn, Associate in Research (0.67 FTE).
[2] Benefits for Associate in Research have changed to 38% for year 3. Time-slip benefits – 16% years 1-2; 11% year 3.
[3] Time-slip wages.
[4] Lab supplies, cell phone charges are allowed; new total includes data loggers (page 4, obj. 3 plans).
[5] Travel to research plots. Travel costs are reduced for year 3 from original amount budgeted.

CONTINUING PROJECT REPORT **Year 1/2**

Project Title: ULV microencapsulated sex pheromones for codling moth
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Objectives:

1. Evaluate the effectiveness of season-long ULV applications of a microencapsulated sex pheromone formulation (MEC) for codling moth and compare its performance against Isomate-C PLUS in replicated apple and pear orchards.
2. Evaluate the use of insecticides and the pear ester with ULV microencapsulated sex pheromone to control adults.
3. Evaluate various factors impacting the deposition and retention of microcapsules.

Significant findings:

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

Methods: Trials were established in eight 20-acre apple orchards situated near Brewster, WA. Orchards were split into two halves and one half was treated with Isomate-C PLUS at 300 dispensers per acre and the other half with five ULV applications (1.25 gallons per acre) of 10 g A.I. Checkmate CM-F (Suterra Inc., Bend, OR) applied every 4 weeks. Orchards were monitored with sex pheromone-baited traps. Orchards were monitored for fruit injury at midseason and prior to harvest. Similar tests were conducted in pear orchards in Medford, OR. Seven 'Comice' and two 'Bartlett' orchards were either treated with four ULV spray applications of pheromone or were treated with 200 Isomate C tt dispensers per acre. Orchards were monitored with pheromone, pear ester, and combo lures. All orchards in both areas also received multiple insecticide applications during the season.

Replicated, small plot (1.0 acre) tests were conducted in three 'Red Delicious' orchards near Moxee, WA. Treatments included the use of the ULV microencapsulated pheromone, ULV Asana, ULV Asana plus pheromone, Isomate C tt dispensers at 200 per acre, and the use of three applications of 24 AKISS per acre on 26 April, 22 June, and 11 August. ULV applications of 0.25% pear ester MEC were included in separate treatments with pheromone and pheromone plus Asana for only the first generation. The Asana treatments were subdivided and treated with two rates of Asana (6 or 10 oz per acre). ULV sprays were applied on 27 April, 11 May, 2 and 24 June, 21 July, and 18 August. The high rate of Asana was changed from 10 to 3.0 oz for the final two applications. Single and group fruit samples were collected and subjected to residue analysis by an independent laboratory. All plots were sprayed with three applications of Guthion (1.0 lb per acre) on 20 May, 25 June, and 6 August. Plots were monitored with traps baited with a pear ester and sex pheromone combo lure. Fruit injury was sampled in late June and mid September. Pest (two-spotted and European Red mite) and predator mites (*Zetzellia mali* and *Galandromus occidentalis*) were sampled on four dates during the season.

Five replicate small blocks (0.3 acre) were established within several apple orchards situated near Orondo, WA. Blocks were a combination of 'Gala', 'Fuji', 'Delicious', and 'Golden Delicious'. Blocks were sprayed with ULV sprays of sex pheromone alone and in combination with Assail (3.4 oz) or Intrepid (16 oz) on 5 and 31 May, 28 June, and 26 July. All orchards had under-tree irrigation. The grower sprayed all blocks with a full-season insecticide program. Mites were sampled in mid-August. Fruit were sampled from 23 August until 15 September.

Studies were conducted to assess the relative deposition of microcapsules marked with a fluorescent dye applied as an ULV spray. Applications were made with the D2 nozzle and with the ATV driving 7.5 mph. Leaves were scanned under an UV-light to count capsules. In the first study, the distribution of microcapsules following a single spray to one side of trees was conducted. Tree height averaged 420 cm and the ULV spray contacted trees at 240 cm. Five leaves were sampled from 25 positions on 10 trees. The second study evaluated the density of microcapsules in seven orchards that varied in mean leaf area. The number of capsules on the top and bottom of 100 leaves were counted. The third study compared the deposition of capsules on the top and bottom of 100 leaves in two orchards when sprays were applied with two different nozzle heights, 1.2 or 1.6 m.

The longevity of the attractiveness of clusters of microcapsules (90 per leaf) was evaluated by field aging treated leaves, and flying male moths to leaves treated either on their top or bottom surface on days 0, 7, 14, 21, and 28 d. Three male moths were flown consecutively to five leaves of each type on each date**.**

Results and discussion:

No significant difference in fruit injury occurred in apple (Table 1) or pear orchards (Table 2) treated with a seasonal ULV pheromone program versus the use of Isomate dispensers. The effect of overhead irrigation was not significant for fruit injury in apple but moth counts were higher in overhead blocks during the first flight (Table 1). Moth counts were higher in both flights in the ULV versus the Isomate treatments. These data support the wider adoption of the ULV approach. Several

programs will be evaluated in 2006 integrating these ULV sprays based on pest pressure and grower's spray programs, e.g. determining the best timings for a four-spray program in low pest pressure situations; evaluating a four spray program with two sprays during each generation timed before and after the peak period of moth emergence; and developing the use of action thresholds based on moth catch or fruit injury to recommend the addition of a ULV pheromone spray to a grower's program.

Significant reductions in moth catch and fruit injury occurred among all treatments compared to the Guthion-alone blocks in the Moxee orchards (Table 3). Treatments including ULV Asana had the lowest levels of fruit injury. The efficacy of the ULV pheromone, Isomate, and AKISS treatments were equivalent. Addition of pear ester MEC did not improve the performance of pheromone or pheromone plus Asana and were discontinued at mid-season. The effectiveness of the ULV Asana sprays appears to be due to both lethal and sublethal effects on adult moths (Table 4). Low rates of Asana can reduce fecundity > 90%, while not preventing mating of moths. Fruit harvested from the six spray 6 oz Asana program did not exceed the allowable threshold for residues (2.0 ppm). Group fruit samples (n = 20 fruits) averaged 0.1 ppm and individual fruits ranged from < 0.01 to 1.67 ppm, mean = 0.21 ppm. The density of pest mites increased in plots treated with Asana until early August and then crashed (Table 5). Predatory mite densities gradually increased during the season. Studies in 2006 will examine softer programs using fewer sprays (3 – 5) and lower rates of Asana (3 oz). The effect of a two-year Asana program on mite populations will be measured.

No significant differences in either moth catches or fruit injury occurred among treatments in the Orondo orchard (Table 6). Pest mite levels remained low all season though the Assail treatment had the lowest level of predator mites. Nevertheless, these data suggested that Assail can be used effectively in ULV sprays. Larger and better replicated studies with Assail will be conducted in 2006. Other registered (Rimon and Imidan) and experimental insecticides (XDE-175, DPX-E2Y45) applied as ULV sprays will also be examined.

Several studies examing the deposition of microcapsules improved our knowledge of this approach. Capsules were distributed throughout the canopy and across to the adjacent row following a single spray from one side of the tree (Table 7). Greater numbers of capsules were deposited on the underside of leaves near the sprayer and higher numbers were deposited on the top than bottom on the opposite side of the canopy and the adjacent row. A high proportion of male codling moths continued to respond to treated apple leaves even after 28 d (Table 8). Following two bouts of precipitation the attractiveness of leaves with capsules on the upper surface declined relative to leaves where capsules were sprayed on the undersurface. A greater number of capsules are deposited on larger than smaller leaves, and the density of capsules per area was fairly consistent across leaf types (Table 9). Raising the height of the spray nozzle did not influence the density of capsules deposited but increased the proportion of capsules on the top of leaves (Table 10).

These data suggest that growers should adjust their spray practices to maximize the distribution of microcapsules on the underside of leaves. Studies in 2006 will further examine the effect of adjusting several application factors to enhance deposition, e.g. stickers, spray pressure, nozzle type, spray angle; and the impact of horticultural factors, such as cultivars, seasonality, and canopy volume and density. Studies to enhance the clustering of capsules will also be conducted. Creating a larger number of attractive point sources in the canopy may be the key to improving mating disruption.

Table 1. Moth catches and codling moth injury in Brewster orchards treated with either ULV MEC sex pheromone sprays or 300 Isomate C Plus dispensers per acre. Orchards were grouped based on irrigation method, overhead (n = 3) or undertree (n = 5).

Treatment	Irrigation	Mean (SE) moth catch per trap		Mean (SE) % fruit injury	
		1st flight	2nd flight	Mid-season	Pre-harvest
ULV	Undertree	32.8 (8.2)	51.7 (22.6)	0.5 (0.3)	1.9 (0.7)
ULV	Overhead	113.8 (29.0)	71.3 (20.2)	1.1 (0.3)	2.9 (0.5)
Isomate	Undertree	21.2 (4.5)	20.2 (6.0)	0.2 (0.1)	2.8 (0.9)
Isomate	Overhead	21.8 (8.4)	20.8 (9.9)	0.4 (0.2)	2.1 (0.2)
ANOVA: (df = 1, 28)					
Treatment		$P < 0.001$	$P < 0.05$	$P = 0.07$	$P = 0.99$
Irrigation		$P < 0.01$	$P = 0.57$	$P = 0.12$	$P = 0.83$
Treatment x Irrigation		$P < 0.01$ [a]	$P = 0.59$	$P = 0.48$	$P = 0.31$

[a] Significant interaction term because moth catch was significantly higher in the overhead ULV orchards than in the other three types during 1st moth flight.

Table 2. Moth catches and injury in 'Comice' and 'Bartlett' orchards treated with either ULV MEC sex pheromone sprays or 200 Isomate C tt dispensers per acre in Medford, OR, n = 9.

Treatment	Seasonal mean moth catch per baited-trap (female moths)			Mean % fruit injury
	Pheromone lure	Pear ester lure	Combo lure	
ULV	4.0	1.7 (1.3)	11.9 (1.6)	0.01
Isomate	2.9	1.6 (1.3)	12.6 (1.9)	0.04

No significant differences occurred between treatments for moth catches or fruit injury, $P > 0.05$.

Table 3. Moth catches and fruit injury among several ULV treatments, 200 Isomate C tt dispensers per acre, or 24 AKISS per acre in small apple plots, Moxee WA, n = 4 – 8.

Treatment	Mean (SE) moth catch per trap		Mean (SE) % fruit injury	
	1st flight	2nd flight	Mid-season	Pre-harvest
ULV PH MEC	43.0 (7.9)b	40.9 (12.2)b	5.3 (1.4)b	12.3 (2.1)b
Isomate C tt	41.4 (18.4)b	26.8 (17.3)b	3.8 (2.5)bc	16.0 (3.2)b
ULV PH MEC + 6 oz Asana	40.8 (10.4)b	19.3 (5.5)b	1.2 (1.1)cd	2.2 (0.5)c
ULV PH MEC + 10 oz Asana				2.2 (0.6)c
ULV 6 oz Asana	102.0 (33.2)b	52.4 (19.9)b	0.4 (0.4)d	4.2 (1.4)c
ULV 10 oz Asana				1.9 (1.1)c
AKISS [a]	59.3 (16.8)b	47.5 (16.8)b	3.4 (1.4)bcd	18.6 (3.4)b
Guthion only	378.0 (152.0)a	254.0 (74.9)a	40.6 (2.1)a	75.0 (4.0)a
ANOVA:	$F = 7.85$; df = 5, 27; $P < 0.001$	$F = 9.37$; df = 5, 27; $P < 0.0001$	$F = 33.6$; df = 5, 22; $P < 0.0001$	$F = 54.6$; df = 7, 43; $P < 0.0001$

Percent fruit injury was high due to very low crop load (50 fruits per tree) from alternate bearing.

[a] AKISS are 0.1 m^2 grids coated with Asana and baited with a combo pheromone and pear ester **lure.**

Table 4. Laboratory bioassays with codling moth adults placed in plastic cups coated with different concentrations of Asana XL. Tests were conducted at 25 ºC for 120 h.

Rate (a.i. ppm)	% adult survivorship @ 72 h	Proportion F mated	# eggs per female	% reduction in fecundity
3.16	0	0.0b	0.0c	100
1.58	20	1.0a	3.4c	97
1.23	65	0.9a	7.0bc	93
0.79	80	1.0a	20.9b	79
0.62	100	0.9a	49.3ab	51
0.31	80	1.0a	79.8a	20
0.00	100	0.9a	99.6a	-

Table 5. Seasonal densities of pest and predatory mites per leaf in Moxee plots treated with six ULV applications of Asana XL or left untreated, n = 5.

Date	Asana applied		No Asana applied	
	Pest mites	Predator mites	Pest mites	Predator mites
Early July	0.8	0.2	0.0	0.9
Mid-July	3.9	0.1	0.0	1.1
Early August	13.2	1.1	0.7	0.5
Late August	6.1	1.3	0.3	0.7

Table 6. Codling moth field trial conducted near Orondo, WA in 2005, n = 5.

Treatment	Mean (SE) moth catch per trap		Mean (SE) % fruit injury	Predator/pest mite ratio
	1st flight	2nd flight		
ULV Pheromone + 3.4 oz Assail	12.2 (2.6)	15.4 (2.0)	0.4 (0.2)	1.3
ULV Pheromone + 16 oz Intrepid	34.0 (10.4)	37.6 (11.3)	0.7 (0.2)	2.7
ULV pheromone	43.2 (7.2)	20.8 (4.3)	0.4 (0.1)	3.0
Untreated	50.4 (18.1)	52.6 (17.8)	0.8 (0.1)	4.3
ANOVA: (df = 3, 12)	$P = 0.12$	$P = 0.17$	$P = 0.39$	

Table 7. Mean (SE) density of microcapsules on the top and bottom of apple leaves distributed at five height classes in the canopy following a standard ULV application.

	Canopy position				
Spray height	Edge + 0 – 60 cm	Edge + 61 – 120 cm	Opposite edge – 61 – 120 cm	Opposite edge – 0 – 60 cm	Next row 400 – 430 cm
Contact	0.02 (0.02)	0.3 (0.1)	1.2 (0.4)*	1.7 (0.6)*	0.3 (0.2)
+ 120 cm	0.1 (0.1)	0.5 (0.2)*	1.3 (0.3)*	0.7 (0.2)*	0.0 (0.0)
Contact	0.8 (0.3)*	0.7 (0.2)*	0.2 (0.1)	0.0 (0.0)	0.5 (0.4)
+ 60 cm	4.6 (1.7)*	2.0 (0.8)*	0.0 (0.0)	0.3 (0.2)	0.02 (0.02)
Contact	0.6 (0.3)*	1.9 (0.5)*	0.3 (0.2)	0.4 (0.2)*	0.2 (0.1)
	3.3 (0.8)*	3.5 (1.1)*	0.1 (0.1)	0.3 (0.1)	0.2 (0.1)
Contact	0.2 (0.1)	0.3 (0.1)	0.8 (0.3)*	0.2 (0.1)	0.9 (0.4)*

– 60 cm	0.04 (0.04)	0.1 (0.04)	0.04 (0.03)	0.3 (0.1)	0.1 (0.1)
Contact	0.3 (0.1)	0.3 (0.1)	0.9 (0.2)*	0.3 (0.1)	0.1 (0.1)
– 120 cm	0.0 (0.0)	0.04 (0.04)	0.1 (0.1)	0.1 (0.1)	0.0 (0.0)

ANOVA: $F = 6.40$; df = 49, 2450; $P < 0.001$. '*' significantly different than 0.0 microcapsules per leaf, LSD test, $P < 0.05$.

Table 8. Proportion of male codling moths responding to field-aged clusters of microencapsulated sex pheromone (90 capsules per leaf).

Age of capsules (d)	Top of leaf		Bottom of leaf	
	Upwind flight	Landing on leaf	Upwind flight	Landing on leaf
0	0.93	0.60	1.0	0.53
7	1.0	0.67	1.0	0.60
14 *	1.0	0.93	1.0	0.80
21*	0.73	0.40	0.93	0.60
28	0.80	0.47	1.0	0.53

* Precipitation occurred on days 12 and 18.

Table 9. The effect of leaf area on the density of microcapsules deposited per leaf.

Mean leaf area (cm ^{2})	Mean # capsules / leaf	Mean # capsules per cm ^{2}
16.0	**6.6**	**0.41**
18.5	**8.9**	**0.48**
20.5	**10.0**	**0.49**
24.0	**7.9**	**0.33**
26.2	**11.4**	**0.44**
27.7	**12.2**	**0.44**
29.7	**15.7**	**0.53**

Budget:

Project title: ULV microencapsulated sex pheromones for codling moth
PI: Alan Knight
Project duration: 2005-2006
Current year: 2006
Project total (2 years): $56,000
Current year request: $28,000

Item	**Year 1 (2005)**	**Year 2 (2006)**
Salaries	14,000	14,000
Benefits 16%	2,250	2,250
Wages	6,000	6,000
Benefits 16%	1,000	1,000
Equipment	1,000	1,000
Supplies	2,000	2,000
Travel (local)	1,750	1,750
Total	28,000	28,000

Supplemental funding has been obtained from both Dupont Agrochemicals and Suterra LLC through 2006.

CONTINUING PROJECT PROPOSAL **YEAR 1/2**

Project Title: Direct control of codling moth with pear ester
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Objectives:

1. Evaluate the effects of the microencapsulated pear ester formulation on the distribution of egg deposition and resulting larval attack of fruit for various apple and pear cultivars.
2. Evaluate the use of the microencapsulated pear ester formulation in combination with insecticides for control of larvae and adults.
3. Evaluate the use of hand-applied dispensers loaded with sex pheromone and pear ester.
4. Evaluate the use of insecticide-treated killing stations (AKISS) baited with combo pheromone / pear ester lures.

Significant findings:

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

Methods:

A 5% microencapsulated formulation of pear ester (DA MEC) was provided by Trécé Inc. (Adair, OK) and was serially diluted in water in all trials. All studies were conducted in apple orchards at either the U.S.D.A. research farm 15 miles east of Moxee or in a leased orchard near Moxee. The influence of applying DA MEC on the location of eggs laid by female codling moth was studied in replicated four-tree plots at the research farm. Each plot consisted of one 'Red Delicious', 'Golden Delicious', 'Gala' and 'Fuji' tree. Sprays were applied with an air blast sprayer at a rate of 100 gallons per acre on 19 May, 9 June, 18 June, and 27 July. Concentrations of 0.001 and 0.01% were evaluated in the first three tests and 0.1 and 1.0% on the final date. Five replicate plots were included with each rate on each date. One 50 cm shoot with fruit was cut from each tree on each sampling date and visually searched for eggs. The distance (cm) of each egg to the nearest fruit on the shoot was measured. One hundred and thirty-seven eggs were sampled.

The influence of applying DA MEC to a fruit cluster on codling moth oviposition was measured by spraying clusters of each of the four cultivars on 12 May, 3 June, 16 June, and 29 June. Approximately 0.9 ml was sprayed on each cluster using a standard spray bottle. Following the first spray application clusters were sampled three weeks later and all eggs were counted. The final three applications were evaluated after a week and only unhatched eggs were counted. Due to the low number of eggs found in these trials the influence of cultivar was not assessed in these tests.

The influence of adding DA MEC as an adjuvant with Guthion was tested at the leased 'Braeburn' orchard beginning on 9 June. All injured fruits were removed from trees prior to spraying and again on 16 June. Trees were sprayed with a handgun using 1.0 lb Guthion per 100 gallons with and without the addition of DA MEC. Twelve replicates of four rates of DA MEC were tested in this study: 0.001, 0.004, 0.01, and 0.1%. Trees were re-sprayed on 29 June, 20 July, and 16 August. All fruits were harvested from trees on 7 September and inspected for fruit injury. Two additional studies were conducted at the research farm. The first study was a two-spray program during the first generation in 'Fuji'. Trees were sprayed using a handgun with 0.5 lb Guthion with and without the addition of the same four rates of DA MEC used in the previous study. Sprays were applied on 27 May and 15 June. Injury was assessed on 3 July. All fruit were picked off the trees and inspected for injury. The third study was conducted in another section of the research farm using four apple cultivars. Teams of three technicians searched all trees and removed all injured fruits prior to the start of this test. Trees were resampled after 7 days to remove fruits injured by previously laid eggs. Four spray applications were made on 10 June, 29 June, 20 July, and 16 August. Samples of 100 fruit per tree were collected from 10-15 September.

Dual choice bioassays were conducted in closed 50 mm-diameter polystyrene Petri plates. Five larvae were placed equidistantly (< 1.0 cm) from two 20 x 3 mm strips of white copy paper impregnated with a 10-µl drop of either DA MEC or a blank MEC formulation. The number of larvae to first touch a particular strip and the duration of larval contact was recorded for a 3 minute period in a dark room kept at 24ºC, 30% RH, and illuminated by red light.

Studies to evaluate a new 'puzzle-piece' dispenser (Trécé Inc., Adair, OK) loaded with sex pheromone and pear ester was conducted in a 36 acre 'Granny Smith' orchard near Brentwood, CA. Two replicate 6-acre blocks were established treated with either 200 Isomate C tt dispensers per acre, 400 Cidetrack CM-pheromone dispensers per acre, or 400 CM/DA combo dispensers per acre. All blocks were also treated with three insecticide cover sprays during the season. Fruit injury was assessed by inspecting 800 fruits per block on 20 August, just prior to harvest. Fruit injury was also measured in an untreated orchard situated 0.25 mile upwind from the study site.

The effectiveness of an insecticide-treated 0.1 m^2 grid (AKISS) baited with the Pherocon CM DA combo lure was evaluated in a 'Granny Smith' orchard near Thornton, CA. The entire orchard was treated with Isomate C tt dispensers (200 per acre) and was sprayed with four insecticide sprays during the season (Imidan and Guthion). Seven and six replicates of the AKISS-treated and untreated plots (2.0 acre) were established. AKISS blocks were treated with 24 grids per acre on 28-29 April and were retreated on 16-17 June using new trees. AKISS were placed in the upper third of the canopy. Fruit injury was assessed in all blocks by inspecting 540 fruit per block from 30 August - 1 September.

Results and Discussion:

Effect on Egg distribution. Eggs were found over a range of 0 – 45 cm from the nearest fruit. No significant effect of the concentration of DA MEC on the distance between eggs and the nearest fruit was found (Table 1). Similarly, no effect was found across four apple cultivars (Table 2). Our data suggests that the application of DA MEC does not affect the distance that codling moth eggs are laid relative to fruit. An earlier study in Italy had suggested that this could be an important mechanism explaining how applications of pear ester in their studies had enhanced larval mortality. Codling moth females detect a number of plant volatiles in addition to pear ester that are likely used as cues to select oviposition sites. It does not appear that this formulation of pear ester at these rates can be used to disrupt the sites where female moths lay eggs. Future studies evaluating this effect are not planned.

Enhanced oviposition. Egg laying by codling moth was enhanced (3-fold) with the application of high rates of DA MEC (Table 3). This suggests it may be possible to use pear ester to improve monitoring of eggs within orchards. Previous laboratory studies found that pear ester released from grey septa could increase egg laying two-fold in both choice and non-choice tests. In comparison, Dr. Broc Zoller increased egg laying by 95-fold by cutting 'Bartlett' fruit during the season. Studies have not quantified the amount of pear ester or other attractive fruit volatiles emitted by cut Bartlett fruit during the season, but this is likely to vary widely as fruit mature. The use of a measured amount of synthetic attractant in the DA MEC spray helps to standardize this response during the season. Our studies in 2006 will examine higher rates of the DA MEC (10 and 100%) and the application of higher spray volumes per cluster (10 ml). Our ability to monitor codling moth eggs, however, is problematic due to the low density of codling moth in most commercial orchards. For example, Dr. Zoller was able to increase the incidence of fruit clusters containing an egg to only 1.0%, while only 0.1% of clusters contained an egg in our orchard, despite a large overwintering population of codling moth. Effective strategies to monitor codling moth eggs using the pear ester will likely involve selective monitoring of high-pressure sites. Nevertheless, the ability to monitor seasonal egg densities can improve our management of this pest and we will continue to investigate this approach.

Adjuvant for larval control. The use of DA MEC at rates of 0.01 – 0.1% significantly improved the effectiveness of low rates of Guthion (Table 4). Lower rates of DA MEC did not reduce fruit injury. Similar results in apple have been found in California, Italy, and Argentina. The effective rate of DA MEC in our studies was higher than the rate recommended by the manufacturer (0.004%). This recommended rate has been effective in walnut trials and ineffective in 'Bartlett' pear. Additional studies will continue to evaluate the use of 0.01 and 0.1% rates of DA MEC in combination with several insecticides, including Imidan and Assail. Studies will also evaluate the effect of these and lower rates in 'Granny Smith' where the pear ester is extremely attractive. Among pear cultivars the pear ester appears to be most attractive in 'Comice' and 'D'Anjou'. Studies in pear will examine the influence of rate on the effectiveness of DA MEC in these important cultivars.

Attractiveness of DA MEC to neonate codling moth. Extremely dilute concentrations of DA MEC were found to be attractive to codling moth larvae in laboratory tests with treated filter paper (Tables 5 and 6). However, only the highest rates were attractive for at least 7 days. These studies are consistent with our field trials. Our studies suggest that the use of DA MEC may enhance larval

wandering prior to attacking fruit and increase their exposure to insecticide residues. Studies are in progress to examine the attractiveness of 14 and 21 d residues in similar tests. Tests this spring will also evaluate the attractiveness of the DA MEC when applied on the surface of leaves of the major apple and pear cultivars. Improving the performance of insecticides with the addition of DA MEC has tremendous value for growers battling both the development of resistance and the reduced effectiveness of new compounds.

Mating disruption using pear ester. Combo 'puzzle-piece' dispensers loaded with pheromone and pear ester were very effective in reducing fruit injury from codling moth (Table 7). High levels of fruit injury occurred in the upwind, untreated block. The highest levels of fruit injury in the treated orchard occurred along the edge of the Isomate C tt-treated block. No injury occurred in the blocks treated with the pheromone/pear ester combo dispenser. This new dispenser will be more widely evaluated during 2006. Lower rates of dispensers per acre will be evaluated. Sex pheromone-based mating disruption continues to be a weak technology. Recent studies have shown that antennal receptors in codling moth for sex pheromone and pear ester interact and a blend of these materials may be a more effective disruptant. Broader demonstration of the effectiveness of this new dispenser could have a major impact on codling moth management.

Killing stations baited with pear ester. Fruit injury from codling moth was significantly reduced in pheromone-treated orchards supplemented with AKISS versus untreated blocks (Table 8). Similar positive results were found in Washington studies during 2005 in 'Red Delicious'. A new AKISS design will be more widely tested in 'Granny Smith' in WA during 2006. The use of AKISS in backyard trees, along orchard borders adjacent to bin piles, and to treat entire blocks will be evaluated.

Table 1. Mean (SE) distance (cm) of codling moth eggs to nearest fruit on apple trees sprayed with DA MEC.

Date sprayed	Date assessed	Concentration of DA MEC sprayed					ANOVA
		0.0	0.001%	0.01%	0.1%	1.0%	*P*-value
19 May	**8 June**	**5.1 (1.0)**	**6.4 (0.7)**	**4.4 (1.2)**	**-**	**-**	**0.43**
9 June	**17 June**	**12.3 (5.7)**	**4.1 (1.1)**	**5.9 (1.4)**	**-**	**-**	**0.09**
18 June	**27 June**	**7.5 (2.2)**	**4.4 (1.4)**	**9.1 (1.5)**	**-**	**-**	**0.16**
27 July	**2 August**	**6.1 (1.2)**	**-**	**-**	**3.3 (0.9)**	**3.8 (1.6)**	**0.20**

Table 2. Mean (SE) distance of codling moth eggs to the nearest fruit on four cultivars of apple sprayed with DA MEC.

Apple cultivar	Concentration of DA MEC sprayed		
	0.0	0.001%	0.01%
Red Delicious	**10.8 (3.1)**	**5.6 (1.7)**	**6.6 (1.3)**
Golden Delicious	**3.9 (1.3)**	**3.8 (1.0)**	**5.4 (1.7)**
Gala	**5.4 (1.1)**	**4.0 (0.6)**	**8.5 (1.0)**
Fuji	**4.1 (2.1)**	**6.4 (2.9)**	**4.3 (1.2)**
ANOVA:	Cultivar: $P = 0.22$	Conc: $P = 0.68$	Interaction: $P = 0.35$

Table 3. Density of codling moth eggs per fruit cluster on clusters treated and untreated with several concentrations of DA MEC.

Date sprayed	Date assessed	No. clusters sprayed per treatment	Concentration of DA MEC sprayed			
			0.0	0.001%	0.1%	1.0%
12 May	**2 June**	**96**	**0.10a**	**0.09a**	**0.13a**	**-**
3 June	**10 June**	**20**	**0.10a**	**0.05a**	**-**	**0.30b**
16 June	**24 June**	**40**	**0.03a**	**-**	**-**	**0.10b**
29 June	**7 July**	**32**	**0.03a**	**-**	**-**	**0.09b**

Row means followed by a different letter were significantly different LSD test, $P < 0.05$. All eggs were counted after the first application, and only unhatched eggs were counted after the three later applications**.**

Table 4. Percent fruit injury from codling moth in three studies evaluating the use of various rates of DAMEC to supplement Guthion.

	Research farm 1	Research farm 2	Leased orchard
Treatment [a]	5/27, 6/15	6/10, 6/29, 7/20, 8/16	6/9, 6/29, 7/20, 8/16
Untreated	24.9a	58.4a	89.1a
Guthion only	7.3b	7.8b	26.1b
+ 0.001% DA MEC	2.8bc	7.6b	17.4c
+ 0.004% DA MEC	5.9b	7.9b	13.3cd
+ 0.01% DA MEC	3.3c	4.9c	9.6d
+ 0.1% DA MEC	0.7d	4.2c	11.2d
ANOVA:	$F = 6.60$; df = 5, 24; $P < 0.001$	$F = 175.7$; df = 5, 62; $P < 0.0001$	$F = 153.0$; df = 5, 66, $P < 0.0001$

Column means followed by a different letter were significantly different, LSD test, $P < 0.05$.
[a] The research farm and leased orchard were treated with 0.5 and 1.0 lb Guthion, respectively. Treatments were single-tree plots replicated 4, 16, and 12 times in the three tests, respectively. The research farm 1 orchard was a 'Fuji' block, the leased orchard was 'Braeburn', and the research farm 2 orchard consisted of four cultivars ('Red Delicious', 'Golden Delicious', 'Gala', and 'Fuji'). The effect of cultivar in this trial was not significant, $P = 0.36$.

Table 5. Mean (SE) proportion of neonates attracted to DA MEC applied to a filter paper strip and aged for 4 to 168 h at 25 °C.

% concentration of DA MEC	Age of residue prior to testing		
	4 h	72 h	168 h
0.00001	0.31 (0.05)*	0.26 (0.05)*	0.20 (0.04)
0.0001	0.36 (0.04)*	0.36 (0.05)*	0.22 (0.05)
0.001	0.40 (0.06)*	0.20 (0.06)*	0.10 (0.05)
0.004	0.48 (0.04)*	0.22 (0.04)*	0.08 (0.04)
0.01	0.44 (0.04)*	0.28 (0.04)*	0.18 (0.04)
0.1	0.46 0.06)*	0.46 (0.06)*	0.20 (0.04) *
1.0	0.58 (0.05)*	0.38 (0.05)*	0.30 (0.04)*

'*' denotes a significant response to the DA MEC versus the untreated strip in choice tests. Five larvae were placed equidistance from a treated and untreated strip. Larvae were observed for three minutes.

Table 6. Mean (SE) time (s) spent by neonate CM larva on filter paper strip treated with DA MEC.

% conc. of DA MEC	Age of residue prior to testing		
	4 h	72 h	168 h
0.0	6.4 (0.4)c	7.1 (0.6)d	6.6 (0.5)d
0.00001	17.1 (3.2)c	9.2 (1.5)cd	7.6 (0.7)cd
0.0001	15.6 (2.1)c	20.3 (3.2)cd	14.1 (2.2)bc
0.001	21.2 (3.7)bc	16.3 (3.5)cd	6.7 (2.7)cd
0.004	15.0 (1.6)c	15.5 (3.4)cd	8.3 (2.7)bcd
0.01	18.0 (2.6)c	25.4 (5.6)bc	12.0 (3.0)bcd
0.1	40.0 (7.4)a	45.7 (9.7)a	17.8 (2.5)b
1.0	35.4 (7.5)ab	38.8 (6.1)ab	25.5 (4.7)a
ANOVA:	$F = 4.42$; df = 7, 181; $P < 0.0001$	$F = 6.37$; df = 7, 123; $P < 0.0001$	$F = 8.15$; df = 7, 64; $P < 0.0001$

Column means followed by a different letter were significantly different, LSD test, $P < 0.05$.

Table 7. The mean percentage of fruit injury by codling moth in two replicate 6.0-acre blocks of 'Granny Smith' treated with different dispenser systems.

Dispenser tested	Border-only	Entire block
Isomate C tt	2.2%	0.3%
Cidetrack CM-PH	0.0%	0.1%
Cidetrack CM/DA Combo	-	0.0%
Untreated	-	88.0%

Table 8. Codling moth fruit injury in blocks of 'Granny Smith' treated with and without two applications of AKISS applied at 24 per acre, Thornton, CA.

Treatment	# replicates	Mean (SE) percent fruit injury
AKISS	7	0.29 (0.10)
Untreated	6	1.36 (0.41)
		ANOVA: $F = 10.1$; df = 1, 11; $P < 0.01$

Budget:

Project Title:	Direct control of codling moth with pear ester	
PI:	Alan Knight	
Project duration:	2005-2006.	
Current year:	2006	
Project total (2 years):	$24,000	
Current year request:	$12,000	
Item	**Year 1 (2005)**	**Year 2 (2006)**
Wages	$8,200	$8,200
Benefits 16%	$1,300	$1,300
Supplies	$2,200	$1,700
Travel (local)	$800	$800
Total	$12,500	$12,000

Funds are not requested for the continued studies on both the use of pear ester for mating disruption and lure and kill. These projects will be supported from base funds and carryover funds from RAMP.

CONTINUING PROJECT REPORT **YEAR 1/3**
WTFRC Project # AE-05-502 Organization Project # 5853525758

PROJECT TITLE: Codling moth granulovirus transmission and autodissemination.

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Cooperators: Alan Knight, USDA-ARS, Wapato, WA;
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ORIGINAL OBJECTIVES:

1. Determine the mode of acquisition of virus (where it is most likely picked up)
2. Determine if acquisition of virus from leaf surfaces result in earlier infection and reduction of stings
3. Determine if virus acquisition can be amplified with phagostimulants or modified by combining with attractants (*e.g.* pear ester)
4. Investigate the potential for autodissemination of virus by codling moth adults including methods for contaminating adults by combining attractants with virus formulations
5. Determine the effect of trap design on potential for contaminating moths with virus in laboratory and field studies.

Revised title: Improving codling moth granulovirus transmission and activity
Revised Objectives:

1. Continue study of attractants (pear ester) and feeding stimulants to improve uptake of virus
2. Substitute continued work on autodissemination with additional work on UV protection of CpGV.

Justification for revision of title and objectives: Findings in Germany reveal the development of tolerance to CpGV in areas where very low dosages of virus have been used for several years (Fritsch et al. 2005). CpGV infections are fairly common in large scale rearing of codling moth where larvae and virus co-exist (Cossentine et al., 2005). These interactions of virus and codling moth populations indicate a selection for virus that allows survival of both codling moth and virus. Under these conditions the virus shows signs of attenuation (Lacey, unpublished observations). Autodissemination of minute amounts of virus, particularly the strain being used in commercial formulations could result in development of tolerance or resistance to the virus. It is our recommendation, therefore, to use virus applications that will result in the highest levels of codling moth larval mortality.

Solar inactivation is the major reason why CpGV must be reapplied at fairly short intervals. Continued work on UV-protection (lignin) encapsulation will address improvement of persistence under orchard conditions.

Significant findings:

- Virus can be transmitted to larvae from treated virus-contaminated eggs, foliage and fruit.
- Several feeding stimulants were evaluated in laboratory and field studies, but none provided more than marginal improvement in CpGV activity.
- Laboratory and field studies with CpGV combined with pear ester indicated that some significant improvement in fruit protection is possible when lower dosages of virus are used.

- Results of autodissemination studies indicate that use of attractants and Exosect wax powder can facilitate contamination of moths with pathogens. Contaminated moths can then spread inoculum to moths of the opposite sex.

Source of virus uptake

Methods. A laboratory study was conducted to quantify different routes of virus uptake for neonate larvae i.e. effect of virus location on likelihood of acquisition. For brevity sake, the methods have been reduced considerably. 4 experimental groups were sprayed with virus (eq. of 1 oz/ac): eggs, leaves, fruit and untreated controls (8 of each). groups sprayed as eggs or leaves were provided fruit; mortality was assessed 10 days later.

Result and discussion

While the conventional wisdom regarding the transmission of codling moth granulovirus is that neonate larvae ingest virus as they make their initial entry into apples that have been sprayed with virus, Table 1 shows that all three sources (eggs, foliage and fruit) contribute independently to larvae infection. However the relative contribution was different, e.g. treated fruit contributed approximately twice as much mortality as treated eggs or foliage. This is important and suggests that efforts to improve virus uptake in the field should focus on all three components. Further studies are needed to confirm this trend and also consider the contribution that adjuvants may have on multiple sources of infection.

Table 1. Effect of source of virus on host location and mortality of neonate codling moth; eggs, leaves or fruit were sprayed with Cyd-X (1 oz/A).

Treated surface	N	% reaching fruit	% mortality (in fruit)
Control	160	21.3	0.0
Eggs	162	22.8	24.3
Leaf	180	13.3	29.2
Fruit	120	14.2	52.9

Use of feeding stimulants.

We evaluated potential feeding stimulants and the pear ester kairomone (E,Z)-2,4-decadienoate to improve virus uptake and efficacy of a commercial preparation of CpGV (Cyd-X) in tests with apple, *Malus domestica* Borkh using leaf dip bioassays, greenhouse field simulations and season long field trials.

In leaf dip bioassays, monosodium glutamate (MSG) at 0.1 mg/ml, L-aspartic acid at 1 mg/ml and pear ester at 10^{-5} vol/vol dilution failed to increase mortality of neonates exposed for 2 hours when compared to virus-only controls. No significant benefits were observed in further tests with a 24 hr exposure period and lower virus concentration, when two commercial products (Pheast and BioEnhancer) were also included at manufacturers recommended rates (Fig. 1 & 2).

In greenhouse studies, pear ester was tested at three rates (1, 10 and 100 ppm) on Delicious branches sprayed with Cyd-X (73 ml/ha) and infested with neonate larvae. No consistent effect of pear ester on host location or larval mortality in fruit was observed from fruit attached to branches and sampled 2 and 10 days post infestation (Fig. 3).

In field tests, pear ester (73 ml/ha) and a phagostimulant treatment were included with weekly applications of Cyd-X applied against natural infestations. No differences were observed after the first larval generation (Fig. 4), but fruit injury was significantly lower in the pear ester/virus combination (39.5%) compared with virus or pear ester alone (48-53%) after the larger second generation (Fig. 5), although overwintering larvae recovered in tree bands were not reduced (Table 2). There were no significant benefits of Pheast (2.34 kg/ha) or BioEnhancer (1.17 l/ha) in tests against

the first and second generation respectively (Fig. 4 & 5). Despite these initial findings, the potential for additives to improve CpGV formulations warrants further attention. Higher rates of pear ester encapsulated formulation with be combined with virus in 2006 and evaluated under orchard conditions.

Figure 1. The effect of formulation additives on mortality of codling moth larvae following 2 hour exposure to leaf discs treated with a 10^{-6} dilution of Cyd-X. Data show mean ± SEM for five replicate tests, 20 larvae per test. Letters indicate Fisher's LSD at $P < 0.05$.

Fig.1

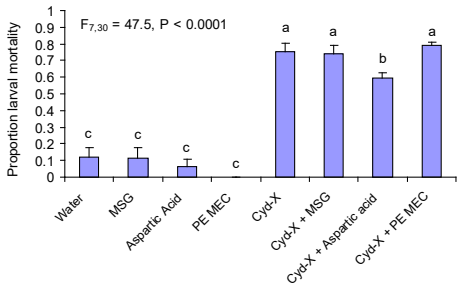


Fig. 2

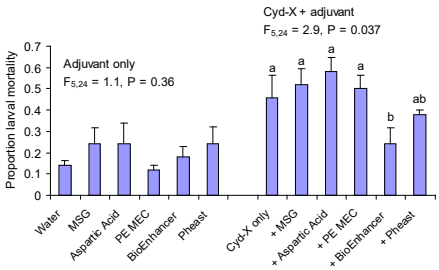


Figure 2. The effect of formulation additives on mortality of codling moth larvae following 24 hour exposure to leaf discs treated with a 5×10^{-8} dilution of Cyd-X. Data show mean ± SEM for five replicate tests, 20 larvae per test. Letters indicate Fisher's LSD at $P < 0.05$.

Figure 3. Greenhouse tests showing effect of pear ester (PE) on host location by neonate codling moth on apple (Delicious) branches sprayed with Cyd-X at a rate of 73 ml/ha. Fruit were sampled 2 and 10 days post infestation, when larval mortality was also assessed. Data show mean ± SEM for tests against (A) laboratory and (B) field strain. Letters indicate Fisher's LSD at $P < 0.05$.

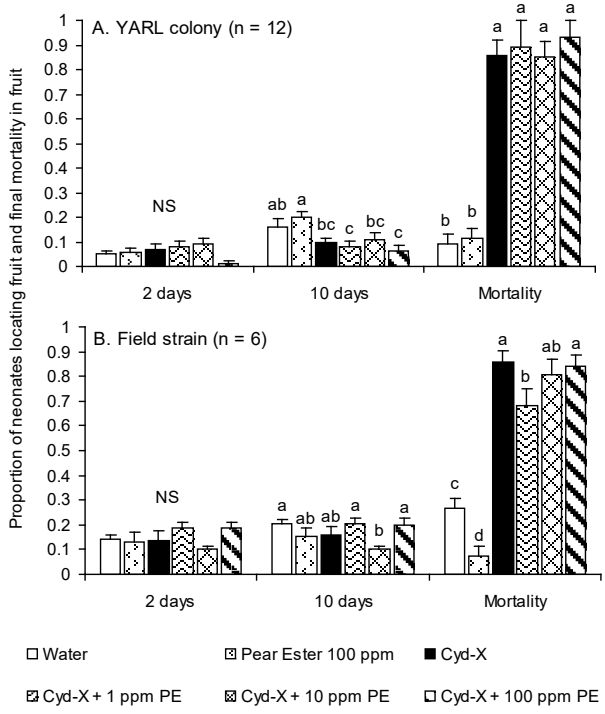


Figure 4. Field tests against first generation codling moth. Formulation additives were pear ester (PE) and phagostimulant (Pheast) (included with seven weekly applications of Cyd-X at 0.22 liter ha^{-1}). Data show (A) proportion fruit injury, (B) # injuries per infested fruit, (C) proportion of deep entries and (D) larval mortality. Bars show average ± SEM for 10 trees and letters indicate Fisher's LSD at $P < 0.05$.

Fig.4 Fig. 5

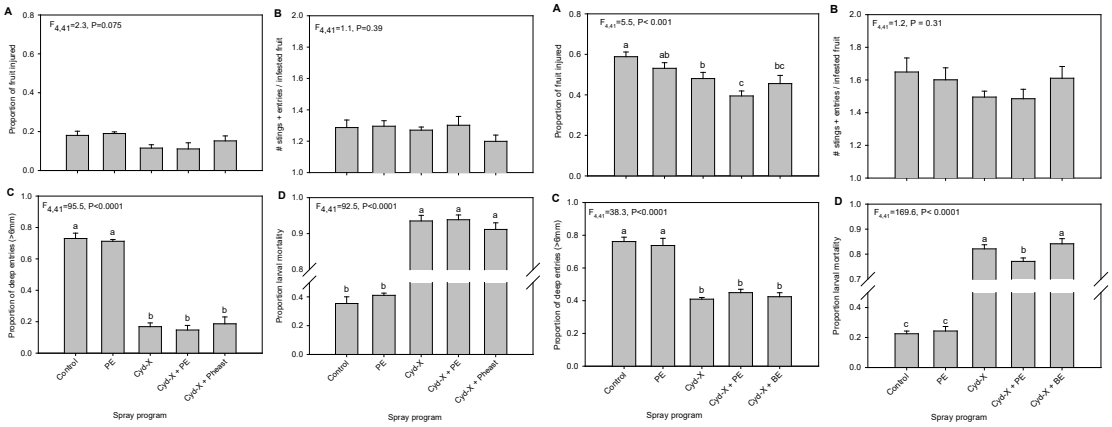


Figure 5. Field trials against second generation codling moth. Formulation additives were pear ester (PE) and phagostimulant (BioEnhancer) (included with six weekly applications of Cyd-X at 0.073 liter ha^{-1}). Data show (A) proportion fruit injury, (B) # injuries per infested fruit, (C) proportion entries > 6mm and (D) larval mortality. Bars show average ± SEM for 10 trees and letters indicate Fisher's LSD at $P < 0.05$.

Table 2. Number of diapause-destined codling moth larvae recovered from bands in Golden Delicious trees treated with granulovirus formulations

Treatment	N	Mean ± SEM[1]
Control	8	95.3 ± 4.5a
Pear Ester only	8	99.3 ± 7.3a
Cyd-X only	10	33.4 ± 3.0b
Cyd-X + Pear Ester	10	34.4 ± 3.8b
Cyd-X + BioEnhancer	10	31.5 ± 4.4b

[1]Data include any additional live larvae removed during orchard assessments. Letters indicate differences at P < 0.05 (Fisher's LSD).

Contaminating adults moths by combining pathogens with powders (carriers) used for autodissemination of virus formulations. A laboratory study was conducted to determine if the incorporation of a pathogenic fungus into the powders used for the autodissemination of the virus would kill the adult moths; (i.e. virus dissemination source would also serve as an attract and infect type trap). Since CpGV does not kill adult insects the additional mortality caused by the fungus would be useful, for example, by preventing egg laying over an extended period while still allowing some of the virus to be disseminated into the environment for a limited period.

Materials and methods: The pathogen used in this study was an isolate of the entomopathogenic fungus *Beauveria bassiana* that was originally isolated from an overwintering codling moth larva. Two carriers (kaolin clay) and carnauba wax that was electro-statically charged.

Results

The results are summarized in Table 2. Contaminated moths died fairly quickly (average survival 3.2 – 4.6 days) and ≥ 87% of dead moths sporulated with *Beauveria*, even at low doses. There was also some sporulation among the controls which were not exposed to the fungus. This suggests there may have been some contamination, most likely in the room where samples were incubated. It was also apparent that the method of exposure (*i.e.* completely coated moths with a large amount of carrier made flight difficult) may have contributed to mortality independently of the pathogen. However, this preliminary test showed that carriers including kaolin and Entostat containing a pathogen such as *B. bassiana* have the potential to infect and kill moths in a relatively short time. However there were some problems with this initial study, and further studies using a more realistic method of exposure, such as exposing adults to traps in flight tunnel studies, would be useful.

Table 3. Mortality of codling moth adults (both sexes combined) exposed to spore suspensions of *B. bassiana* diluted in two carriers at different concentrations in glass vials

Carrier	Dosage (% a.i. w/w)	Average survival (days)	% sporulating
Entostat	20	4.0	100
	2	4.3	100
	0.2	4.5	100
	0.02	4.6	95
Kaolin	20	3.8	87
	2	3.8	100
	0.2	3.5	98
	0.02	3.2	100
Control	n/a	8.2	46

Potential for autodissemination of virus by codling moth adults by combining moth attractants and carriers containing virus. We were interested in the possibility of virus autodissemination into the orchard by attracting adult moths (using various lures) towards a source of virus (typically contained in a powdered carrier that readily adheres to the moths scales). Contaminated moths would then fly off and spread virus principally through (1) mating, (2) other moth-to-moth contact and (3) contamination of eggs.

Results and discussion (flight tunnel studies)

In our initial attempts we were unfortunately unable to get moths to respond (i.e. fly) under flight tunnel conditions and therefore did not complete our listed objectives. Further experience with this study system and modifications with air flow, position of attractants, lighting and acclimatization to reverse photoperiod are needed. In initial tests we obtained moths (both sexes < 48 hrs old) from the YARL colony and placed in reverse photoperiod conditions to allow studies to be conducted during work hours. To speed up the 'moth conditioning' process, one modification would be to place pupae directly under reverse-photoperiod conditions.

Field studies

The study was in the middle of a 21A commercial orchard near Zillah, WA (20 year old Delicious planted at 202 trees/A) that was heavily infested with codling moth. Five Exosect powder stations (containing Entostat with a florescent dye incorporated) were placed high in the canopy along a single row at 288 ft. intervals on 22 April as outlined (figure 1). A few days later ten monitoring stations (Pherocon® VI wing-type traps baited with 1 mg codlemone and DA pear ester lures) were placed at various distances from the nearest powder station. Moths captured on sticky cards in monitoring stations were removed from the orchard on 2, 9, 25, 31 May and 21 June. Powder stations were renewed with fresh powder (which blew out of stations during windy conditions) on 25 May.

Results and discussion (field studies)

Moths removed from the orchard were stored individually in 1ml of 95% ethanol in 1.5 ml microcentrifuge vials and analyzed by fluorometric analysis. The dye added to the Entostat powder can be detected at minute quantities with this method. The proportion of orchard-collected moths that had been exposed to the powder at the station or had been contaminated via exposure to contaminated foliage or other moths carrying the powder could thus be estimated. The results of an initial batch of moths that were collected from traps farthest away (54') showed that males had 0.21micrograms of powder on 50% of those captured while females had 0.033 micrograms of powder on 12% of those captured (Table 4). This is interesting as it suggests the powder is being transferred to both sexes during courtship, or from alighting on contaminated surfaces. No virus was included in the powder in the initial studies. Further studies would measure virus infection in the orchard at various distances from the dissemination traps.

Table 4. Analysis of coding moths captured in orchard containing Entostat powder

	Males	Females
Mean contamination (μg)	0.2	0.033
% contaminated	50	12

Budget:

Project title: Codling moth granulovirus transmission and autodissemination.

PI. Lawrence A. Lacey, USDA-ARS, Wapato, WA
Project duration: 2005-2007 (3 years)
Project total (3 years): $81,500
Current year request: 26,500

	year 1 (2005)	**year 2 (2006)**	year 3 (2007)
Salaries and wages (includes benefits)			
Salary, technician, partial support for GS-5	$20,000	$20,000	$20,000
wages, summer help, GS-3, 1 FTE (3 mos.)	5,000	5,000	5,000
chemicals, plasticware, misc. materials	1,500	1,500	3,500
Total	$26,500	$26,500	$28,500

[1] chemicals, plasticware, misc. materials

CONTINUING PROPOSAL **YEAR 1/3**
WTFRC Project #AE-05-504 **WSU Project #13C-3643-3190**

Project title: Management of leafrollers in apple orchards
PI: Jay F. Brunner
Organization: WSU Tree Fruit Research and Extension Center
Address, phone, e-mail: 1100 N. Western Avenue, Wenatchee, WA 98801
(509) 663-8181 ext. 238; jfb@wsu.edu

Co-PIs and affiliation: John Dunley and Mike Doerr, WSU Tree Fruit Research and Extension Center
Contract administrators: Mary Lou Bricker (mdesros@wsu.edu, 509-335-7667; Sally Ray (saray@wsu.edu, 509-663-8181 x221)

Objectives:

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

Significant findings:

1. Based on a diet incorporation study, we found that two IGR products affect OBLR in very different ways. There was a clear concentration of OBLR larvae (third-fourth instars) to novaluron (Rimon). All concentrations above 1 ppm prevented the successful completion of development to the pupal stage. At 0.3 and 1.0 ppm, 22 and 44% of larvae, respectively, completed development to the pupal stage, but those that pupated did so at roughly the same time following exposure as the untreated control (between 7-14 days); that is; there was no observed delay in development. OLBR larvae exposed to pyriproxyfen (Esteem) showed a concentration-based response. However, development to the pupal stage was delayed in relation to increasing concentration. For example, all pupae were produced in the check by 28 days after the beginning of the study, but at 1 ppm pyriproxyfen it took 70 days before all pupae were produced. No pupae were produced in pyriproxyfen concentrations above 10 ppm, and only 2% were produced at this concentration.
2. OBLR larvae exposed to a low concentration of pyriproxyfen (0.3 ppm) for different periods of time showed a delay in development that was of the same duration as the time exposed to the treated diet. There was only about 15% mortality based on numbers reaching the pupal stage. The study shows that young larvae, third-fourth instars, that escape pyriproxyfen residues (move to new shoots) can successfully develop to the pupal stage. This shows the importance of proper timing with this product; that is, it must be timed against the last-stage larvae just prior to pupation.
3. OBLR larvae (third-fourth instars) fed different doses of pyriproxyfen showed a delay in development mortality that was directly related to the dose. A single dose of 1.0 μg reduced successful pupation by about 30% but successful adult emergence by more than 60%. Thus, exposure to low doses of pyriproxyfen affects development rate, pupation rate and adult emergence.
4. Baseline data for susceptibility to novaluron were established for neonate OBLR larvae. The LC_{50} values for 7- and 14-day assessments were 27.2 and 5.8 ppm, respectively, and were statistically different. Baseline data for susceptibility to novaluron were established for neonate PLR larvae.

The LC_{50} values for 7- and 14-day assessments were 358.6 and 38.9 ppm, respectively, and were statistically different. OBLR appear to be inherently more susceptible to novaluron than PLR. For example, estimated percent mortality of PLR larvae at the dilute field concentration of novaluron is 28.4% after 7 days, while it is 68.0% for OBLR larvae.

5. A survey of OBLR populations from three sites showed significant levels of resistance to azinphosmethyl (Guthion) and methoxyfenozide (Intrepid). Two of the three sites showed significant resistance levels to spinosad (Success, Entrust) though the levels of resistance were only two- to threefold higher than the susceptible colony. The three field populations showed no resistance to two new insecticides soon to be registered, emamectin benzoate (Proclaim, Syngenta – 2006) and Exp A (2008-2009).
6. In three field trials spinosad (Success), an experimental insecticide (Exp A), and emamectin benzoate (Proclaim) provided good control of OBLR larvae in the spring. Results with novaluron were more variable, in part due to the slow-acting nature of this product. All products tested would be expected to provide good control of OBLR under most field situations.

Methods:

Diet incorporation bioassay **-** The effect of adding difluorobenzamide (Diamond 7.5WG, Crompton Corp.) and pyriproxyfen (Esteem 35WP, Valent USA Corp.) to artificial pinto bean leafroller diet (Shorey and Hale 1965) was evaluated for its effect on third- to fourth-instar OBLR larvae (source, TFREC colony). Five third- to fourth-instar OBLR larvae were placed in the treated diet cups. Ten cups were used for each treatment (50 larvae/treatment). The bioassay was evaluated every 7 days until all OBLR larvae emerged as adults or died at some earlier stage. OBLR mortality, pupal formation and number of emerged adults were recorded at each evaluation date. In a second experiment, OBLR larvae were treated as described above but only one concentration was used, 3.0 ppm; and instead of larvae being left on the treated diet throughout the duration of the study they were removed after exposure at different intervals, 0, 1, 7, and 21 days. One group of larvae served as a positive control and was left on the treated diet for the entire duration of the study, 63 days. OBLR larval mortality, pupal and adult formations were noted as above. Statistical analysis was as described above.

Leaf disk bioassay **-** A measured dose of pyriproxyfen (Esteem 35WP, Valent USA Corp.) was evaluated for its effect on third- to fourth-instar OBLR larvae. The dose was administered by leaf disk. OBLR larvae (source, TFREC colony) were starved for 24 hours prior to onset of the bioassay. A series of concentrations (1000, 300, 100 ppm) of pyriproxyfen was prepared by diluting the appropriate amount of insecticide in 1 liter of water. A 0.1 ml drop from the dilutions was dried on a small leaf disk (8.5 mm diameter). This resulted in a known dose of 1.0, 0.3 and 0.1 µg per disk. A leaf disk was presented to individual larvae after their starvation period. Only those larvae that consumed the entire leaf disk were used in this bioassay. After consuming the entire leaf disk, larvae were transferred to an untreated artificial pinto bean diet (Shorey and Hale, 1965). Five larvae were treated at each dose, and the entire test was replicated five times (25 larvae/dose). The bioassay was evaluated every 7 days until all OBLR larvae emerged as adults or died at some earlier stage (42 days). OBLR mortality, pupal formation and emerged adults were recorded at each evaluation date.

Novaluron bioassay **-** The effect of novaluron (Rimon 0.83EC, Chemtura Corp.) on OBLR and PLR neonate larvae was evaluated using a leaf disk bioassay. Treatments were prepared by diluting the appropriate amount of insecticide in 500 ml water in a glass beaker. Untreated apple leaves were collected from 'Delicious' trees at the WSU Tree Fruit Research and Extension Center, Wenatchee. Leaves were dipped into a series of dilutions and then allowed to dry. Two disks (2.3 cm diameter) were taken from each leaf. Four leaf disks were placed in a petri dish (Falcon 1006, 50x9 mm, and five 2- to 5-day-old OBLR or PLR larvae were placed on the leaf disks using a camel's-hair brush. Ten dishes were used for each treatment and each species (50 larvae/treatment). The bioassay was evaluated after 7 and 14 days and larval mortality recorded. Mean mortality data were corrected by Abbott's formula (Abbott 1925), then analyzed using a one-way analysis of variance. Mean

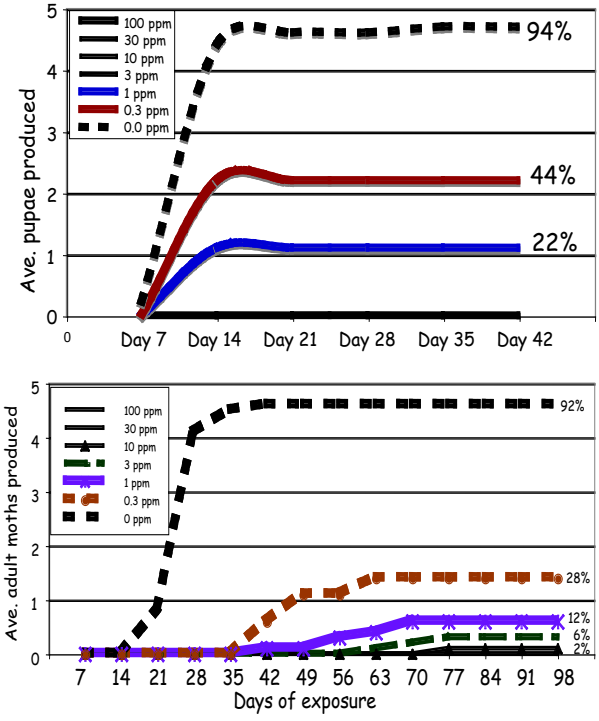
separations were determined by Student's *t* test (P=0.05) using JMP statistical software (JMP IN v. 5.1.2, SAS Institute Inc., 2004). Probit regression lines and LC_{50} values were estimated using the probit option of POLO-PC (LeOra Software 1987). Probit models from each bioassay were then used to calculate toxicity indices relative to field use rates. A relative toxicity ratio LC_{50}: MFR (maximum field rate, the highest labeled concentration for use on apples, assuming a dilute spray) was presented. Expected mortality was calculated using each probit model at 1× and 0.1× MFR (maximum field rate, the highest labeled concentration for use on apples, assuming a dilute spray). A comparison of lethal concentrations was calculated according to Robertson and Priesler (Lethal Ratio Significance Test, 1992).

Survey of field-collected populations - In 2005, populations of OBLR larvae were collected from orchards and assessed for their susceptibility to azinphosmethyl (Guthion 50WP, Bayer CropScience), spinosad (Success 2SC, Dow AgroSciences), emamectin benzoate (Proclaim 5WG, Syngenta Crop Protection, Inc.), methoxyfenozide (Intrepid 2F, Dow AgroSciences) and an experimental insecticide (Exp A) using the leaf disk bioassay technique. Three OBLR populations were collected from commercial orchards that were experiencing high OBLR densities despite intensive spray programs targeting leafroller. The field populations were collected as third- to fifth-instar larvae from the overwintered generation. Larvae were reared following the methods of Shorey and Hale (1965). For field-collected populations, neonates of the F_2 generation were assayed. Bioassays were not conducted on the F_1 generation because we felt it was necessary to rear through an additional generation to ensure sufficient individuals for a complete series of dose-mortality bioassays. Treatments were prepared and data collected and analyzed as described above for the leaf disk bioassay. In this test expected mortality was calculated using each probit model at 100% and 10% of the maximum field use rate for each insecticide, assuming a dilute spray of 400 gal/acre.

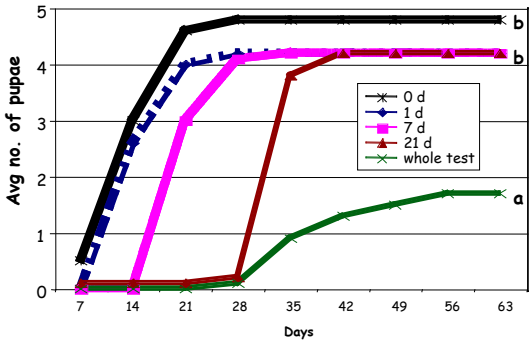
Field trials - Three field tests were conducted evaluating the efficacy of spinosad, novaluron and emamectin benzoate against overwintering larvae. Treatments were applied by handgun sprayer to replicated multiple-tree plots. Assessment was made by counting live and dead larvae.

Results and discussion:

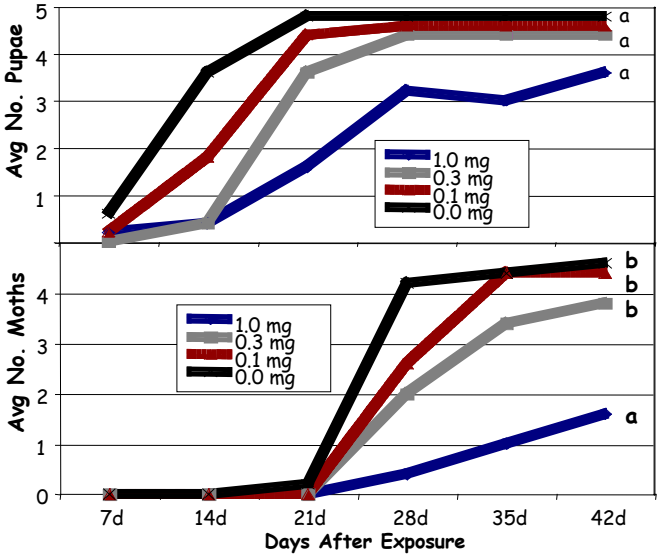
IGR and leafroller development - A statistically significant concentration-based response with novaluron (Rimon) was noted in the mortality evaluation at each sample date. All treatments except 0.3 ppm caused statistically significant mortality of OBLR larvae after 7 days relative to the untreated control. The 3.0-ppm rate and higher caused a high level of mortality at 14 days, with no difference among those treatments. All novaluron treatments at rates higher than 1.0 ppm prevented OBLR survival to the pupal stage. The lower rates produced significantly fewer pupae than the untreated control. No adults emerged from pupae exposed to the 1.0-ppm treatment, and only 18.2% of adults emerged from pupae produced by larvae exposed to the 0.3-ppm treatment (Fig. 1). Novaluron is highly toxic to OBLR larvae, and where larvae survived exposure to lower concentrations very few adults emerged. We do not know if any of these adults could reproduce, but it is probable that they would be sterile.



OBLR larvae required constant exposure to pyriproxyfen (Esteem) for 35 days before significant mortality was noted in any treatment (0.3 ppm). It was not until 42 days of exposure that mortality was noted in most treatments. Very few adult moths emerged from larvae exposed to concentrations of pyriproxyfen greater than 0.3 ppm (Fig. 2). Pyriproxyfen acted to delay the development of OBLR larvae by at least 21 days compared to the untreated control. In addition, OBLR larvae completed extra molts, becoming much larger than those in the untreated control.



In the second experiment, a significant exposure-time effect was noted for the mortality (DF 4, SS 56.08, *F* 19.902, *P*=0.0001) and adult emergence (DF 4, SS 108.0, *F* 37.9, *P*=0.0001) measures. Only the Whole Test treatment resulted in significantly fewer pupae than the untreated control (DF 4, SS 58.8, *F* 32.31, *P*=0.0001). Pupal development was first noted in the untreated control at 7 days. Pupal development was delayed in all pyriproxyfen treatments. After removal of third- and fourth-instar larvae from the treated diet they began producing pupae within 7 days (Fig. 3). Almost all pupae produced by larvae removed from the treated diet produced moths. The only exception was the larvae left exposed to treated diet during the entire study period where an average of 1.8 pupae were produced compared to 4.7 in the 0 day treatment and only 0.7 (of 5 possible) adults were produced.



Leaf disk bioassay – Significant OBLR mortality was not noted in any pyriproxyfen treatments until day 42. At that time, only the 0.1-mg dose resulted in more mortality than the untreated control (DF3, SS 14.95, *F* 5.86, *P*=0.007). Pupal development was significantly delayed in all treatments at day 14. However, by day 42 no treatments resulted in a reduction in the number of pupae relative to the untreated control (DF 3, SS 4.15, *F* 2.12, *P*=0.14). Adult emergence was significantly delayed in all pyriproxyfen treatments relative to the untreated control at day 28. However, by day 42 only the 0.1-mg dose resulted in a significant reduction in adult emergence (DF 3, SS 28.4, *F* 9.23, *P*=0.001). Dosing larvae in the manner described above offers another potential bioassay method to compare efficacy against populations. Higher doses, possibly given later in larval development, may offer more contrasting results.

Novaluron bioassay - A statistically significant concentration-based response was noted with novaluron against OBLR at both 7 and 14 days. Probit analysis indicated a good model fit for both data sets. It was apparent that novaluron results in delayed mortality, as a significantly lower LC_{50} was noted after 14 days than at 7 days. At 7 days only 68.0% mortality would be expected, while at 14 days 96.2% mortality would be expected at the MFR. Since this test was evaluated for only 14 days it is not clear if further mortality would be noted with time or if sublethal effects from this insect

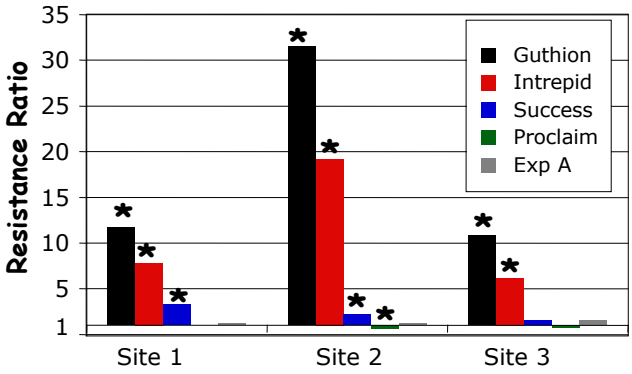
growth regulator would be important. However, it was apparent that, for this insecticide, the time to kill is delayed and thus must be taken into account when evaluating its efficacy in field trials.

A statistically significant concentration-based response was also noted with novaluron against PLR at both 7 and 14 days. Probit analysis indicated only a marginal model fit at 7 days, as the slope was rather flat. However, a good model fit was noted at 14 days. It was apparent that novaluron results in delayed mortality, as a significantly lower LC_{50} was noted after 14 days than at 7 days. At 7 days only 28.4% mortality would be expected at the MFR, while at 14 days 68.8% mortality was expected at the MFR. These data still suggest a significantly lower acute toxicity of novaluron to PLR compared to OBLR.

	Pandemis leafroller		Obliquebanded leafroller	
	7 day	14 days	7 day	14 days
Year	2005	2005	2005	2005
n	350	350	350	350
Slope (SE)	0.8 (0.2)	1.4 (0.2)	1.1 (0.2)	1.6 (0.3)
Intercept (SE)	-2.1 (0.4)	-2.3 (0.5)	1.1 (0.2)	1.6 (0.3)
LC_{50} (95% CI)	358.6 (155.2-1852.5)b	38.9 (21.0-61.1)a	27.2 (14.9-44.1)b	5.8 (2.4-9.7)a
Max Field Rate (ppm)	75.0	75.0	75.0	75.0
Ratio LC_{50}: MFR	4.773	0.519	0.363	0.077
% Mort at 1.0 MFR	28.4	68.8	68.0	96.2
% Mort at 0.1 MFR	7.9	15.4	27.6	57.3

n = number of larvae in bioassay.
MFR = maximum field rate concentration on apples assuming dilute application.
Lethal concentrations followed by the same letter are not significantly different (P=0.05, Lethal Ratio Significance Test, Robertson and Priesler, 1992).

Survey of field-collected populations - All three populations of OBLR showed high levels of resistance to azinphosmethyl (Guthion), 10- to 31-fold, and were resistant to methoxyfenozide (Intrepid), 6- to 19-fold (Fig. 4). This pattern has been observed in the past and reflects the cross-resistance between organophosphate insecticides and methoxyfenozide reported by Dunley et al. (2005). Two of the three sites showed resistance to spinosad (Success) but the level of resistance was low. Two insecticides yet to be registered showed no resistance in the three field populations. This is encouraging since it indicates that these products would not suffer from cross-resistance to OP insecticides.



Field trials - In the first test an experimental insecticide provided control of OBLR larvae at three rates comparable to that of spinosad. In the second test novaluron provided good control of OBLR larvae at the low rate, but live larvae were still found at the high rate at the 14-day evaluation. In the third test emamectin benzoate provided excellent control of OBLR larvae, similar to spinosad. In this test novaluron did not provide good suppression of OBLR larvae, but the evaluation was done at 7 days and likely did not allow enough time to capture the full effect of this product. Two products that appear to be very good prospects for leafroller control could be registered in the relatively near future. This is good news given the slight increase in tolerance that some populations of OBLR have shown to spinosad as well as methoxyfenozide.

Budget:

Project title: Management of leafrollers in apple orchards
PI: Jay F. Brunner
Project duration: Three years (2005-2007)
Current year: 2006
Project total (3 years): $80,442
Current year request: $26,922

Year	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Total	$25,898	**$26,922**	$27,622

Current year breakdown

Item	Year 1 (2005)	**Year 2 (2006)**	Year 3 (2007)
Salaries (0.5 GRA); (AR-0.25); (AP-0.083)[1]	0 (GRA) 7,048 (AR) 4,603 (AP)	**0 (GRA)** **7,401 (AR)** **4,817 (AP)**	0 (GRA) 7,697 (AR) 5,010 (AP)
Benefits (0.5 GRA); (AR-49%); (AP-34%)[1]	0 (GRA) 3,242 (AR) 1,565 (AP)	**0 (GRA)** **3,626 (AR)** **1,638 (AP)**	0 (GRA) 3,772 (AR) 1,703 (AP)
Wages [2]	5,400	**5,400**	5,400
Benefits (10%)	540	**540**	540
Equipment	0	**0**	0
Supplies [3]	1,500	**1,500**	1,500
Travel [4]	2,000	**2,000**	2,000
Miscellaneous	0	**0**	0
Total	25,898	**26,922**	27,622

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

[2] Summer labor to assist with rearing of leafrollers - $9/hour for 600 hours.

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

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

NOTE: A proposal was funded by the Washington Commission on Pesticide Registration in the amount of $24,605 per year, primarily to support a Ph.D. graduate student. This information is provided to the Commission for informational purposes only. This information is not presented as formal cost sharing and therefore does not constitute a cost-sharing obligation on the part of Washington State University. Moreover, there is no requirement for WSU to document this information as part of any cost-share or matching obligation.

CONTINUING PROJECT REPORT **Year 1/2**
WTFRC Project # AE-05-505

Project Title: Distribution of flower thrips eggs in apple blossoms
PI: David Horton
Organization: USDA-ARS, Yakima Agric. Research Lab., Wapato, WA
509-454-5639
Horton@yarl.ars.usda.gov

Co-PI: Gene Miliczky
USDA-ARS, Yakima Agric. Research Lab., Wapato, WA

Cooperators: Elizabeth Beers, WSU, Wenatchee
Steve Cockfield, WSU, Wenatchee

Contract Admin.: Janet Tsukahira, jtsukahira@pw.ars.usda.gov, (510) 559-6019

OBJECTIVES:

Describe egglaying preferences of western flower thrips among non-damaging and potentially damaging areas within the flower/fruitlet cluster in apples. Assess whether preference changes with apple phenology.

SIGNIFICANT FINDINGS:

- Developed new methods to survey tissues for presence of thrips eggs in apple tissues that avoid difficulties in earlier published methods
- Showed that adult thrips numbers in blossoms peaked at full bloom. Egg and nymphal counts lagged adult phenology
- Showed that adults were absent or at very low densities at pink, petal fall, and post-petal fall
- Showed that almost no eggs were deposited in damaging areas before petal fall. The calyx was highly preferred until well after petal fall
- As the calyx dried following petal fall, there appears to have been a shift of egglaying to the stem and developing fruitlet, but because adults had mostly disappeared from the orchard at this time, only very few damaging eggs were actually found
- To confirm this shift in egglaying preferences, we introduced egglaying adults onto apple clippings in the laboratory. The developing fruitlet became highly preferred as the fruitlet reached the 15-25 mm stage

METHODS

Sampling methods for adults and nymphs followed techniques developed earlier by Miliczky (swishing of clusters in soapy water). Assessment of egg densities is being done using methods developed in Horton's laboratory in 2005. Briefly, the tissues are immersed in a warmed solution of white distilled vinegar and blue food coloring. The vinegar and warming allows us to easily tease away the top layer of the plant tissues, exposing the (bluish-colored) oviposition scar and associated egg. Samples are taken at pink, king bloom, full bloom, petal fall, and 15-25 mm fruitlet. Clippings having cut ends placed in water are infested with adult thrips in the laboratory to obtain egglaying at those phenological stages in which adult thrips are absent from clusters in the field.

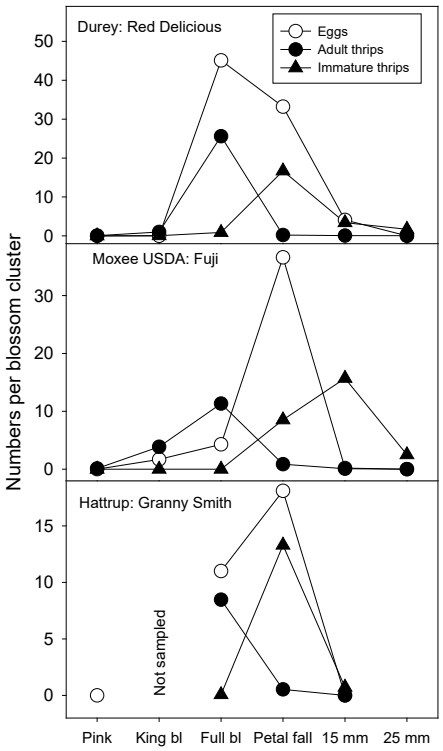
New 2006. We obtained potted Granny Smith trees (donated by Van Well Nursery), and will artificially infest blossoms at different timings to assess damage. Trees will be fumigated following

known intervals of exposure to adult thrips, to kill ovipositing thrips. Fruitlets will be allowed to develop, and assessed for damage.

RESULTS AND DISCUSSION

Figure 1 shows densities of adults, nymphs, and eggs of flower thrips at the three orchards that were monitored. Egg and nymphal population curves trail adult curves, as is expected. Peak egg numbers occurred at full bloom and petal fall. Adults disappeared from clusters at petal fall.

Figure 1. Numbers of thrips per blossom cluster at each of three orchards



Egg distribution among tissues (combined across orchards) is summarized in Table 1. No or few eggs were found at pink, king, and 15-25 mm fruitlet (as shown also in Figure 1), due to low densities of adult thrips at these times. The calyx was heavily preferred until after petal fall. Eggs deposited in potentially damaging areas were first obtained at full bloom (0.4% of the total eggs deposited), peaking at petal fall when 3.4% of the eggs had been deposited in potentially damaging areas.

Because few eggs were present in field material at pink, king, and the 15-25 mm stage, preference could not be reliably assessed. We artificially infested clippings in the laboratory to assess egglaying preferences during these phenological stages (Table 2). At pink and king stages, we obtained large numbers of eggs. None were deposited in potentially damaging areas, suggesting that even if adults happened to be present in the orchard at those times, eggs would not to be deposited in damaging areas. However, at the 15-25 mm stage, a very high percentage (67.6) of the 71 eggs that we found had been deposited directly on the developing fruitlet. Thus, if egglaying adults happen to be present in clusters at this phenological stage, there appears to be considerable potential for damage.

Table 1. Distribution of thrips eggs on field-collected material. Orchards combined.

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	0	--	--	--	--	--
King bloom	16	0	74.2	19.4	6.4	0
Full bloom	286	0.8	84.0	13.0	1.8	0.4
Petal fall	513	4.4	67.2	20.4	4.6	3.4
15-25 mm	31	0	8.5	90.0	0	1.5

*** Potentially damaging eggs

Table 2. Distribution of thrips eggs on artificially infested material. Includes mix of Red Delicious, Golden Delicious, Fuji, and Granny Smith.

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	769	0	63.7	25.6	10.7	0
King bloom	405	0.4	59.3	29.1	11.2	0
15-25 mm	71	0	31.0	1.4	0	67.6

******* Potentially damaging eggs

PRESENTATIONS

Miliczky, E., D. Horton, S. Cockfield and E. Beers. 2005. Spatial and temporal distribution of western flower thrips eggs in apple tissues. 101st Annual Meeting, Washington State Horticultural Assoc., Wenatchee, WA. (Poster)

Miliczky, E., D. Horton, S. Cockfield and E. Beers. 2006. Spatial and temporal distribution of western flower thrips eggs in apple tissues. 80th Annual Conference, Western Orchard Pest and Disease Management, Portland, OR. (Poster)

BUDGET

Project Title: Distribution of flower thrips eggs in apple blossoms

PI: David Horton
Co-PI: Gene Miliczky
Project duration: 2005-2006
Current year: 2006
Project total (2 years): $30,000
Current year request: $15,000

Year	Year 1 (2005)	**Year 2 (2006)**
Total	15,000	**15,000**

Current year breakdown

Item	Year 1(2005)	**Year 2 (2006)**
Salaries[1]	15,000	**15,000**
Total	15,000	**15,000**

[1] Partial support for Gene Miliczky.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC PROJECT #: 2005 AE-04-426

Project title: Alternate Hosts of Apple Maggot as a Threat to Apples
PI: Wee Yee
Organization: USDA-ARS, Wapato, WA
Collaborators: Pete Landolt, USDA-ARS, Tom Sandoe, WSDA, Yakima, WA, various growers and homeowners in western and central Washington

Objectives (2004-2006):
1) Determine apple maggot abundance and prevalence on alternate and normal host trees, including hawthorn, in different regions in Washington, with emphasis in central Washington.
2) Determine effects of fruit maturity and spatial relationships between alternate host trees on apple maggot infestations.
3) Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies in apple or hawthorn.

Goals and Activities and Anticipated Accomplishments for 2006:
1) Determine frequency of infestations of hawthorn species and apple varieties
2) Determine abundance of hawthorns and alternate hosts near representative commercial orchards.
3) Determine acceptance of apple varieties by apple maggots reared from hawthorns &alternate hosts.

2005 Significant Findings:
● Despite extensive samples of apples and hawthorns in Ellensburg, Nile Valley, and Yakima, only one hawthorn sample produced one confirmed apple maggot pupa; thus, infestations levels in central Washington are currently very low.
● Rose maggot pupae but not apple maggot pupae were recovered from apple samples in Ellensburg.
● Apple maggot flies infested apricot, Midland hawthorn, a cotoneaster not previously recorded as a host, and possibly mountain ash, all new host records for the state. Thus, apple maggot flies have an even wider host range in Washington than previously thought.
● In western Washington, apples and hawthorn were attacked in similar frequencies, suggesting that flies here do not prefer one over the other, but still unknown if host races occur in the state.
● In western Washington, early varieties of apples were more frequently infested; late, hard apples were less infested; thus, not all apples may be equally threatened.

Methods for 2006:
1) *Determine frequency of infestations of hawthorn species and apple varieties*. This study will be conducted in western and central Washington. Black, English, Midland, and other hawthorns and different varieties of apples – early, middle, and late developing - will be collected and placed in tubs for larval emergence.
2) *Determine abundance of hawthorns and alternate hosts near representative commercial orchards.* Based on previous years' collections, main alternate hosts – pear, plum, Asian pear, and apricots – will also be sampled or their abundance recorded near commercial orchards throughout western and central Washington.
3) *Determine acceptance of apple varieties by apple maggots reared from hawthorns and alternate hosts*. Flies reared from hawthorn, Asian pear, and other alternate hosts will be provided early and late varieties of apples inside cages. Observations will be made of flies probing the apples over 15-30 min periods. Numbers of eggs laid will be counted.

2005 Results and Discussion:
1) *Determine apple maggot abundance and prevalence on alternate and normal host trees*. In Yakima (Table 1), no infestations by apple maggots were found in the numerous apple and hawthorns sampled. However, apple maggot does occur in Yakima and vicinity, based on adult catches and larval finds (WSDA, Tom Sandoe, personal communication). That infestations were not detected

indicates that below a certain fly density, infestations are extremely difficult to detect without sampling very high numbers of trees and fruit.

In Ellensburg (Table 2), as in Yakima, no apple maggot larvae were detected despite the fact that they have been found in Ellensburg in the past (Mike Klaus, WSDA, personal communication, specimens reared to adulthood by W. L. Y). No apples have been found to be infested with apple maggot in Ellensburg. Apple was infested by rose maggot (*R. basiola*) in Ellensburg, and although this was a rare event, it is important because this species can be confused with apple maggot.

In the Nile Valley (Table 3), results were different than in Yakima and Ellensburg, as more plants were used by apple maggot. Black hawthorn was infested by one apple maggot pupae, further confirming establishment of this species in Yakima County. The low infestations of apple maggot in black hawthorn in this area suggest a recent introduction or that some environmental factor in this area, such as low moisture, has prevented the population from increasing to higher levels. The high abundance of trees and hawthorn fruit suggest the population is not limited by breeding sources. Although apples were negative, apple maggot were reared from apples in this area in 2004 (Mike Klaus, personal communication).

In Puyallup (Table 4), sample sizes were small, but there were clear differences in patterns from those at the three central Washington sites. Significantly, European mountain ash was infested by some fruit fly larvae, perhaps of apple maggot. All ornamental hawthorns and apples were infested, indicating high populations of apple maggots exist in this area. Another significant finding was that Asian pear was infested in 2004 and 2005, making it five consecutive years that this plant (as a host species, not necessarily individual trees) has been used by apple maggot, suggesting establishment on this host.

In Vancouver (Table 5), results were different from other sites in that several new state or general host records of apple maggot were determined. New host records were: Midland hawthorn, apricot, cotoneaster, and possibly western mountain ash. Apple maggot was also observed stinging cherry plum, but oviposition was not confirmed.

2) *Effects of fruit maturity and spatial relationships between alternate host trees and normal trees on maggot infestations*. Observations in 2004 and 2005 indicated that the following patterns occurred in western Washington: the early developing variety of black hawthorn was less frequently attacked than the later developing variety. English hawthorn matured late, but was frequently attacked. Early apples such as "Early Transparent' were frequently infested, whereas late varieties (e.g., 'Gravenstein'), were infrequently infested. Early-developing pear and Asian pear were more frequently attacked than later varieties. In contrast, early-developing plums were less frequently attacked than later-developing plums because flies are not abundant when the early varieties ripen. Infestations of alternate hosts appear to be more common near infested apples or hawthorns, but flies appear to be capable of persisting on alternate hosts for many years.

3) *Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies*. In a first test, apple maggot flies were placed inside outdoor pyramid cages enclosing *Cotoneaster dammeri*. Fruit were collected, but they produced no pupae. In a second test, 20 one-gallon pots of *C. dammeri* were placed in or near a feral orchard that was known to be infested with apple maggot in 2004. Ten were placed between apple trees in a north-south row within the orchard. Ten more were placed north-south 20 ft outside the orchard. Fruit were removed, but none produced pupae. In a third test, 20 one-gallon pots with *C. dammeri* were placed in screened containers in the laboratory. Five pairs of apple maggot were placed inside the containers for 3 weeks. Coupling was observed and on several occasions females probed the fruit. However, fruit did not produce any pupae. Likely reasons for the negative results in all three tests were that the cotoneaster species used developed ripe fruit too late in the season and that they are

difficult to penetrate. Earlier developing species or varieties of cotoneaster may lead to different results. However, cotoneasters were naturally used by apple maggots (Table 5).

Summary of 2004 and 2005 data.

Apple maggot uses about 14 species of hosts in Washington (Table 6). At least five questions need to be answered to assess the importance of some of these alternate hosts as threat to apples: 1) How frequently are they used and are apple maggots established on them? 2) Are these hosts common? 3) Are they found near orchards? 4) Do flies reared on alternate hosts attack apples? and 5) do flies in nature fly from alternate hosts to apples? Data from 2004 and 2005 have mostly answered questions 1 and 2: flies are frequently found on them in western but not central Washington, flies show the capability of establishing on some of them, and these hosts are common. In 2006, questions 3-5 need to be answered.

Significance to the Industry and Potential Economic Benefits. The research is significant for the apple industry because it may help reduce the possibility of quarantines being imposed in areas now free of apple maggot, specifically by identifying which plants can be infested by the fly and which therefore should be treated. Apple maggot is now found in the Nile Valley, Naches, Yakima, and Wenatchee areas. If an apple maggot is found within ½ mile of an apple orchard, even on an alternate host, the orchard is threatened and inspection of the fruit is required, at costs in time and money to the grower. Apple maggot establishment on alternate hosts near or in commercial orchards may increase numbers of spray applications that lead to additional costs to growers. Identifying whether flies of alternate host origin will attack apple will help determine the risk of the alternate hosts being sources of flies as threats to commercial apples.

Table 1. Plants sampled and numbers of *Rhagoletis* fruit fly pupae and adults from various fruit collected in Yakima and vicinity, Yakima County, WA, June to September 2005

Plant Species	No. Plants	No. Fruit	No. Pupae	% Trees Pos.	Likely Species
Black Hawthorn	39	4,643	0	0	---
English Hawthorn	25	9,667	0	0	---
Washington Hawthorn	3	120	0	0	---
Apple	30	1,446	0	0	---
Crabapple	74	12,499	0	0	---
European Pear	2	6	0	0	---
Garden Plum	6	381	0	0	---
Sweet/Sour Cherry	20	3,025	2,428	95.0	WCFF
Choke Cherry	16	3,608	0	0	---
Roses	29	2,900	130	31.0	RM
Japanese Quince	3	32	0	0	---
Serviceberry	4	384	0	0	---
Mountain Ash	13	2,644	0	0	---
Cotoneaster	1	400	0	0	---
Common Snowberry	10	600	77	100.0	SBM
Elderberry	9	1,800[a]	0	0	---
Red Osier Dogwood	6	2,892	0	0	---
Golden Currant	14	2,953	2	14.3	CF
Tall Oregon-Grape	34	10,520	60	20.6	OG
English Walnut	36	1,080	7,835	97.2	WHF
Holly	1	145	0	---	---
Juniper	1	25	0	---	---
Highbush-Cranberry	1	145	0	---	---

[a]Estimated; AM, Apple maggot, *R. pomonella*; WCFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; CF, *R. ribicola*; DF, *R. tabellaria*; WHF, *R. completa*.

Table 2. Plants sampled and numbers of *Rhagoletis* fruit fly pupae and adults from various fruit collected in Ellensburg, Kittitas County, WA, June to September 2005

Plant Species	No. Plants	No. Fruit	No. Pupae	% Trees Pos.	Likely Species
Black Hawthorn	15	1,450	0	0	---
English Hawthorn	30	5,580	0	0	---
Apple	139	6,950	3	0.7	RM
Crabapple	108	13,800	0	0	---
European Pear	48	1,645	0	0	---
Garden Plum	32	960	0	0	---
Sweet Cherry	1	500	103	---	WCFF
Choke Cherry	14	5,300	0	0	---
Apricot	6	180	0	0	---
Roses	53	8,000	179	43.4	RM
Serviceberry	3	300	0	0	---
Peach	4	110	0	0	---
Common Snowberry	9	1,520	88	55.6	SBM
Elderberry	11	2,200[a]	0	0	---
Tall Oregon-Grape	10	1,000	93	80.0	OG
Red Berries	4	550	0	0	---
Juniper	3	225	0	0	---

[a]Estimated; AM, Apple Maggot, *R. pomonella*; WCFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; CF, *R. ribicola*; DF, *R. tabellaria*; WHF, *R. completa*.

Table 3. Plants sampled and numbers of *Rhagoletis* fruit fly pupae and adults from various fruit collected in the Nile Valley, Yakima County, WA, June to September 2005

Plant Species	No. Plants	No. Fruit	No. Pupae	% Trees Pos.	Likely Species
Black Hawthorn	46	18,425	1	2.2	AM
Apple	73	2,905	0	0	---
Crabapple	4	642	0	0	---
European Pear	5	125	0	0	---
Garden Plum	6	350	0	0	---
Sweet/Sour Cherry	6	1,161	0	0	---
Bitter Cherry	23	3,947	76	21.7	WCFF
Choke Cherry	41	8,200	73	12.2	WCFF
Roses	52	5,200	548	69.2	RM
Serviceberry	22	3,293	0	0	---
Common Snowberry	23	1,557	522	100.0	SBM
Elderberry	41	8,200	0	0	---
Golden Currant	12	2,985	28	50.0	CF
Red Osier Dogwood	20	7,959	4	20.0	DF
Tall Oregon-Grape	2	254	0	0	---

[a]Estimated; AM, Apple maggot, *R. pomonella*; WCFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; CF, *R. ribicola*; DF, *R. tabellaria*; WHF, *R. completa*.

Table 4. Plants sampled and numbers of *Rhagoletis* fruit fly pupae and adults from various fruit collected in Puyallup, Pierce County, WA, June to September 2005

Plant Species	No. Plants	No. Fruit	No. Pupae	% Trees Pos.	Likely Species
English Hawthorn (rd)	3	600	219	100.0	AM
English Hawthorn (ov)	3	600	71	100.0	AM
Apple	13	820	816	100.0	AM
Asian Pear	2	35	25	100.0	AM
Garden Plum	2	100	0	0	---
Roses	3	70	0	0	---
European Mountain Ash	1	100	13	---	AM
Salal	2	200	0	0	---

AM, Apple maggot, *R. pomonella*; WCFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; CF, *R. ribicola*; DF, *R. tabellaria*; WHF, *R. completa*.

Table 5. Plants sampled and numbers of *Rhagoletis* fruit fly pupae and adults from various fruit collected in Portland, Multnomah County, OR, Vancouver, Clark County, WA, and Skamania, Skamania County, WA, June to September 2005

Plant Species	No. Plants	No. Fruit	No. Pupae	% Trees Pos.	Likely Species
Black Hawthorn	17	6,558	331	70.6	AM
English Hawthorn	54	33,165	2,393	87.0	AM
Midland Hawthorn	1	272	188	---	AM
Apple	47	1,733	1,032	93.6	AM
Crabapple	1	189	6	---	AM
Apricot	4	146	44	100.0	AM
European Pear	7	268	0	0	---
Garden Plum	7	381	5	14.3	AM
Sweet Cherry	3	625	203	100.0	WCFF
Cherry Plum	3	201	28	66.7	WCFF
Cherry Laurel	6	696	75	50.0	WCFF
Woods' Rose	3	827	15	66.7	RM
Roses	16	2,081	264	62.5	RM
Western Mountain Ash	1	552	8	---	AM
European Mountain Ash	1	643	0	---	---
Cranberry Cotoneaster	1	132	5	---	AM
Cotoneaster	3	1,361	3	33.3	AM
Common Snowberry	18	5,151	574	94.4	SBM
Elderberry	5	5,045	0	0	---
Orange Honeysuckle	1	38	0	---	---
Highbush-Cranberry	1	372	0	---	---
Cornelian Cherry	1	541	0	---	---
Pacific Dogwood	2	2,875	0	0	---
Oyster Dogwood	1	694	0	---	---
Cherry Olive	1	446	0	---	---
Tall Oregon-Grape	1	547	0	---	---
Dull Oregon-Grape	1	252	82	---	OG
English Walnut	1	62	454	---	WHF
Black Walnut	2	116	370	100.0	WHF
Blueberry	3	1,663	0	0	---
Grape	2	1,060	0	0	---
Cascara	4	1,352	4	50.0	WCFF
Bittersweet Night Shade	1	571	0	---	---
English Yew	1	228	0	---	---

Strawberry Madrone	1	57	0	---	---
Pacific Madrone	1	1,320	0	---	---

AM, Apple maggot, *R. pomonella*; WCFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; CF, *R. ribicola*; DF, *R. tabellaria*; WHF, *R. completa*.

Table 6. Complete updated list of 14 confirmed or suspected apple maggot developmental hosts in nature in Washington, as confirmed by studies in 2002-2005

Common Name	**Scientific Name**	**Host Status**
Apple	***Malus domestica***	**Common all areas**
Black Hawthorn	***Crataegus douglasii***	**Common all areas**
English Hawthorn	***Crataegus monogyna***	**Common, more abundant than BH**
Midland Hawthorn[a]	***Crateagus laevigata***	**Too rare to know**
Crabapples	***Malus* spp.**	**Common all areas**
Asian Pear[b]	***Pyrus serotina***	**Common, but relatively few trees**
Bitter Cherry[b]	***Prunus emarginata***	**Rare, only west of Yakima Valley**
Common Pear[b]	***Pyrus communis***	**Moderately used, mostly early varieties**
Garden Plum[b]	***Prunus domestica***	**Moderately used**
Cranberry Cotoneaster[b]	***Cotoneaster apiculatus***	**Rare**
Cotoneaster[b]	***Cotoneaster bullata*?.**	**Rare**
Apricot[b]	***Prunus armeniaca***	**May be moderately used near apples**
European Mountain Ash[c]	***Sorbus aucuparia***	**Too few samples to be certain**
Western Mountain Ash[c]	***Sorbus scopulina***	**Too few samples to be certain**

[a]New hosts in Washington identified 2005.
[b]New hosts in Washington identified in 2002-2004.
[c]Suspected hosts, awaiting emergence of adults for confirmation in 2006.

Project title: Alternate Hosts of Apple Maggot as a Threat to Apples
PI: Wee Yee
Project duration: 2004-2006
Current year: 2006
Project total (3 years): $78,000
Current year request: $26,000

Item	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries	19,500[1]	19,500[1]	**19,500[1]**
Benefits	2,000	2,000	**2,000**
Wages	0	0	**0**
Benefits	0	0	**0**
Equipment	0	0	**0**
Supplies	2,500[2]	2,500[2]	**2,500[2]**
Travel	2,000[3]	2,000[3]	**2,000[3]**
Miscellaneous	0	0	**0**
Total	26,000	26,000	**26,000**

[1]Two-three GS-3 to GS-5 for 3-6 months; Traps, tubs, screening, and shrubs and trees from nurseries.
[3]Gasoline for travel to and from field sites.

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC PROJECT#: 2005 AE-04-427

Project title: Control of Apple Maggot Using Bait Spray Insecticides and Traps
PI: Wee Yee
Organization: USDA-ARS, Wapato, WA
Collaborators: Tom Sandoe, WSDA, Yakima, WA, WSU personnel, growers, and homeowners in Vancouver and Puyallup

Objectives (2004-2006):
1) Determine release of ammonia and other volatiles from bait sprays.
2) Determine attraction to bait sprays and feeding behaviors on baits.
3) Determine effects of bait sprays under different habitat types.
4) Determine effects of bait sprays and trapping methods on apple maggot control.

Goals and Activities and Anticipated Accomplishments for 2006:
1) Determine effects of different concentrations of GF-120 and Mazoferm bait mixed with ammonia compounds, spinosad, and imidacloprid on attraction and feeding by and mortality of apple maggot in the laboratory
2) Determine effects of GF-120 and Mazoferm bait sprays mixed with spinosad and imidacloprid on control of adults and larvae of apple maggot in the field

2005 Significant Findings:
● GF-120 bait mixed with ammonium carbonate was more attractive than GF-120 mixed with ammonium acetate when placed with traps, suggesting ammonia form is important.

● In the field, more flies were attracted to and fed on GF-120 mixed with ammonium carbonate and ammonium acetate than on GF-120 alone, suggesting GF-120 attractiveness can be increased, although overall responses were low.
● In the laboratory, the percentages of flies feeding on GF-120 with or without ammonium carbonate or ammonium acetate were low, suggesting low attractiveness of even enhanced bait.
● Inconsistent with attraction and feeding tests, no reduction in larval infestations in apples were seen when GF-120 alone or with ammonium carbonate and ammonium acetate was sprayed on apple trees.
● Larval infestations of apples were reduced equally by Entrust (spinosad alone), GF-120, and Mazoferm + spinosad, but were not reduced by Nulure + spinosad, suggesting GF-120 and the much less expensive Mazoferm may be substituted for GF-120.

Methods for 2006:
1*) Determine effects of GF-120 and Mazoferm baits using spinosad and imidacloprid on attraction to and toxicity of apple maggot flies*

In the laboratory, toxicity of Entrust, GF-120, and Mazoferm with spinosad and imidacloprid will be tested by exposing drops to flies in the lab for 1 h, 8 h, and 24 h and determining mortality at 1, 2, and 3 days. Treatments will be: 1) control, 2) Entrust, 3) GF-120, and 4) Mazoferm. GF-120 will be tested at 20% and 40% concentrations. Mazoferm will be tested at 40% and 80% concentrations. GF-120 and Mazoferm will also be enhanced with 5 and 10% ammonium carbonate (AC), ammonium acetate (AA), or various concentrations of uric acid (a component of bird feces).

In the field, attraction and feeding on GF-120 and Mazoferm with AC, AA, and uric acid with spinosad or imidacloprid will be determined by applying them on leaves and then observing flies that visit and feed on the droplets.

2) *Determine effects of GF-120 and Mazoferm bait sprays mixed with spinosad and imidacloprid on control of adults and larvae of apple maggot*

Replicated spray tests will be conducted to complement the laboratory findings in orchards and rural settings in western and in central Washington, if possible. Numbers of adults caught on traps and larval infestations in apples will be determined in at least two sites in Puyallup and two sites in the Vancouver area (where fly densities are high), as well as in the Nile Valley and vicinity in central Washington (where fly densities are low). Surrounding trees will be sprayed to reduce chances of immigrating flies. Depending on the outcome of laboratory attraction tests, test 1 will consist of 1) control, 2) Entrust, 3) 40% GF-120, 4) 40% Mazoferm, and 5) 80% Mazoferm. Also depending on the outcome of laboratory tests, test 2 will consist of a control and 4 of the following treatments: 1) GF-120, 2) Mazoferm, 3) GF-120 + AA, 4) GF-120 + AC, 5) Mazoferm + AA, 6) Mazoferm + AC, 7) GF-120 + uric acid, and 8) GF-120 + uric acid. In Test 3, blank GF-120 will be mixed with imidacloprid and compared with regular GF-120, and Mazoferm with spinosad will be compared with Mazoferm with imidacloprid. To test the materials rigorously, a spray volume of 30-50 ml per tree will be applied.

2005 Results and Discussion:

1) *GF-120 bait mixed with ammonium carbonate was significantly more attractive than GF-120 mixed with ammonium acetate when placed with sticky yellow panel traps.*

The attraction of apple maggot to yellow panel traps baited with 40% GF-120 + 10% AC suggests that flies can be drawn to a bait from moderate distances, as it was assumed that flies trapped were not in the immediate vicinity of traps, but flew to them either from other parts of the tree or from neighboring trees. Traps baited with 40% GF-120 alone caught more flies than controls, although not significantly, suggestion perhaps a slight attraction. GF-120 + 10% AC was more attractive than GF-120 + 10% AC, suggesting AC is more attractive than AA when the two occur in equal amounts. Significantly, AA is found in small quantities in GF-120 alone, so possibly the use of AA in the GF-120 may have little or no effect on attraction. Results suggest that 10% AC in GF-120 retains its attractiveness over the entire season. It is unclear why AC in GF-120 was so superior to AA in GF-120 for attracting flies to traps. One possibility is that AA is repellent and another is that the AC simply released more ammonia than the AA in our tests even though the amounts used were equal.

2) *In the field, more flies were attracted to and fed on GF-120 mixed with ammonium carbonate and ammonium acetate than on GF-120 alone, but over responses were low.*

Despite the responses of flies to GF-120 mixed with AA and AC on traps, responses to the baits sprayed on apple foliage were low. The low responses suggest the odor levels, in particular those of ammonia, that emanated from the drops were much lower than from lures, and that it takes a longer than the 30-min observation periods for the flies to find the bait when odor levels are low. Point sources of odor may be difficult to duplicate in spray deposits on leaves. However, similar to the tests using traps, adding 10% AC to GF-120 seemed to increase fly attraction compared with GF-120 alone, but unlike those tests, the GF-120 + 10% AA seemed to be equally attractive. Perhaps in traps the AA was repellent, whereas sprayed as drops, the concentration was too low to have this effect. Females possibly were more attracted to drops than males.

3) *In the laboratory, the percentages of flies feeding on GF-120 with or without ammonium carbonate or ammonium acetate were low.*

In the laboratory, the overall low responses to GF-120 + AC and AA were generally consistent with those in the field, but patterns differed. Unlike in the trapping test and field feeding tests, the responses to GF-120 + 10% AC and 10% AA were no greater than to 13% sugar alone. Flies fed on the GF-120 with 10% AC, but few fed and only for short periods (< 1 min), suggesting perhaps a repellent effect, although further testing to determine this is needed. Because responses were no greater than to water or 13% sugar, it appears that the flies found the GF-120 through normal foraging and that given enough time and food deprivation, the flies would have found the GF-120 droplets without necessarily being attracted to them. The field results suggest some weak attraction. Females

fed as long on sugar as on GF-120 + AA, suggesting the other treatments did not stimulate flies enough. Although results are somewhat inconsistent, both indicate low responses to GF-120 and that the attraction to GF-120 and mixed ammonia compounds is weak.

4) There was no reduction in larval infestations in apples when GF-120 alone or with ammonium carbonate (AC) and ammonium acetate (AA) was sprayed on apple trees.

Adding AC and AA to GF-120 did not significantly increase control of flies and larval infestations. So despite the greater attraction of flies to traps and leaves baited with GF-120 + AC or AA this did not translate into better control. Possibly flies did not feed on it any more than on the other treatments. Adding AC and AA may draw in flies from surrounding trees, even though it is generally assumed the range of attraction is fairly short. The lack of control cannot be attributed to the toxicity of GF-120, albeit in the absence of ammonia compounds. The results could be due to the low volume of 100 ml/tree used, and also due to immigrating flies moving into test trees, as in 2004. Because Entrust is apparently unattractive, it is possible flies fed found it through normal, wide-ranging foraging. The results with Entrust suggest that a closer look needs to be made as to whether spinosad requires addition of bait to be effective.

5) *Larval infestations of apples were reduced equally by Entrust (spinosad alone), GF-120, and Mazoferm + spinosad, but were not reduced by Nulure*.

The numbers of adult flies caught in traps placed in trees treated with Entrust, GF-120, Nulure + spinosad, and Mazoferm + spinosad were lower than in control traps (Table 6). Numbers of larvae per apple (41-109 picked per tree) were lower in the Entrust, GF-120, and Mazoferm + spinosad treatment than in the control, but not in the Nulure + spinosad treatment. The results suggest that contrary to the hypothesis that all baits will work, GF-120 and Mazoferm were fed upon more by the flies. Because the spray volume and amount of active ingredient (spinosad) were the same for all treatments, the chances of flies randomly encountering the drops should have been the same. Therefore the differences are probably due to preferentially feeding, although not necessarily greater attraction. Since Entrust was as effective as the baits, it is questionable whether adding the baits has any advantage over it, as the same amount of spinosad insecticide was used. More tests are needed to confirm these results, but they may result in wiser use of spray materials. For example, Mazoferm costs only 10% as much as GF-120, even after taking into account the Entrust added to the Mazoferm.

Brief Summary of findings in 2004

(1) Aging GF-120 under central Washington clearly reduced toxicity after only 3 or 7 d, suggesting breakdown in control can occur if flies do not find the bait before that time. When flies do find GF-120 and ingest it, high mortality will occur. Perhaps even with brief feeding, the high toxicity of spinosad may be sufficient to cause mortality. Fresh GF-120 was most effective, but 3-day old residues were nearly as effective in killing flies before they can oviposit. If sprays are shortly after flies emerge and before flies are gravid, spray intervals of 7 d may be sufficient.

(2) Volumes of GF-120 sprays had no differential effect on larval infestations, although results differed in the two apple habitats. It was not expected that the treatments would give complete control because surrounding trees were likely sources of infestations, but significant suppression was expected. However, this was seen only in isolated trees, suggesting that there were fewer immigrating flies that infested these than the trees in the orchard. The sprays likely cannot protect apples from flies that carry fertilized eggs, perhaps explaining why infestations were present in both habitat types.

(3) Combining red sphere traps with GF-120 at the highest spray volume did not increase the control levels, but red spheres and GF-120 alone were effective in single trees. GF-120 sprays

apparently killed many flies before they oviposited, whereas the baited red spheres removed many gravid flies. Traps may be useful for lowering populations one year so that GF-120 can further reduce or eliminate populations the following year.

Summary of 2004 and 2005 results

Overall results suggest that GF-120 is not highly attractive to apple maggot flies, and that when even made more attractive with ammonia compounds, control is not improved when fly densities are high. Thus the relationship between fly attraction to GF-120 and control remains unclear. Residual toxicity of GF-120 does not seem to be an explanation for the lack of control. Rather, it seems relatively few flies found the GF-120 early enough to result in control, except where GF-120 at the highest volume was used as a cover and not bait spray. The spray experiments in this study were conducted under severe infestation conditions not likely to be encountered in central Washington, where fly populations are low, trees are isolated or occur in patches, and immigrating flies are unlikely to be a problem. Here, GF-120 alone might be sufficient for control. In small-scale backyard apple operations in western Washington, the addition of spheres may be a suitable approach to help increase the effectiveness of GF-120 by first reducing population densities.

***Significance to the Industry and Potential Economic Benefits*:** Apple maggot flies have recently been found breeding in commercial apple-growing areas. The results are significant to the apple industry in that they show GF-120 and Mazoferm with spinosad can potentially be used to manage apple maggot in feral or backyard trees. GF-120 and other baits with spinosad appear to be safer alternative to the organophosphates used in the past to control the flies and thus are more desirable to use near residential areas and creeks and rivers. Results also show the potential of mass trapping to reduce larval infestations, suggesting baited red spheres may be useful in feral and backyard trees. By suppressing fly populations using bait sprays, the risks of flies spreading into commercial apple-growing areas and of these areas being placed under quarantine are reduced. When a fly is caught $\leq$ ½ mile from an apple orchard, the orchard is considered threatened; larval finds result in quarantines. Apples from such an orchard need to be inspected and permits need to be issued for their movement. Much less expensive baits such as Mazoferm mixed with spinosad or imidacloprid seem promising; if they are as effective as GF-120, they can be used in place of it and can save growers substantial money in application costs.

Table 1. Mean total numbers of apple maggot flies trapped over the season per yellow panel trap with GF-120 treatment lures, 2005

	Test 1: Vancouver	Test 2: Skamania
Treatment	19 July-22 August	28 July-22 September
Control	1.2a	9.0a
10 g AC	15.5c	--
17% GF-120	1.8a	17.3a
40% GF-120	1.8a	66.0a
40% GF-120 + 10% AC	7.0b	220.3b
40% GF-120 + 10% AA	2.8b	41.8a

AC, ammonium carbonate; AA, ammonium acetate
Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Table 2. Total numbers of apple maggots feeding on apple leaves or not feeding but < 15 cm away from baits in the field, 2005

	Tests 1 and 2		Test 3	
Treatment	Feeding	<15 cm, no feed	Feeding	<15 cm, no feed

Water Control	---	---	0	1
13% Sugar Control	1	0	0	1
Spinosad Only	---	---	0	1
17% GF-120	1	1	0	5
40% GF-120	1	1	0	12
40% GF-120 + 10% AC	8	6	---	---
40% GF-120 + 10% AA	3	12	---	---
40% GF-120 + 2.5% AC	---	---	2	15
40% GF-120 + 2.5% AA	---	---	2	16

AC, ammonium carbonate; AA, ammonium acetate
Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Table 3. Percent of apple maggot flies feeding on bait or on apples with bait over 1-h observations in the laboratory, 2005

	Males			Females		
Treatment	N	% Fed	% on Apple	N	% Fed	% on Apple
Water Control	18	0	16.7	22	0	9.1
13% Sugar Control	20	15.0	25.0	27	33.3	48.1
17% GF-120	21	4.8	28.6	25	20.0	44.0
20% GF-120	18	11.1	22.2	22	27.3	59.1
40% GF-120	24	16.7	20.8	27	7.4	18.5
40% GF-120 + 10% AC	20	5.0	30.0	21	9.5	42.9
40% GF-120 + 10% AA	10	10.0	60.0	12	0	25.0

Table 4. Feeding durations and duration (min) spent on apples by apple maggot flies on apples over 1-h observations in the laboratory, 2005

	Males		Females	
Treatment	Fed	On Apple	Fed	On Apple
Water Control	0	1.3	0	46.9
13% Sugar Control	3.5	8.6	15.0bc	23.2
17% GF-120	0	10.1	4.4ab	16.7
20% GF-120	7.1	4.1	1.8a	18.2
40% GF-120	20.2	4.7	20.2c	4.7
40% GF-120 + 10% AC	0.4	13.7	1.3	11.4
40% GF-120 + 10% AA	0.2	31.9	0	17.8

Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Table 5. Effects of GF-120 treatments on numbers of apple maggot flies trapped on unbaited yellow traps and larval infestations, 2005

	Test 1: Woodland		Test 2: Vancouver		Test 3: Puyallup	
Treatment	Adults	Larvae/ Apple	Adults	Larvae/ Apple	Adults	Larvae/ Apple
Control	0.2	0.225	2.0	1.712	35.2c	1.225b
Spinosad Only (Entrust)	---	---	---	---	14.0ab	0.049a
17% GF-120	0.8	0.205	4.0	0.415	---	---
40% GF-120	0.5	0.091	3.3	0.302	11.6a	0.070a
40% GF-120 + 10% AC	0.2	0.311	3.8	0.302	---	---
40% GF-120 + 10% AA	0.2	0.079	2.8	0.842	---	---
40% GF-120 + 2.5% AC	---	---	---	---	28.2c	0.085a

40% GF-120 + 2.5% AA	---	---	---	---	17.8b	0.163a

Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Table 6. Effects of spinosad and bait sprays with spinosad on total numbers of apple maggot flies caught per yellow trap and on numbers of larvae per apple, Puyallup, WA 2005

Treatment	Adults	% Reduction	Larvae/Apple	% Reduction
Control	43.8b	---	0.846b	---
Spinosad Only (Entrust)	14.8a	66.2	0.108a	87.2
40% GF-120	12.3a	71.9	0.095a	88.8
40% Nulure + spinosad	13.5a	69.2	0.746b	11.8
40% Mazoferm + spinosad	18.0a	58.9	0.104a	87.7

Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Table 7. Effects of GF-120 sprays and red sphere traps on larval infestations, 2004

Test 1: Puyallup			Test 2: Woodland		
Treatment	Adults	Lv/Apple	Treatment	Adults	Lv/Apple
Control	---	0.580b	Control, Panel	41.7	1.795b
GF-120	---	0.011a	GF-120, Panel	12.2	0.027a
6 Red Spheres	220.4	0.059a	6 Red Spheres	113.5	0.167a
GF-120 + 6 Red Spheres	180.2	0.096a	GF-120 + 6 Red Spheres	44.5	0.023a

Means followed by the same letter within columns with the same letter are not significantly different (ANOVA, Fisher's LSD test, $P > 0.05$).

Budget:
Project title: Control of Apple Maggot Using Bait Spray Insecticides and Traps
PI: Wee Yee
Project duration: 2004-2006
Current year: 2006
Project total (3 years): $83,100
Current year request: $27,700

Item	Year 1 (2004)	Year 2 (2005)	**Year 3 (2006)**
Salaries	22,000[1]	22,000[1]	**22,000[1]**
Benefits	2,200	2,200	**2,200**
Wages	0	0	**0**
Benefits	0	0	**0**
Equipment	0	0	**0**
Supplies	2,000[2]	2,000[2]	**2,000[2]**
Travel	1,500[3]	1,500[3]	**1,500[3]**
Miscellaneous	0	0	**0**
Total	27,700	27,700	**27,700**

[1]Two GS-5, for 6 months, One to two GS-3, 3 months.
[2]Traps and spray equipment and insecticides; [3]Gasoline for travel to and from field sites.

FINAL REPORT
WTFRC Project #: ARS-Wapato Organization Project # ARS-YARL

Project Title: Biological control of leafrollers through habitat modification
PI: Tom Unruh
Organization: USDA-ARS
Address 5230 Konnowac Pass Rd.
Wapato, WA 98951
509-454-6563; unruh@yarl.ars.usda.gov

CO-PIs Jay Brunner, WSU-TFREC, Wenatchee
Dave Horton, USDA-ARS, Wapato

OBJECTIVES:

1) **Establish new and expand existing rose gardens and establish *Ancylis* and *C. florus***
2) **Measure parasitism of and damage by leafrollers at different distances along transects from rose plantings into apple orchards.**
3) **Monitor the seasonal phenology and stability of alternate host populations in rose gardens and associated parasitism of SLR by *C. florus* and other parasitoids**
4) **Conduct field-day demonstrations for establishing and maintaining rose plantings and widely disseminate information from project to grower community**

SIGNIFICANT FINDINGS

- **High parasitism by *C. florus* in spring and summer was observed at orchards near gardens that harbored significant numbers of Ancylis and C. florus.**
- **The strawberries *Fragraria virginiana* collected at various localities under pine trees in the Cascade Mountains and the commercial *Fragraria* var. Quinault were excellent supplements to the gardens composed of *Rosa woodsii***
- **Ancylis did not establish or quickly went extinct at some gardens due to unknown factors that may include intense predation or pesticide spray drift.**
- **Re-infestation of gardens with *Ancylis* at several sites resulted in successful establishment and subsequent high parasitism of leafrollers in spring and summer by *C. florus***
- **In orchards adjacent to productive gardens parasitism by *C. florus* accounted for up to 95% of spring and summer parasitism. In orchards adjacent to poor gardens (few Ancylis) tachinids dominated parasitism but parasitism is usually lower, especially in spring. In the Royal slope, Frenchman hills the close relative of *Colpoclypeus, Sympiesis* spp., is often dominant in summer.**

Budget: This proposal was funded for 2 of proposed 3 years; subsequent matching funds were received from Western SARE for $105,000 over 3 years (2005-2007).

2003	2004	WTFRC Total
52,400	51,700	104,100

Objective 1. Establish and expand gardens

Since the inception of this work in 2001, eighteen growers and the PI's lab group have planted 35 gardens of multi-floral rose and strawberries (34 single gardens plus 50 small gardens around one 500 acre orchard) throughout the major pome fruit centers of the Pacific Northwest. Four new gardens were planted in 2005 and six older gardens were re-infested with *Ancylis.* The objective of having many gardens over a wide geographic range is to test that this habitat manipulation is robust to geographic variation in climate and other biological and physical factors. At each garden we have attempted to establish the Strawberry leafroller, *Ancylis comptana,* document the use of this host by the parasite *Colpoclypeus florus* and to determine the impact of the availability of this alternate and overwintering host on the parasitism of pest leafrollers in targeted orchards in spring and summer. The positions of the 35 gardens are shown in Figure 1**.**

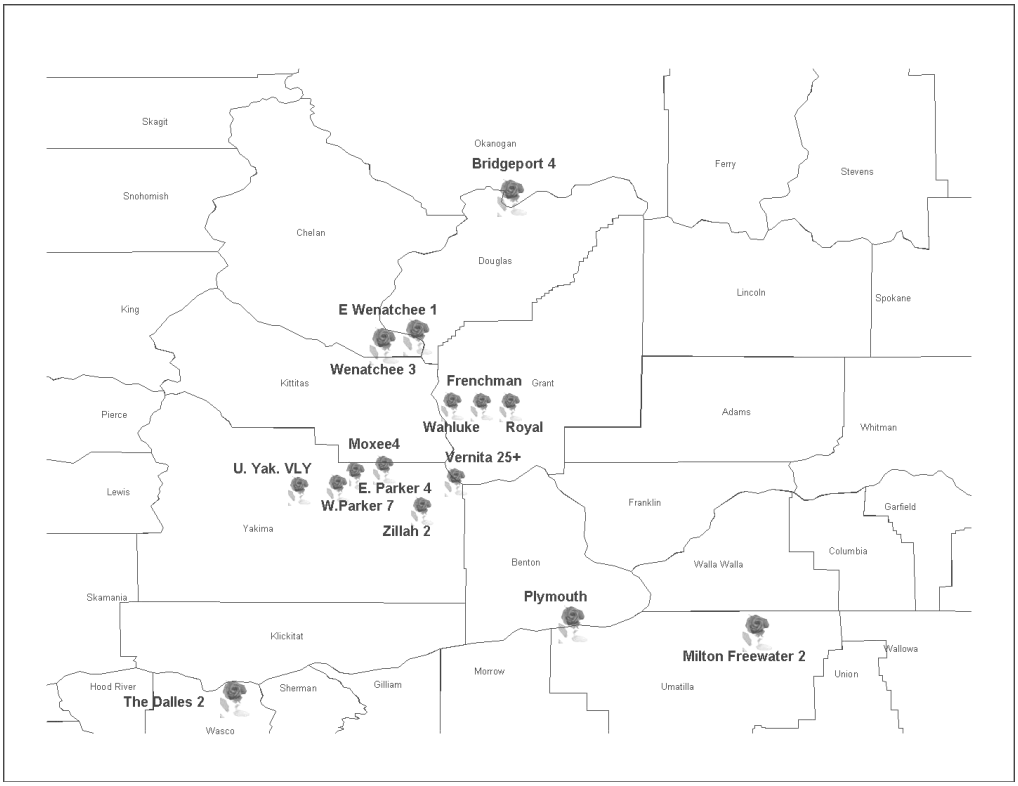


Figure 1. Locations of gardens of the multi-floral rose, *Rosa woodsii,* planted adjacent to pome fruit and cherry orchards in Central Washington and North-Central Oregon are depicted. Gardens were planted at Wahluke, Plymouth, Upper Yakima Valley, and one in Zillah in 2005. In all cases these gardens were established by growers; we provided consultation on placement, help in planting in some cases, and infested them with *Ancylis*, and monitored parasitism in the orchards nearby.

To assess the value of strawberries in the gardens we conducted common garden experiments begun in 2002 and assessed in 2004. We planted 19 accessions of strawberries including 3 species and multiple varieties including commercial hybrids in 5 gardens. Similarly we planted 11 sources of Rosa spp. including *Rosa woodsii* and *Rosa nutkana*. We found locally collected *Fragraria*

virginiana (from the Cascade Mountains under pine trees) ranked highest in cover and spreading followed closely by several accession of the *F. chiloensis*. The commercial variety Quinalt followed by the wild Cascade species ranked highest in producing/supporting *Ancylis* through the season. However, at many of our gardens strawberry appears to be slowly disappearing because of the combined pressures of encroaching over-story of the roses and weed growth. At all gardens roses have prospered once they make it through the first year and an adequate water supply continues.

Objective 2. Evaluate parasitism

We monitored parasitism by *C. florus* and other parasitoids in orchards next to gardens using sentinel leafroller larvae which since 2003 we put directly onto tree branches (4th instar OBLR produced in the laboratory; 25 larvae/ branch, 10-20 branches/site, 3 sites/orchard) using a barrier of steel wool to prevent migration throughout the tree. Larvae are deployed at 3 specific times: in spring and summer (when pest leafrollers are in susceptible larval stages) and in fall (when parasitism of sentinel leafrollers indicates that *C. florus* are seeking overwintering hosts).

Parasitism was measured at 21 orchards one or more times in 2004 (not shown) and at 142 sites in 27 orchards in 2005 (see below). Attempts to measure parasitism in Arrowhead (Okanagon) and The Dalles generally failed because of very poor recovery of deployed insects. In Arrowhead we ascribe this to a combination of overhead sprinklers and pesticide use. Parasitism results are depicted in 3 ways: first is the season-long pattern of parasitism across all sites; second is the proportion of parasitism due to *C. florus* across all sites and all dates through mid August represented as a plot of *C. florus* versus total parasitism; third is the pattern of parasitism seen at 9 sites where parasitism was monitored in spring, summer, and fall. Figure 2 plots season-long parasitism in 2005 showing that parasitism increases significantly throughought the season and that *C. florus* dominates the parasitism through most of the year, with two parasitic flies (Tachinidae) accounting for most of the remaining parasitism. Figure 3 plots spring and summer parasitsm by *C. florus* against total parasitsm. It shows that overall *C. florus* is responsible for 70% of all parasitism despite many sites/dates where most parasitism was due to tachinid flies.

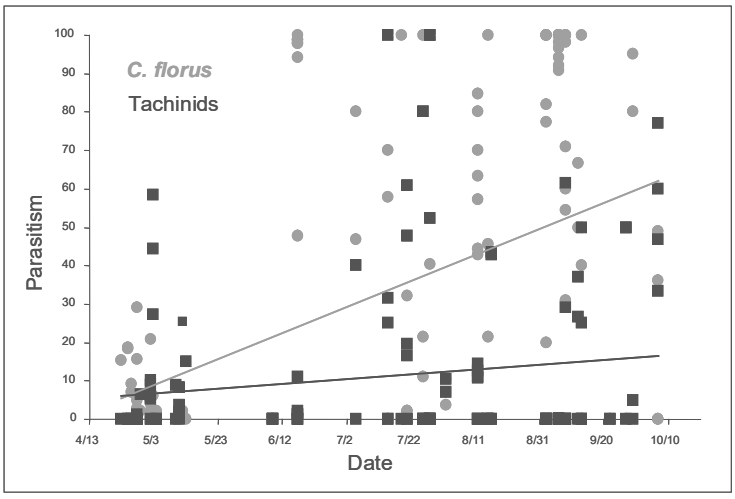


Figure 2. 2005 season-long parasitism by *C. florus* (light circles) and tachinid flies (dark squares) plotted against collection date of sentinel OBLR deployed 2-3 weeks before. Pest leafrollers are not present in orchards at the appropriate stage for *C. florus* or Tachinid attack after late August; parasitism by *C. florus* of sentinel leafrollers late in summer and especially in fall represents a measure of it seeking hosts on which to overwinter. The overwintering hosts of the tachinids are unknown.

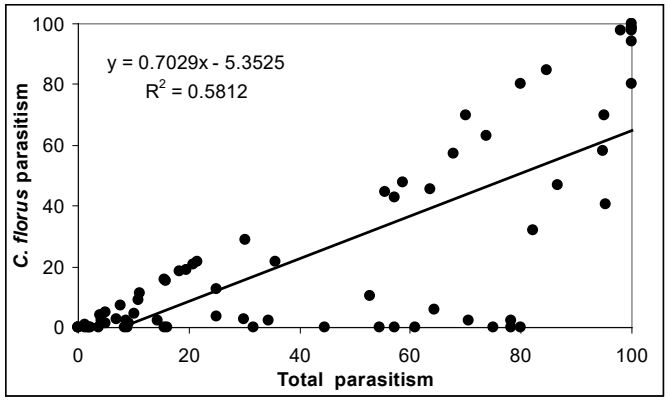


Figure 3. The percentage of parasitism due to *C. florus* is plotted against total percent parasitism for collection dates from early May until mid August. There are two patterns of parasitism: at some sites and dates tachinid flies dominate parasitism and that due to C. florus is low (points close to x axis); at other sites and dates parasitism is dominated by C. florus (points close to the fitted line). Overall, *C. florus* accounts for roughly 70% of all parasitism.

Parasitism of our experimentally depoyed sentinel hosts remained very high in late summer into fall (after mid-late August), when suitable stages of pest leafrollers are not found in or near orchards. This parasitism of fall deployed sentinels is coincident with *C. florus* parasitism of *Ancylis* leafrollers in the gardens. The overwintering hosts and biology of the tachinid flies remains unknown but their abundance after mid-August also suggests they may be subject to manipulation if a suitable overwintering host and habitat were found. Figure 4 provides some detail on seasonal pattern of parasitism at 9 sites where we were able to measure parasitism at spring, summer and fall periods.

In several gardens *Ancylis* failed to establish or were extirpated by pesticide drift or unknown cause (possibly intensive predation). Six such gardens were reinfested with *Ancylis* in 2005. Still to be analyzed are samples from 12 rose gardens taken in late November-December designed to provide estimates of *Ancylis* abundance and parasitism by *C. florus* that help us generate expectations for parasitism in the orchards in the coming spring . These samples will be analyzed through February and results, redictions, and tests of predictions will be reported next year.

More importantly, *Ancylis* abundance in gardens was low in the winter of 2004-2005 (not shown) and this presaged lower spring parasitism by *C. florus* than in previous years. This resulted in modest parasitism in spring as seen in Figure 4. Low spring parasitism was observed at older sites (UP7, Moxee) as well as newer sites (DJM, Ostensen). Fortunately during the 2005 year *Ancylis* populations and *C. florus* populations rebounded resulting in strong parasitism in summer and exceptionally highparasitism in fall. We expect much higher spring parasitism at all sites (excpt those where it is already very high such as UP3). A significant exception to spring parasitism leading to higer summer parasitism was observed at several sites (e.g. UP4 in Figure 4) and these are reliable associated with spray patterns.

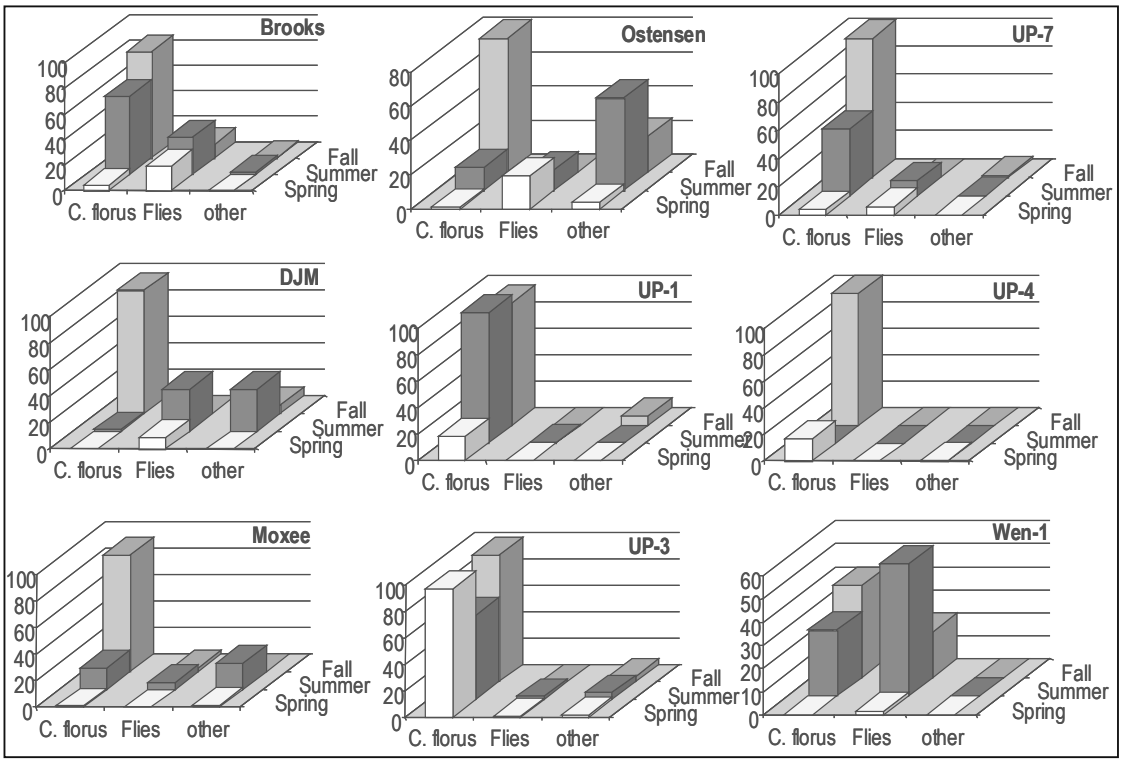


Figure 4. Pattern of parasitism by *C. florus*, tachinids and other wasps in 9 sites in 2005.

Objective 3. Monitor abundance and parasitism of *Ancylis* in rose gardens

As discussed under objective 2, abundance of *Ancylis* in gardens is critical to the success of this habitat manipulation. In some gardens, despite repeated attempts to colonize it, the leafroller has not established. In other gardens, it is very abundant every year. Figure 5 depicts the abundance of *Ancylis* across years for 3 gardens and the parasitism of these *Ancyli*s. Please note that *Ancylis* density (in fallen leaves) is presented on a logarithmic scale and varies between 13/m^2 to over 3000/m^2! Despite high variability, in most years, parasitism of *Ancylis* by *C. florus* exceeded 30%. It is also important to note that strawberries are also planted and established in most gardens. They contribute significantly to the abundance of *Ancylis* (roughly 75% of Ancylis overwinter in strawberry opposed to the rose) but C. florus much prefers to parasitize *Ancylis* in roses. We discovered that this preference is unrelated to the plant species but the height of the strawberries versus the rose. Specifically when strawberry plants are elevated to be the same height as rose plants, the use of the Ancylis hosts in the strawberries goes up. This is depicted in Figure 6. Overall, these results suggest that strawberries add stability to the garden system, providing abundant Ancylis to go up and also infest the roses, while at the same time protecting some subset of Anclis from overexploitation by parasitoids.

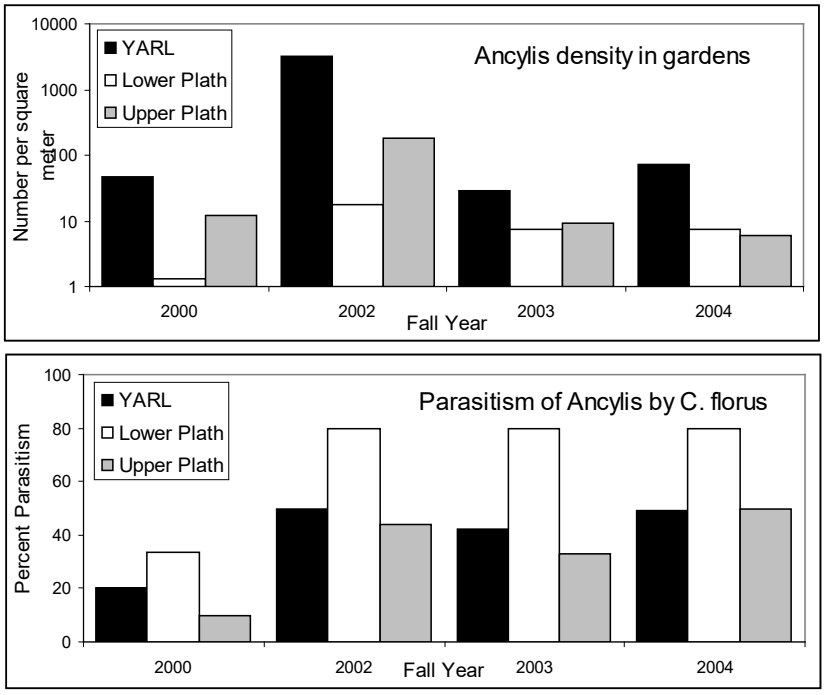


Figure 5. Highly variable Ancylis density in gardens (logarithmic scale of abundance) in upper panel and the more consistent pattern of parasitism by C. florus across four years.

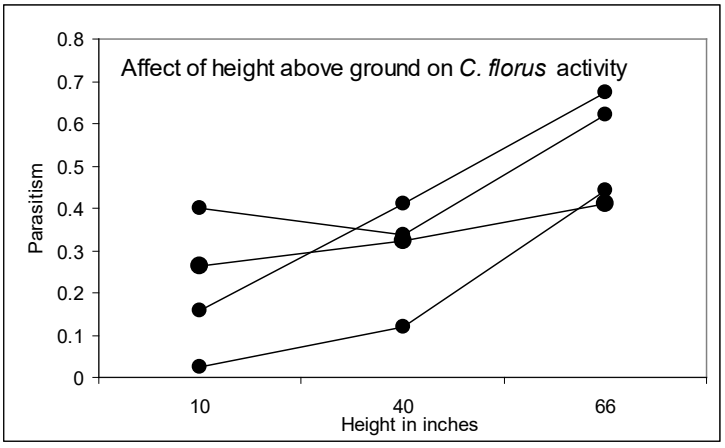


Figure 6. *C. florus* parasitism of *Ancylis* larvae feeding on strawberry plants when plants were deployed at different heights above the ground. Different lines depict replicate vertical transects on the north, east, south and west sides of a rose garden.

Objective 4. Share information about gardens with growers

To date we have presented our results at the third National Organic Tree Fruit Symposium held in Chelan WA in June 2005, at the Washington Horticultural AssociationConvention in 2001-2005, and from 2001 to 2005, in 2003,4, and 6 at the Western Orchard Pest Management and Disease Conference, and at the WTFRC research reviews yearly since 2001. In addition the work has been covered by the press in the Columbia Basin Journal, ARS magazine, and Western Fruit Grower among others. We have created web pages describing how to implement gardens from how and where to plant and maintain the roses and strawberries, where to find infested roses for transferring *Ancylis* to your own garden, and with overviews of our results for the last 5 seasons. The website is not yet connected but will be so in the coming months. Completion of this website represents an important milestone to be met before the end of 2006 under our WSARE funding.

Many in the grower community are familiar with the concept of using rose gardens to enhance leafroller parasitism based on the above presentations. A measure of this grower awareness are the contacts by growers to consult us on how to establish gardens and our subsequent help in getting the strawberry leafroller established in the gardens they plant. Such was the path taken for the 4 new gardens planted in 2005. We will continue to provide such help, including onsite visits, phone consultations, provision of insects etc. throughout the duration of our SARE funding. Furthermore, we believe the web site will further grower confidence in striking out on their own to create these gardens in the future.

This is a long-term ecological experiment and our work is far from completed. We have several deficiencies under this objective that need to be met. The most important is identifying the cost to growers of creating and maintaining a garden. We know the materials and supplies (black irrigation hose, couplers, ¾ " PVC for risers, 5 sprinkler heads, 20-50 *Rosa woodsii* seedlings, weed cloth, buildozing a clean area to plant, connection to the orchard irrigation) can be less that $200 for a sizable garden, but we need to formalize these estimates and make them available to the grower community.

Conclusions

This remains a work in progress and it should be clear to growers an scientists alike that habitat manipulations are long-term ecological manipulations. Early work, reported to WTFRC in the past has demonstrated the principle that rose habitats can greatly enhance spring and summer parasitism of orchard leafrollers by *C. florus* by providing a nearby overwintering host for this wasp, namely *Ancylis comptana* overwintering as full sized larvae in the rose gardens. The continued work will include studies of dispersal of C> florus from the gardens with Dr. Vince Jones, and continued monitoring of the population trends in gardens planted by my lab and collaborating growers, and finally, continued study of spring and summer parasitism of pest leafrollers in orchards near gardens. Much if this later work will be supported through 2007 by WSARE but the WTFRC will receive updates.