Washington Tree Fruit Research Commission 2004 Apple Entomology Research R
22 January 2004 Wenatchee, WA

Time	Page	PI	Organization	Title
9:00		**McFerson**		**Introduction**
9:05	1	Brunner	WSU - Wenatchee	Stink bug behavior and control
9:10	6	Walsh	WSU - Prosser	Lygus bug thresholds, cultural, and biological control
9:15	12	Cockfield	WSU - Prosser	Thrips biology, migration, and management
9:20	17	Cockfield	WSU - Wenatchee	Feeding, thresholds, and pheromone trapping of Campylomma
9:25	22	Brunner	WSU - Wenatchee	Behavioral-based control tactics for apple pests
9:30	28	Judd	PARC	Codling moth larval aggregation pheromone for IPM
9:35	33	Landolt	USDA - ARS, Wapato	Host plant kairomones as attractants for codling moth
9:40	38	Miliczky	USDA - ARS, Wapato	Extra-orchard host plants and habitats for natural enemies
9:45	44	Unruh	USDA - ARS, Wapato	Habitat modification to control leafrollers
9:50	48	Unruh	USDA - ARS, Wapato	Effects of new insecticides on natural enemies of apple pests
9:55	53	Unruh	USDA - ARS, Wapato	Genetic markers to identify pests
10:00	59	Felsot	WSU - Richland	Pesticides exposure and drift - alternative sprayer technology
10:05	65	Brunner	WSU - Wenatchee	Sensor-webs to monitor and improve pest management
10:10		**BREAK**		
10:30	71	Yee	USDA - ARS, Wapato	Optimizing ammonia with traps to manage apple maggot
10:45	81	Yee	USDA - ARS, Wapato	Re-evaluation of host use by apple maggot
11:00	85	Jones	WSU - Wenatchee	Sampling plans for leafrollers and their natural enemies
11:15	94	Jones	WSU - Wenatchee	Protein markers to determine movement of pests
11:30	101	Landolt	USDA - ARS, Wapato	Insect response to induced defensive apple & pear chemistry
11:45	107	Landolt	USDA - ARS, Wapato	Bait stations for Leafrollers
12:00-1:30		**LUNCH**		
1:30	111	Jones	WSU - Wenatchee	Mechanisms of mating disruption in codling moth and leafroller
1:45	120	Hebert	WSU - Prosser	Chemical release behavior of mating disruption products
2:00	126	Dunley	WSU - Wenatchee	Codling moth OP resistance and associated tolerances
2:15	130	Knight	USDA - ARS, Wapato	Codling moth behavior
2:30	135	Knight	USDA - ARS, Wapato	Monitoring codling moth with the DA lure
2:45	145	Knight	USDA - ARS, Wapato	Removing female codling moths from orchards
3:00	151	Lacey	USDA - ARS, Wapato	Control of codling moth using granulovirus and nematodes
3:15	161	Brunner	WSU - Wenatchee	New pest management program
3:30				POSTER SESSION

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CONTINUING PROJECT REPORT **YEAR 3/3**
WTFRC Project #AE-02-221 **WSU Project #13C-3643-4094**

Project title: Stink bug behavior and control in orchards

PI: Jay F. Brunner

Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee, WA; (509) 663-8181; jfb@wsu.edu

Co-PI and affiliation: Christian Krupke, Tree Fruit Research and Extension Center, Wenatchee, WA

Objectives:
1. Determine the suitability of orchard cover crop plants as hosts that will mature stink bugs.
2. Determine if control programs directed at orchard cover crops would be a practical management strategy for stink bugs without disrupting integrated mite management.
3. Evaluate systems of monitoring stink bugs in orchards (border or internal) that predict arrival of immigrants in late summer and/or occurrence of new adults in the orchard ground cover.
4. Implement a border management program with combinations of aggregation pheromone, attractive plants and feeding stimulants.
5. Determine the potential for attracting stink bugs away from orchards to "trap crops" as a means of reducing orchard invasion or killing stink bugs prior to orchard invasion.
6. Evaluate new candidate pesticides as controls for stink bugs.

Significant findings – 2003:
1. Stink bugs were able to complete development on mullein, common mallow and white clover but not on grass, lamb's quarter or dandelion.
2. D-Vac collections from the orchard failed to indicate that stink bugs were present in the orchard, and this was further supported by fruit injury patterns occurring primarily on orchard borders and not on the interior of orchards.
3. The negative impact of Danitol applied in 2001 on integrated mite management carried over into the spring of 2002 with extreme spider mite densities requiring miticide applications.
4. Danitol applied to the border rows did not reduce damage as much as applications made to the entire orchard.
5. Prototype aggregation pheromone lures provided by commercial companies were tested for the second year.

Methods:
Pyrethroids applied to borders – fruit damage: Three synthetic pyrethroids (Danitol, Warrior, Asana) were applied to orchard border rows at four dates during the period of peak stink bug injury (July 15, July 29, August 12, August 27) and evaluated using counts of damaged fruit at harvest.

Pyrethroids applied to borders – effects upon mite populations: Populations of pest and beneficial mites were recorded before and after insecticide applications in all blocks.

Trapping systems in orchards: Three variations (2, 3 or 4 feet in height) of a pyramid trap were tested during the growing season to determine their relative efficacy in attracting and retaining stink bugs.

Lure evaluations: We evaluated two commercial lures in the field. Lures were attached to mullein plants, and bugs were counted and removed twice weekly. We tested both fresh lures and field-aged (3-week, 6-week) lures.

Attract-and-kill: Using observational experiments, we conducted studies with an attract-and-kill formulation similar to that developed commercially for use against codling moth (Last Call CM). We tested pheromone concentrations of 1%, 3%, 10% and 20% in attracticide formulations.

Results and discussion:
In light of extensive research in 2002 indicating that stink bug damage occurs primarily on orchard border rows, we confined insecticide treatments to these rows only. Counts of damaged fruit indicated no significant differences between any of the treatments and the unsprayed control blocks (Fig. 1). The levels of fruit injury observed in 2003 were much lower than in previous years, suggesting that the natural populations of stink bugs were lower, and this may have influenced the lack of discrimination of treatments in this test. Injury was even lower than that observed in orchards in the same region (Fig. 2) but not part of the study. It is possible that the untreated areas were not large enough and that stink bug populations in these areas were affected by the insecticide-treated areas. Studies in previous years have demonstrated that all of the synthetic pyrethroids used in these trials have high acute toxicity to stink bugs; however, the residual toxicity may not be sufficient to protect the crop at the intervals tested (14 days).

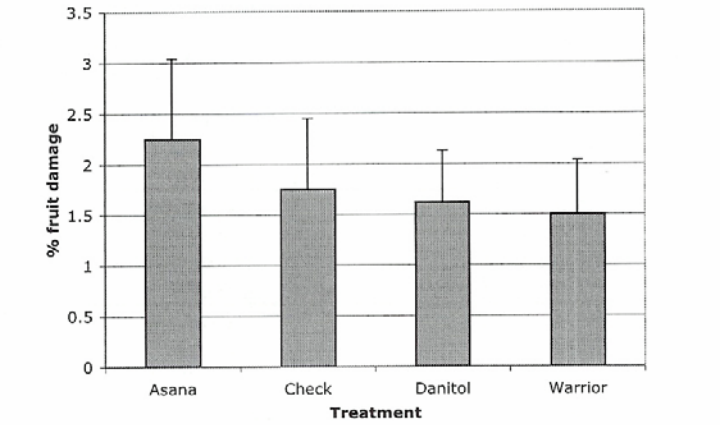


Figure 1. By-treatment distribution of stink bug injury in pyrethroid-treated blocks at harvest; data represent pooled results of four orchards surveyed. No significant differences detected.

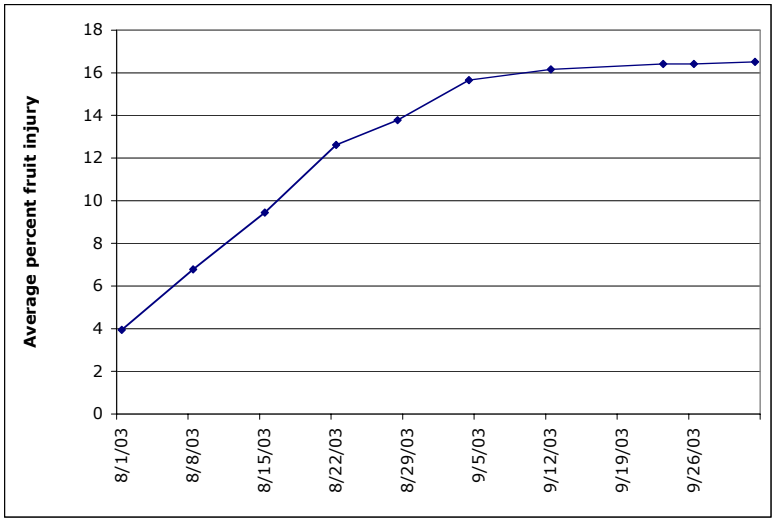


Figure 2. Average percent fruit injured by stink bug feeding on border trees.

We found few significant effects of pyrethroid treatments upon in-season populations of pest and predator mites (Table 1). However, as shown in 2001-2002 research, the disruptive effect may be more apparent in the season following pyrethroid applications, and this should be monitored in 2004.

Three variations of a stink bug trapping system were tested in orchards in 2003. The standard pyramid trap sold by IPM Technologies measures 4 feet in height. However, this trap proved cumbersome and unstable for use in orchards, due to sandy terrain and high winds. Our study found no significant differences in stink bug catch associated with trap height (Fig. 3), indicating that 2-foot traps would be as efficacious for stink bug trapping as full-sized traps. In a separate experiment, unbaited traps were tested in comparison with traps baited with the aggregation pheromone to assess the contribution of the pheromone to trap capture. Pheromone-baited traps captured significantly more stink bugs than unbaited traps in this study (Fig. 4).

Table 1. Comparison of effects of in-orchard insecticide applications to border rows upon populations of pest and predator mites. Significant differences within each date category followed by an asterisk.

DATE	SITE	Ratio of spider mites/predator mites/leaf
07/16/03 (Pre-count)	Check	1.40 : 0.05
	Asana	1.20 : 0.05
	Danitol	1.95 : 0.40
	Warrior	1.65 : 0.10
07/23/03	Check	1.50 : 0.45
	Asana	2.80 : 0.55
	Danitol	1.30 : 0.25
	Warrior	1.75 : 0.95
08/05/03	Check	2.90 : 0.20
	Asana	1.45 : 0.20
	Danitol	0.85 : 0.00
	Warrior	2.50 : 0.00
08/25/03	Check	3.08 : 0.00
	Asana	4.50* : 0.00
	Danitol	0.90 : 0.10
	Warrior	3.25 : 0.00
09/17/03	Check	1.45 : 0.20
	Asana	3.95 : 1.00*
	Danitol	0.75 : 0.10
	Warrior	2.35 : 0.05

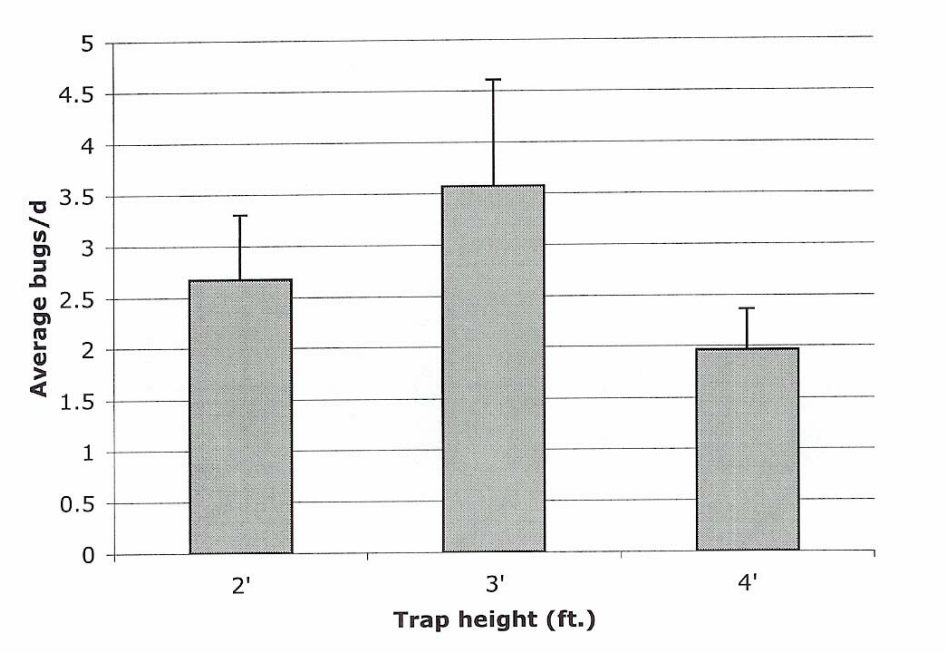


Figure 3. Mean capture of *E. conspersus* in pheromone-baited pyramid traps of three heights. No significant differences detected.

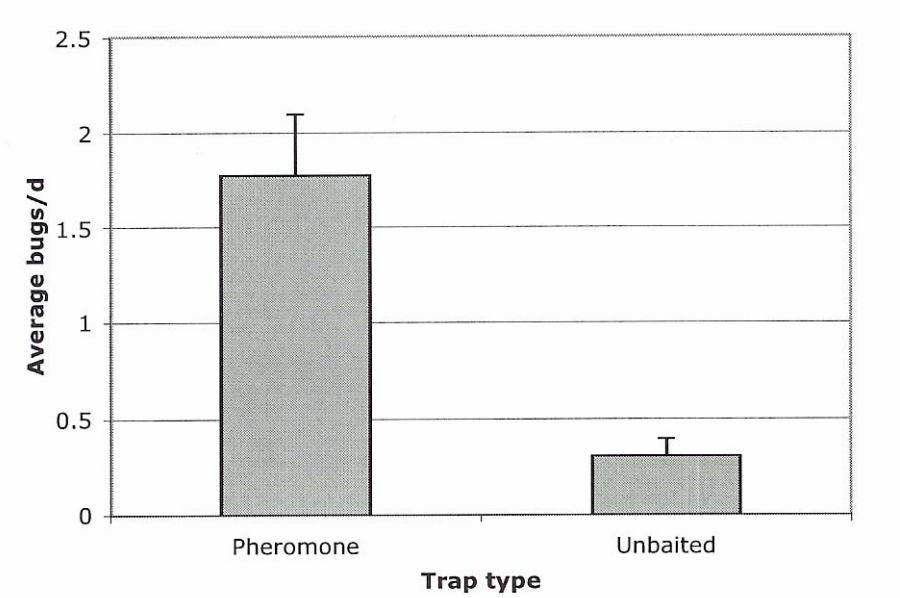


Figure 4. Mean capture of *E. conspersus* in pheromone-baited and unbaited pyramid traps.

In 2003 we continued to test pheromone release devices to develop an optimal lure type for use in monitoring and trapping programs. Two commercial lures were tested, the bubble lure produced by PheroTech Inc., and a polyethylene vial produced by IPM Technologies Inc. Both lures exhibited similar attractiveness when placed on mullein plants (Fig. 5), with a decline in attractiveness of both lures between three and six weeks. Either of these lures would be suitable for a management/monitoring application, such as combination with pyramid traps for in-orchard monitoring or for use in mass trapping initiatives.

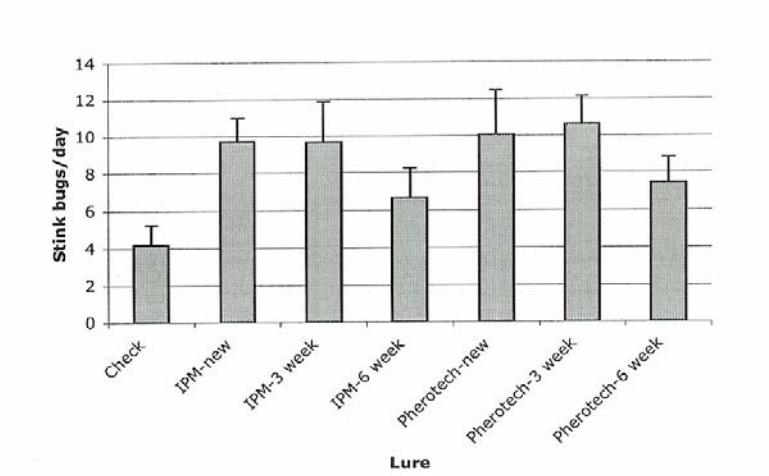


Figure 5. Comparison of field attractiveness of two different lure types placed on mullein plants and comparison with unbaited control plant. No significant differences detected.

In an attempt to exploit stink bug attraction to aggregation pheromone, we assessed the efficacy of an attract-and-kill product that combined the pheromone with a synthetic pyrethroid (permethrin) in a paste formulation. This method does not appear to hold much promise. In extensive field testing stink bugs were shown not to be attracted to any of the concentrations of attract-and-kill droplets tested. This may be due to competition on the plant from other stink bugs producing pheromone.

Budget:
Project title: Stink bug behavior and control in orchards
PI: Jay F. Brunner
Project duration: 3 years (2004 final year)
Current year: 2004
Project total (3 years): $83,798
Current year request: **$**27,847

Item	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Salaries [1] (Krupke)	$12,609	$13,113	**$13,638**
Benefits	1,308	1,361	**1,429**
Wages [1]	8,000	8,000	**8,000**
Benefits (16%)	1,280	1,280	**1,280**
Equipment [4]	1,500	0	**0**
Supplies [2]	1,000	1,500	**1,000**
Travel [3]	2,500	2,500	**2,500**
Total	$28,197	$27,754	**$27,847**

[1] C. Krupke will complete his Ph.D. program in 2004. He will work full time on this project in 2004 while writing his thesis on his own time. Supplemental funding for Krupke is being picked up by IFAFS/RAMP or, if successful, from a new WSU program funding GRAs.

[2] These items involve pheromone, traps and lures, rearing materials to maintain a stink bug colony. Cell phone charges are allowed.

[3] Pays for a vehicle for six months used full time on this project plus fuel and maintenance costs.

[4] D-vac sampling device for collecting stink bugs from cover crops and native habitats.

CONTINUING PROJECT REPORT **YEAR 2/3**
Organization Project # 13C-3343-3123

TITLE: ***Lygus*** **bug thresholds, cultural and biological control in Washington State apple orchards**

PI: D.B.Walsh, Agrichem./Environ. Educ. Spec., WSU- Prosser

Cooperator(s): M. Bush, Extension Agent, Yakima Valley Region
H. Ferguson, Extension IPM Coordinator
R. Zack, Associate Professor, WSU Entomology
T. Waters, Research Assistant, WSU Entomology

OBJECTIVES:

1. Develop economic thresholds for *Lygus* on apples.
2. Evaluate cover crops/ indigenous plants for their ability to increase survivorship/ effectiveness of Lygus parasitoids.
3. Survey conventional and organic apple orchards for the presence of Lygus parasitoids.
4. Conduct innoculative releases of *Peristenus* spp. into established refugia in apple growing regions where *Peristenus* has not been detected.
5. Conduct orchard floor treatments with formetanate hydrochloride and several candidate synthetic pyrethroid insecticides

SIGNIFICANT FINDINGS- ***LYGUS***

1. ***Lygus*** **Economic Thresholds.** Branch cage studies were conducted for a 3rd year to determine the period of time at which Lygus feeding resulted in the most damage. Previous studies in 2001 and 2002 helped quantify proportional *Lygus* abundance to fruit damage. From these studies we determined that Lygus feeding can result in sub-surface superficial feeding injury at any point between fruit set and harvest, but that early to mid-spring feeding is cosmetically more damaging then summer feeding. Our 2003 results confirm our previous results.

2. Cover Crops/ Indigenous Plants. Replicated plots of 14 cover crop blends were established on the Roza unit at WSU IAREC in May 2003. We have documented significant differences among cover crop blends in their potential to build populations of Lygus bugs. We also established replicated stands of indigenous native and exotic plants and we will begin evaluating these plants for their ability to serve as complete hosts for Lygus in 2004.

3. Biological Control/ Orchard Surveys for Lygus Parasitoids. A parasite *Peristenus* spp. attacks the nymph stages of Lygus and keeps individuals from reaching sexual maturity by emerging in the late instar nymph or early adult stage. Extensive surveys conducted by Walsh in 2002 and 2003 determined the presence of Lygus parasitism by *Persitenus* spp. in several important fruit production regions in Washington State. However, the results of the survey were disappointing in that levels of parasitism were low or not detected in several important stone fruit growing areas

4. Inoculative Releases of ***Persitenus*** **into refugia.** We have identified several candidate research sites of interest from our 2002 and 2003 *Peristenus* spp. surveys. In June 2004 we will be capturing Lygus nymphs from areas surrounding the Touchet area. This is the area from which we have detected the greatest incidence of parasitized Lygus nymphs. The captured nymphs will be released into refugia at our candidate sites.

5. Orchard Floor Treatments. We have now received support and cooperation from Gowan Co. for testing the efficacy of orchard floor treatments with formetanate hydrochloride. In 2004 we

will compare the efficacy of formetanate hydrochloride with lambda-cyhalothrin, zeta-cypermethrin, and permenthrin as orchard floor treatments. The additional private funding we have received will be used to evaluate the efficacy of these products against western flower thrips.

METHODS

1. **Lygus economic thresholds.** Sleeve cages were sewn in 2001 that covered 1 meter lengths of apple branch. Fruit was thinned so that constant ratios of Lygus to apples can be maintained. Adult *Lygus* were introduced into the sleeve cages at ratios of 0.25, 0.17, 0.125, 0.083, 0.056, 0.033, and 0.015 Lygus per fruit. Each cage treatment was replicated 4 times on Fuji fruit set in April and mid-season in July 2001. These same trials were repeated on May 24, 2002. Cages were left on the trees for 2 weeks and when they were removed the branches were treated with acephate. A damage assessment was taken just prior to commercial harvest. Additional cage studies were conducted in 2002 and 2003 in which 3 replicate cages were established weekly from June through August in which fruit was thinned to 10 and in each cage 10 Adult Lygus bugs were placed. This was to determine if damage impacts from Lygus feeding changed over time as the growing season progressed.
2. **Orchard floor cover crops.** Replicated plots of 14 cover crop blends were established on the Roza unit at WSU IAREC in May 2003. Irrigation was applied by handline sprinklers and irrigation was applied to mimic recommended orchard management practices. Sweep net surveys were conducted every 2 weeks and the number of Lygus, thrips and spiders captured was quantified and calculated.
3. **Biological Control.** A parasite *Peristenus* spp. attacks the nymph stages of Lygus and keeps individuals from reaching sexual maturity by emerging in the late instar nymph or early adult stage. Extensive surveys conducted by Walsh in 2002 and 2003 determined the presence of Lygus parasitism by *Persitenus* spp. in several important fruit production regions in Washington State. However, the results of the survey were disappointing in that levels of parasitism were low or not detected in several important stone fruit growing areas.

RESULTS/ DISCUSSION LYGUS-

***Lygus* Damage.** *Lygus* feeding has been likened to chemical injury. *Lygus* feeding damage in apple orchards is a significant concern after fruit set, however feeding damage can result in fruit disfigurement during the fruit growing season. Branch cage studies in 2001 and 2002 have helped quantify proportional *Lygus* abundance to fruit damage. Three sets of sleeve ages were placed on branches of Fuji trees. Fruit was hand thinned within each cage and specific numbers of adult *Lygus* were added to each cage to produce specific ratios of fruit to *Lygus* bug in each respective cage. Ratios of fruit to Lygus per cage included 0, 4, 6, 8, 12, 18, 30, & 60 fruit per Lygus. Cages were left on for 2 weeks at each cycle in April and July 2001 and May 2002 and then removed. Each caged tree branch was then treated with acephate (Orthene) to prevent subsequent feeding injury from occurring. On August 30, 2001 an.d September 10, 2002 ten fruit were removed from each cage site and peeled with a paring knife. Lygus damage was noted if necrotic feeding spots were present below the fruit skin surface. Our estimates for fruit damage are much higher then typical consumer standards. A majority of *Lygus* feeding damage was not observable above the fruit skin surface. However, April feeding injury was greater then feeding damage in May or July (Figures 1, 2, & 3) . In 2002 and 2003 we also designed a sequential sample experiment in which we established cages weekly from June through August. One meter sleeve cages were placed over tree branches on which fruit had been thinned to a total of 10. Ten adult Lygus bugs were placed into 3 replicate cages each week. In September ten fruit were removed from each cage site and peeled with a paring knife. Lygus damage was noted if necrotic feeding spots were present below the fruit skin surface. Lygus feeding damage was greatest in May or June in both years (Figures 4 and 5). Damage for every other week appeared to be fairly consistent.

Figure 1. April 2001 Feeding Injury

Figure 2. July 2001 Feeding Injury

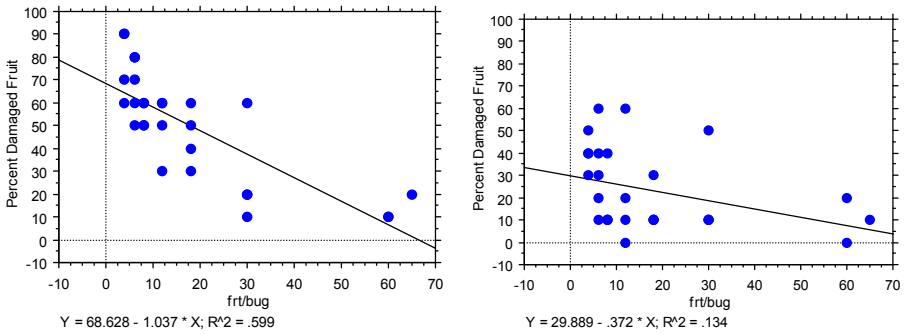


Figure 3. May 2002 Feeding Damage

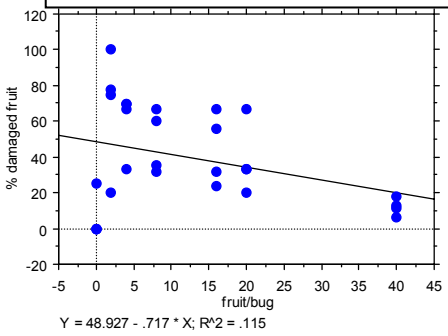


Figure 4. Sequential feeding injury 2002

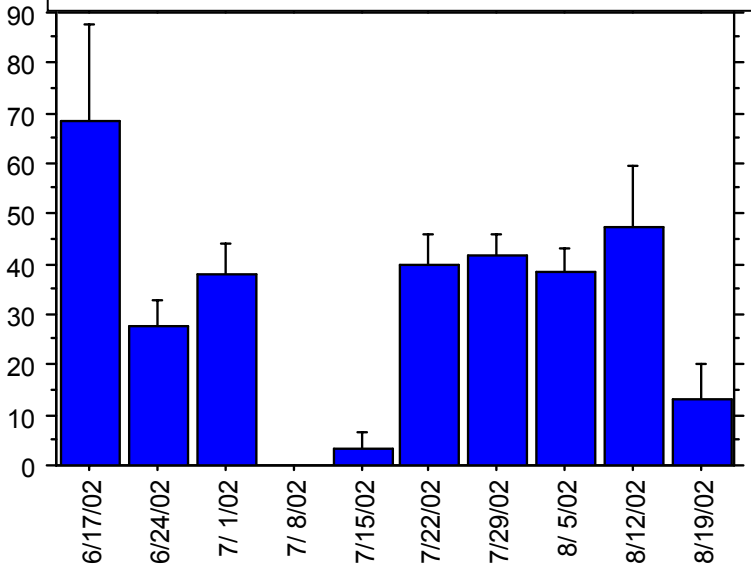
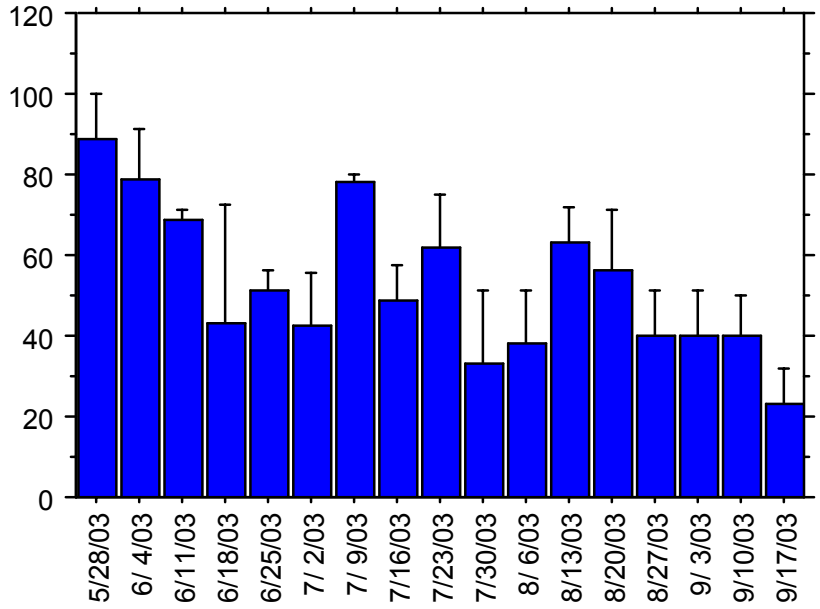
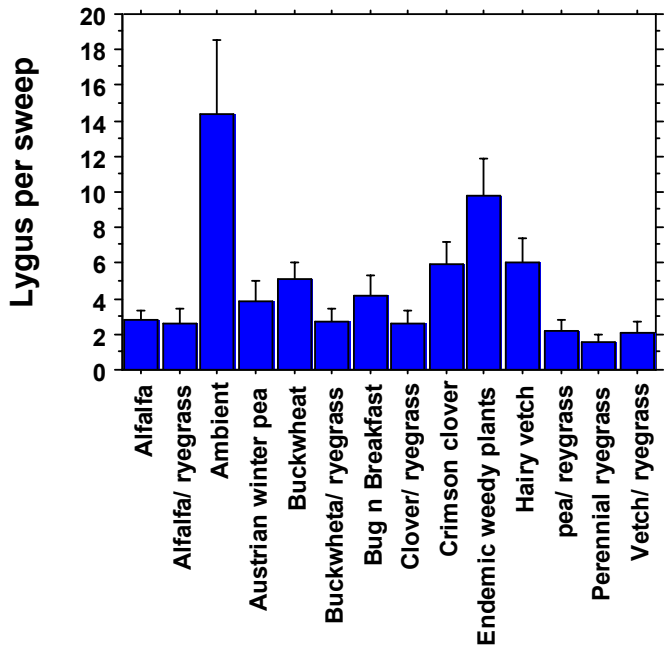


Figure 5. Sequential feeding injury 2003



Orchard floor cover crops. Replicated plots of 14 cover crop blends were established on the Roza unit at WSU IAREC in May 2003. Cover crop blends included perennial rygrass, buckwheat, buckwheat/ryegrass, alfalfa, crimson clover, hairy vetch, alfalfa/ryegrass, clover/ryegrass, vetch/ryegrass, Austrian winter pea, pea/ryegrass, Bug n Breakfast, and naturalized and endemic weeds. Irrigation was applied by handline sprinklers and irrigation was applied to mimic recommended orchard management practices. Sweep net surveys were conducted every 2 weeks and the number of Lygus, thrips and spiders captured was quantified and calculated. In total the cover crop plots were sampled 6 times on 30 June, 14 July, 29 July, 12 August, 9, September and 22 September respectively. Analysis of variance demonstrated that there were no significant differences in Lygus populations among the sample dates so all the dates were pooled.

Lygus bugs per sweep- all sample days pooled



In 2003, the "Ambient" and "Endemic weedy plants consisted primarily of pigweeds and barnyard grass. These plots are essentially the same but have extra plots will prove helpful in the future as we expand these studies. We can conclude that all of the cover crops were superior to no weed control in reducing populations of Lygus bugs as estimated by sweep net samples.

Biolgical control. A parasite attacking Lygus spp. was discovered in 1995 in Washington State and subsequent collections in Parma, Idaho in 1996 and 1997 showed that the parasite was present (Mayer unpublished data). The parasite has been described as *Peristenus howardi* Shaw (Hymenoptera: Braconidae), a new species . Previously, *Peristenus pallipes* Curtis was reported from Idaho. However, recent taxonomic work on the genus indicates that these may have been misidentified. *Peristenus* spp. attacks the nymph stages of Lygus and keeps individuals from reaching sexual maturity by emerging in the late instar nymph or early adult stage.

Collections made in 2000 (Mayer, unpublished data) did not document the parasite's presence beyond the Touchet, Washington and Parma, ID regions. Extensive surveys conducted by Walsh (AE News 2003) in 2002 and 2003 determined the presence of Lygus parasitism by Persitenus spp. in several important fruit production regions in Washington State. However, the results of the survey were disappointing in that levels of parasitism were low or not detected in several important fruit growing areas Extensive surveys in 2002 and 2003 determined the presence of Lygus parasitism by *Persitenus* spp. In total over 75 sites were surveyed and over 8,000 Lygus were dissected to determine if *Peristenus* spp were present. Parasitism by of Lygus by *Peristenus* was greatest in areas that were less disturbed by human activity.

BUDGET:

Project title: *Lygus* bug thresholds, cultural and biological control in Washington State apple orchards

PI: D.B.Walsh, Agrichem./Environ. Educ. Spec., WSU- Prosser

Cooperator(s): M. Bush, Extension Agent, Yakima Valley Region
H. Ferguson, Extension IPM Coordinator
R. Zack, Associate Professor, WSU Entomology
T. Waters, Research Assistant, WSU Entomology

Project duration: 2003 – 2006

Current year: 2004

Project total (3 years): $71,416

Current year request: $24,000

Year	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Total	23,416	**24,000**	24,000

Current year breakdown

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3(2005)
Salaries	8,766 Admin Prof 0.20 FTE	**12,360** **Admin. 0.25 FTE**	12,855 Admin Prof. 0.25 FTE
Benefits (31%)	2,717	**3,708**	3,857
Wages	5,000	**4,011**	3,456
Benefits (16%)	800	**642**	553
Equipment			
Supplies	3,233	**379**	379
Travel	2,900	**2,900**	2,900
Miscellaneous			
Total	23,416	**24,000**	24,000

OTHER SUPPORT OF PROJECT: Walsh drafted a companion proposal to the WSCPR that was funded in November 2003 to support this project in 2004. An additional proposal will be submitted to the WSCPR for consideration at their November 2004, funding meeting for continuation of this project though its final year in 2005. An additional proposal to the WSU Ag Research Center for support of a Graduate Research Assistant (Tim Waters) will be submitted in January 2004 to support his graduate studies through 2005.

CONTINUING PROJECT **YEAR 2/3**
WTFREC 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

Co-PIs and affiliations:
Stephen D. Cockfield, Associate in Research, WSU Tree Fruit Research and Extension Center, Wenatchee, WA
David Horton, Research Entomologist, USDA-ARS, Wapato, WA
Gene Miliczky, Research Associate, USDA-ARS, Wapato, WA
Vince Jones, Associate Entomologist, WSU Tree Fruit Research and Extension Center, Wenatchee, WA
John Dunley, Associate Entomologist, WSU Tree Fruit Research and Extension Center, Wenatchee, WA
Rich Zack, Assistant Entomologist, WSU Dept. of Entomology, Pullman, WA

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 ground cover 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. Populations of thrips in apple flowers were highest on the orchard border next to sagebrush steppe, indicating significant migration from dry uncultivated areas into the orchard.
2. Milk and egg whites show promise for a method of marking thrips as they visit different flower hosts.
3. Rubidium was effective in permanently marking thrips on the host plant from which they originated.
4. In the first year of comparisons between thrips populations in weedy and weed-controlled blocks, very little reduction in thrips was measured. Management of thrips by control of weeds may be successful after many seasons.

Methods:

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

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

This investigation was done jointly by personnel from WSU-TFREC, Wenatchee, and USDA-ARS, Yakima.[1] Orchards had one or more edges bordered by uncultivated land. Seven blocks were selected to include a variety of climates. 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 pink, king bloom, full bloom and petal fall at the edge, 100, 200 and 300 feet into the block. Twenty-five open apple flowers and 25 dandelion

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

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 flowering, and total adult thrips were counted at petal fall. Data were analyzed with Analysis of Variance to detect if a population density gradient corresponded to the distance from the wild habitat.

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

In 2003, a number of greenhouse studies were conducted to find effective methods of marking thrips. The objective was to select methods that could be used in field trials in 2004 and 2005. Two categories were tried, protein sprays and Rubidium. Proteins were used as an inexpensive, external marker that can be detected by ELISA, a common assay technique used in medical diagnosis. Three non-toxic protein products were used: soy milk, egg whites and cow's milk. Products were sprayed on a number of common plants from the orchard and the surrounding sagebrush steppe, as well as on plants that could be kept in bloom in the greenhouse. In the second technique, Rubidium was incorporated into a plant or insect in place of Potassium. It becomes a permanent, internal marker that can be detected with specialized equipment. On blooming greenhouse plants, both Rubidium and protein products were applied, and thrips were periodically monitored to test the longevity of the marks. Rubidium chloride in a 500 ppm solution was applied to the soil of dandelions and marigolds in two separate trials. Soy milk in a 10% or 20% solution was sprayed on dandelion, bitterbrush flowers, arrowleaf balsamroot, sweet alyssum and marigold. Egg whites in a 10% solution and milk in a 15% solution were sprayed on dandelion, sweet alyssum and marigold. Plants were confined in a large insect cage, and yellow sticky cards were placed above the plants to catch adult thrips. Thrips were removed and tested individually with ELISA for the presence of proteins. About 30 thrips were assayed together at each sample time for the level of Rubidium accumulated internally. Some of the trials have been completed at the time of writing, and others will continue to be done throughout the winter months.

2. Management of resident WFT populations by reducing alternative hosts within the orchard.

Four orchards were selected with the following cultivars and locations: 'Braeburn' in Quincy, Pateros and Brewster and 'Granny Smith' in Bridgeport. Each orchard was approximately 5-10 acres. One-half of each block received regular herbicide treatments to eliminate broadleaf weeds over time. The other half received herbicides only in the tree rows, as the grower normally managed the block. This was the first year of the management regimes. The plots will be managed and sampled for two years to measure the reduction in WFT over time (2003-2005). At approximately 3-5 m intervals, ten 1-m squared areas were randomly selected and marked in the drive, or middle, row of each treatment block. Within these areas, dandelion plants were counted every month beginning in April. The number of dandelions in flower was also recorded. Each month beginning in April, dandelion plants were collected at random locations within the middle row. Four plants without flowers and four plants with flowers (if available) were selected. In May during full bloom and before insecticides were applied, 25 open apple blossoms were selected from eight trees within the middle row. In June, July and August 10 buds were collected from each of eight trees. Plant tissue samples were washed with soapy water to dislodge adult and immature thrips. Representative specimens were slide-mounted for identification.

3. Susceptibility of apple bloom stages to WFT damage.

This experiment is scheduled to begin in the spring of 2004. Preparations were begun in 2002, when 'Braeburn' trees on dwarf rootstock were planted in pots. These plants were stored in a cold room for the winter of 2002-2003 and moved outdoors in March. Trees bloomed about two weeks later than local orchards. The trees were grown for another year in 2003 and will be ready to bloom for the experiments in 2004.

Results and discussion:

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

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

The distance into the orchard was a significant factor in explaining the variance in samples, after first accounting for the differences in population between orchards (ANOVA, 3 df, $P<0.005$). According to results of a multiple range test on the means, the numbers of thrips collected at the border were significantly higher than the numbers collected inside the orchard (LSD test, significance level = 0.05, 18 df.; Fig. 1). Apparently most of the thrips entering the orchard from surrounding sagebrush stay within 100 feet of the border. The time of the migration may be spring or fall. The strong and encouraging results of this experiment indicate that further study is necessary, and the sampling will be continued for another year.

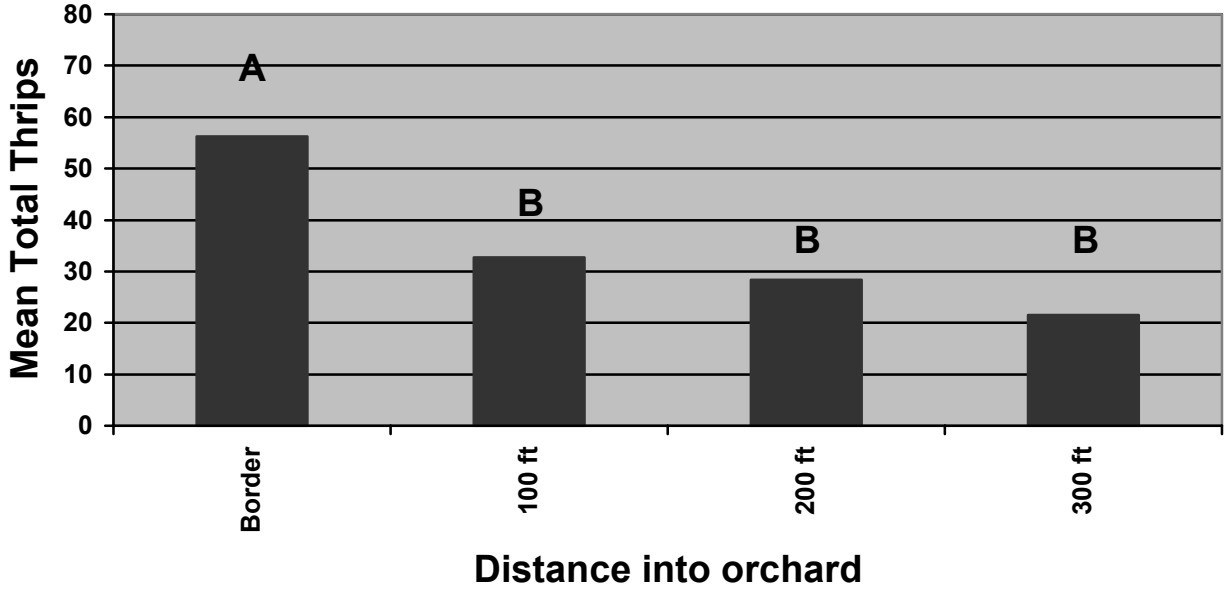


Fig. 1. Mean total adult thrips collected in samples from pink to petal fall in seven orchards. Means followed by the same letter are not significantly different (LSD test, significance level = 0.05, df = 18).

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

At the time of writing, one-third of the samples had not been processed. Throughout the year, Dr. Vince Jones worked to develop the ELISA technique, and development is continuing. From the completed work, the proteins appear to be a good external mark for thrips. Milk and egg whites are the most promising. Protein solutions can be sprayed successfully on many of the plant species inside

and bordering an orchard and, with some of the proteins, a great majority of thrips will retain the mark. The Rubidium technique can be used successfully with thrips feeding on small plants such as dandelion. Adult thrips incorporate some Rubidium within a day of application. The strongest mark results from larvae feeding on Rubidium-fertilized plants throughout their development. Rubidium could be used, for example, to follow overwintering adults in the fall from their larval host to apple flowers the next spring. Proteins can be used to follow the migration of those adults as they visit various flower hosts, on the orchard floor or outside the orchard, before moving to apple.

2. Management of resident WFT populations by reducing alternative hosts within the orchard.

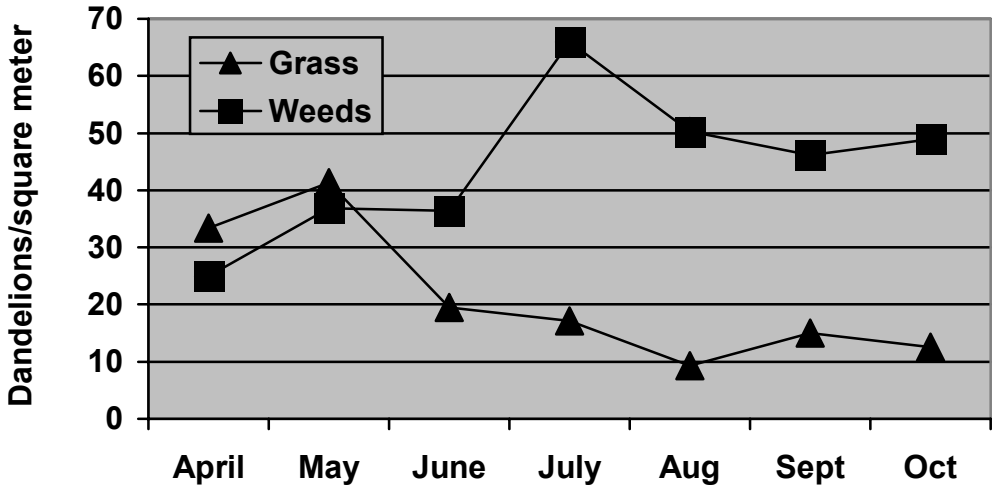


Fig 2. Mean dandelion populations in paired herbicide-treated (grass) and non-treated (weeds) blocks within four orchards, 2003.

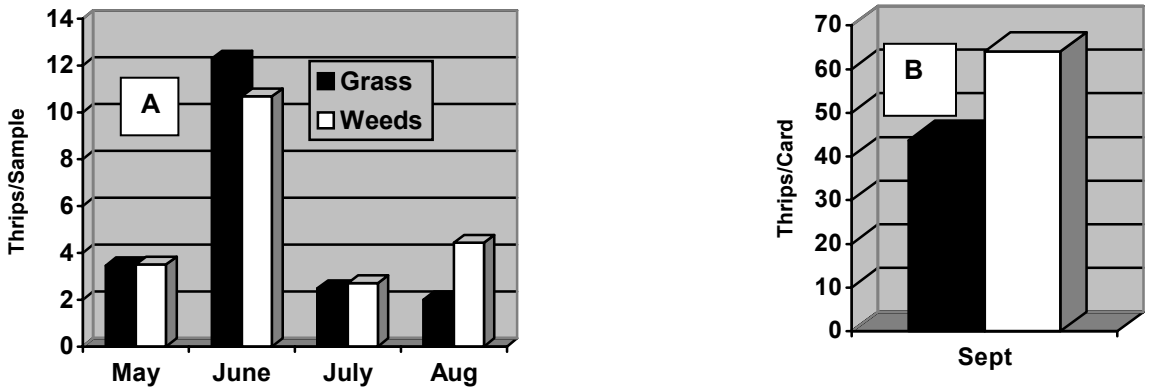


Fig 3. (A) Mean thrips per 25 flowers (May) and 10 shoots (June-Aug) in herbicide-treated (grass) and non-treated (weeds) blocks, 2003. (B) Mean thrips caught per sticky card in the same blocks.

Dandelion populations were similar in each half of each orchard at the start of 2003. Most of the orchards required two or more herbicide applications to reduce weeds. By August, dandelions were

reduced in the treated half at most sites (Fig. 2). However, new seedlings had emerged by September, and herbicides will need to be reapplied throughout 2004. Dandelions are an indicator species; other thrips hosts, such as white clover, were also reduced. Thrips populations in the apple trees in treated blocks were not greatly altered in 2003, except perhaps at the end of the summer and fall (Fig. 3 A,B). By remaining a host for thrips throughout the summer, the apple trees might overwhelm any effects of host plant populations on the orchard floor. This experiment will continue for another two years, during which time thrips populations may be reduced in non-weedy blocks.

3. Susceptibility of apple bloom stages to WFT damage.

Preparations for this experiment were completed in 2003. Data will be gathered in 2004 and 2005.

Budget:
Project title: Biology, migration, and management of Western flower thrips in apple orchards
PI: Elizabeth H. Beers
Project duration: 2003 through 2005
Current year: 2004
Project total (3 years): $108,414
Current year request: $ 35,131

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Salary[1] (0.60 FTE, 12 mo)	$21,300	**$22,378**	$23,273
Benefits (34%)	7,029	**7,609**	7,913
Wages[2]	3,900	**900**	900
Benefits (16%)	624	**144**	144
Equipment[3]	600	**600**	600
Supplies[4]	1,500	**1,500**	1,500
Travel[5]	2,000	**2,000**	2,000
Miscellaneous	-	**-**	-
Total	$36,953	**$35,131**	$36,330

[1] Salary for Steve Cockfield.
[2] Time-slip wages.
[3] Computer (60%).
[4] Lab and field supplies. Cell phone charges are allowed on this grant.
[5] Local travel to plots for sampling.

CONTINUING PROJECT **YEAR 3/3**
WTFREC Project #AE-02-220 **WSU Project #13C-3643-4386**

Project title: Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci*

PI: Elizabeth H. Beers, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee

Co-PI and affiliation: Stephen D. Cockfield, Associate in Research, WSU Tree Fruit Research and Extension Center, Wenatchee

Objectives:
1. Modify and validate fall pheromone trap sampling as a method of identifying high-risk orchards for spring sampling.
2. Determine the relative susceptibility to campylomma damage of apple cultivars other than 'Delicious' and 'Golden Delicious.'
3. Develop provisional treatment thresholds on susceptible apple cultivars that currently have none.

Significant findings:
1. Pheromone traps used the previous fall in 'Delicious' orchards were equally as effective as tap samples in the spring for detecting injurious population levels. Pheromone traps were more effective than spring tap samples in 'Golden Delicious' orchards.
2. The economic injury level for campylomma on 'Gala' appears to be near one nymph per tap.
3. Injury studied on 'Gala' appeared as russet, tiny dark spots, or as a combination of bumps and dimples. Most injury grew less visible as the apple matured.
4. From trial results in 2003, just as in 2002, the susceptibility of the cultivars 'Gala,' 'Granny Smith' and 'Fuji' was similar to that of 'Delicious' and lower than that of 'Golden Delicious.' The susceptibility of 'Cameo' was similar to that of 'Delicious' in 2002 but similar to that of 'Golden Delicious' in 2003.

Methods for 2003 research:
1. Apple cultivar susceptibility to campylomma feeding.
Trees of the cultivars 'Golden Delicious,' 'Delicious,' 'Fuji' and 'Gala' were selected in a block at TFREC, Wenatchee, Washington. The trial on 'Granny Smith' was located in Omak, and the trial on 'Cameo' was located in Bridgeport. At king bloom, campylomma nymphs were collected in an orchard in Orondo. Second instar nymphs were placed in 15x20-cm sleeve cages placed over flower clusters at each site. Flowers in the cages were pruned to a single king bloom and two leaves. Flowers were pollinated before closing the cages, and nymphs were allowed to feed for a week. Each cultivar had 20 cages containing two campylomma nymphs, and there was a corresponding number of check (empty) cages. Fruit injury was assessed at petal fall and the presence of campylomma nymphs recorded. Damaged fruits were photographed at harvest. Data were analyzed using two-way analysis for comparisons of damage/no damage frequency. Data for each cultivar were compared with the cultivars 'Golden Delicious' and 'Delicious.'

2. Development of an economic threshold for 'Gala.'
Ten 'Gala' trees were selected at TFREC, Wenatchee, Washington, for artificial infestations. Cages were placed on these trees on April 29. Cages consisted of a fabric tube slipped over a branch with blossom clusters approximately at king bloom. Caged portions of the branches corresponded to the width of a beating tray (45 cm). Blossoms were pollinated with crabapple flowers before closing. Each of the four cages received 0, 10, 20, 30 or 40 nymphs.

Cages were removed at petal fall, and tap samples were taken of each branch. Fruits were evaluated for campylomma feeding injury in May and after June drop. In May, all injury was counted. By July, most of the injury was barely visible, and only economically significant damage was counted. A final evaluation was done at harvest in August. Using linear regression, the initial density of nymphs was compared with the proportion of fruit injured.

3. Pheromone traps to determine risk level for spring sampling of campylomma.
Ten 'Delicious' and 10 'Golden Delicious' orchards were selected from Quincy to Omak, Washington, to serve as test subjects of the fall pheromone trapping technique. Each block consisted of 10 acres and was divided into four 2.5-acre sections. Pheromone trap procedures were followed according to Reding (2000). Four delta traps, loaded with campylomma pheromone lures, were each placed 2 m above the ground in the center of a 2.5-acre quadrant of a 10-acre block. Traps were set out before August 1, and males were counted every 4-6 weeks when the trap liners and lures were changed. Traps were collected after November 1, and the total number of males caught per trap was recorded.

The risk of encountering a high spring population of nymphs was calculated according to the thresholds for delta traps. The thresholds were 175 nymphs per trap for 'Delicious' and 125 for 'Golden Delicious.' In October secondary pest populations were assessed in each block. Twenty-five spurs were randomly selected and examined under a microscope for ERM eggs. One hundred shoots were examined in the field for signs of aphid colonies.

The following spring, 25 tap samples were taken around the area of each trap, for a total of 100 tap samples per block. Samples were taken as far into the egg hatch period as possible during the time of fruit susceptibility. Most samples on 'Delicious' were taken at petal fall, while on 'Golden Delicious' most of the samples were taken the day before the blocks were sprayed for campylomma, usually between full bloom and petal fall. Each block was determined to be above or below threshold according to procedures outlined in Beers et al. (1993). In the summer, 400 fruits were examined per block, and the percentage with significant campylomma damage was determined.

Results and discussion:
1. Apple cultivar susceptibility to campylomma feeding.
As in 2002, none of the 'Braeburn' fruits in the trial at TFREC had any damage, and there was no damage to any of the fruits in the check (empty) cages. The percentage of 'Golden Delicious' fruit damaged was higher than that of 'Delicious.' Results of the trials on 'Gala,' 'Fuji,' and 'Granny Smith' were the same as in 2002; that is, damage was significantly lower than that of 'Golden Delicious' but not 'Delicious' (2X2 χ^2, α=0.05) (Fig. 1). In 2003 'Cameo' was damaged at about the same frequency as 'Golden Delicious' but not 'Delicious.' Results for 'Cameo' were different in 2002.

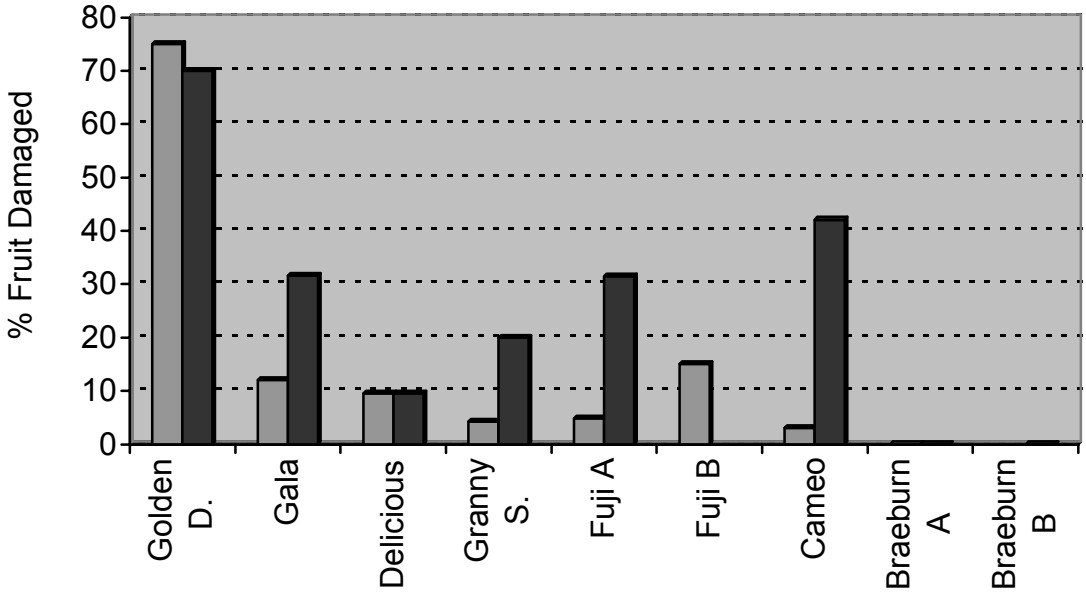


Figure 1. Percentage of fruit damaged by campylomma in 2002 (gray bars) and in 2003 (black bars).

2. Development of an economic threshold for 'Gala.'

Initial nymph densities ranged from 1.02 to 2.28 per flower. Tap samples taken at petal fall, when cages were removed, ranged from 3-36 nymphs per tap. Tap samples recovered 42% of the nymphs introduced to the cages. The branches had an average of 15 flowers along the 45-cm branch length. Of these, an average of 9/branch had formed fruit in late May, and 5.8/branch remained on the tree by late July.

Most of the injuries caused by nymphs were single, raised growths or rough areas smaller than 0.5 mm diameter. By July, many of these were barely visible, less than 1 mm, and resembled an abnormal lenticel. A few injuries were larger than 0.5 mm initially and expanded to a wider area by harvest. Significant injuries sometimes resembled russet or large bumps. Many were surrounded with a dent in the apple surface or caused extreme growth reduction around the feeding site. Some fruits had multiple injuries.

A preliminary regression of initial nymph density and economic injury at harvest yielded the following equation:

Proportion of fruit injured = 0.00467 (nymphs/branch)

With an R-squared of 0.69.

Based on the regression equation, a nymph density of 2.14 per 45-cm branch length would result in 1% fruit injury. Given that 42% of the nymphs would be recovered by tap samples, the tap sample threshold would be 0.89 per tap. To avoid injury above 3%, the threshold would be 2.7 per tap. Published thresholds for 'Golden Delicious' and 'Delicious' are 1.0 (3% fruit injury) and 4.0, respectively (Beers et al. 1993). More data will be gathered in 2004, and the entire data set will be analyzed.

3. Pheromone traps to determine risk level for spring sampling of campylomma.
Some blocks of both cultivars were rated high risk by the pheromone trap data, although most of the 'Golden Delicious' blocks were in this category (Table 1). Most of the trap results for both 'Delicious' and 'Golden Delicious' were accurate: 9 of the 10 'Delicious' blocks and 5 out of the 10 'Golden Delicious' blocks. Many of the blocks had high populations of secondary pests, such as ERM eggs. Campylomma females may selectively lay eggs on branches with potential prey items for their young, e.g. European red mite. It had been previously stated in the literature (Edwards 1998) that the lack of prey items during late summer and fall may discourage oviposition in the orchard and thus cause false positive results. Similarly, spring applications of chlorpyrifos and oil may cause false positive results in the risk assessments. One of the 'Delicious' blocks and five of the 'Golden Delicious' blocks had false positive results for pheromone trap data. All of these blocks received chlorpyrifos, and all of the "Golden Delicious' blocks received a treatment for campylomma, such as Carzol.

Tap samples, the only sampling method in current, widespread use, were far less accurate than trap results in 'Golden Delicious' blocks (Table 1). Three out of 10 blocks had tap samples below threshold and failed to warn of significant fruit injury. In these blocks, however, the pheromone traps did warn of high risk. The tap samples used in the research (100 taps/block) were more extensive than samples taken by consultants (often 25 taps/block). Data will be gathered for another flowering season, and it will remain to be seen whether tap samples will continue to yield such unreliable results.

Table 1. Results of pheromone traps, tap samples, and fruit inspections, first trapping season 2002-2003.

Cultivar	Location	Block	Risk[1]	Tap sample results[2]	injury[3]
'Delicious'	Brewster	1	low	no	no
		2	low	no	no
		3	low	no	no
		4	low	no	no
		5	low	no	no
		6	low	no	no
		7	low	no	no
	Orondo	8	high	yes	yes
	Quincy	9	high	no	no
		10	low	no	no
'Golden Delicious'	Omak	1	low	yes	no
		2	high	no	no
	Brewster	3	low	no	no
	Quincy	4	high	no	no
		5	high	no	no
		6	high	no	yes
		7	high	no	yes
		8	high	no	yes
	E. Wenatchee	9	high	no	no
		10	high	no	no

[1]Based on pheromone traps operated from August 1 to November 1, 2002. Low is below threshold catch for cultivar, high is above threshold catch.
[2]100 tap samples taken per block at petal fall or one day before spray date, 2003. "Yes" is above threshold for cultivar; "no" is below threshold.
[3]Based on 400 fruit samples taken per block during the summer 2003. "Yes" is above 3% injury; "no" is at or below 3%.

Literature cited:

Beers, E.H., J.F Brunner, M.J. Willett, and G.M. Warner. 1993. Orchard pest management: a resource book for the Pacific Northwest. Good Fruit Grower, Yakima, WA.
Edwards, L. 1998. Organic tree fruit management. Certified Organic Associations of British Columbia, Keremos, B.C.
Reding, M. E. 2000. Biology, monitoring, and management of *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) in Washington apple orchards. Ph.D. Thesis, Washington State University, Pullman.

Budget:

Project title: Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci*
PI: Elizabeth H. Beers
Project duration: 2002 through 2004
Current year: 2004
Project total: $80,131
Current year request: $26,591

Item	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Salary (0.40 FTE, 12 mo)[1]		$14,918	**$15,515**
Benefits (34%)		5,967	**6,206**
Wages[2]		1,440	**1,440**
Benefits (16%)		230	**230**
Equipment[3]		400	**-**
Supplies[4]		1,200	**1,200**
Travel[5]		2,000	**2,000**
Miscellaneous			
Total	$27,385	$26,155	**$26,591**

[1] Salary for Steve Cockfield, Associate in Research.
[2] Time-slip wages.
[3] Partial cost of computer.
[4] Lab and field supplies. Cell phone charges are allowed under this grant.
[5] Local travel to plots for sampling.

CONTINUING PROJECT REPORT **YEAR 3/3**
WTFRC Project #AE-02-222 **WSU Project #13C-3643-4093**

Project title: Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm.
PI: Jay F. Brunner
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Avenue, Wenatchee, WA; phone 509-663-8181; fax 509-662-8714; jfb@wsu.edu
Co-PIs and affiliations: Betsy Stutzman (formerly Valdez), Associate in Research, Tree Fruit Research and Extension Center, Wenatchee, WA;
Peter Landolt, USDA-ARS Yakima

Objectives:

1. Evaluate sprayable pheromone systems for behavioral control of codling moth.
2. Evaluate fiber pheromone formulations for behavioral control and attract & kill possibilities for leafrollers and codling moth.
3. Evaluate a formulation of an attract & kill technology, LastCall, for leafrollers in large field trials for efficacy and rate effect.
4. Develop a bait & kill system for control of lacanobia fruitworm and assess the impact on other noctuids in orchards.

Significant findings – 2003:

1. Suterra CM-F sprayable pheromone contributed to suppression of codling moth (CM) activity when combined with supplemental insecticides in low-pressure orchards. Higher rates of CM-F sprayable pheromone performed as well in low-pressure orchards when compared with hand-applied dispensers at half rates. CM-F sprayable pheromone does not perform as well as the full rate of hand-applied dispensers in moderate- to high-pressure orchards.
2. The Scentry fiber pheromone showed promise in large field trials for suppression of CM and leafroller adult flight activity when applied with the modified ground applicator. An "attract & kill" product, when combining the fibers with permethrin, looked promising in field trials this year, particularly for OBLR.
3. The IPM Technologies' OBLR and PLR LastCall attract & kill formulation reduced moth activity significantly in large field studies in both generations. The reduction of adult moth activity could not be directly correlated with larval populations.
4. The female OBLR traps used in the Scentry fiber and LastCall trials in the first generation showed a low percentage of female recovery, either due to predation or escape. The modification in the second generation allowed for a high recovery rate of females, but percentage of mated females and males captured was very low overall. A system for assessing female leafroller mating status remains an issue.

Methods:

Suterra CM sprayable pheromone, Wapato: Suterra CM-F was compared to Checkmate XL1000, a hand-applied dispenser product, in an orchard in west Wapato. The treatments were applied to a 32-acre orchard with each treatment replicated four times (8-acre blocks). Suterra CM-F was applied at rates of 10-20 g ai/acre while Checkmate XL-1000 was applied at a rate of 200 dispensers per acre (dpa). All blocks were monitored with delta-style traps loaded with Suterra BioLure 10x, Suterra BioLure 1x, Pherotech SuperLure or Trécé DA kairomone lures. Pheromone applications were made on May 7, May 25, June 2, July 15 and August 6. Fruit injury was evaluated at the end of each generation by examining 15 fruits per tree from 52 trees in all treatment areas.

Suterra CM sprayable pheromone, Chelan: This moderate- to high-pressure 4-acre site was treated with Suterra CM-F sprayable pheromone at 7 g ai/acre every 7-10 days starting on April 19. A 3-acre adjacent block treated with Isomate-C Plus dispensers at a rate of 400 dpa and served as comparison. Monitoring consisted of delta-style traps baited with Pherotech SuperLure, Trécé 1x red

septa or Trécé DA kairomone lures. Hand-applied dispensers were in place prior to Biofix. Fruit injury was evaluated at the end of first and second generations by examining 20 fruits per tree from 100 trees in both blocks. Both blocks received supplemental insecticide applications.

Suterra CM sprayable pheromone, Pateros: Sprayable pheromone, Suterra CM-F, or Isomate-C Plus was applied to large blocks of approximately 8 acres, each replicated three times. The first application of sprayable pheromone was made on April 28 at a rate of 20 g ai/acre plus Nu-Film 17 at 8 oz./acre. Subsequent applications were made May 22, June 23 and July 23 at a rate of 10 g ai/acre plus Nu-Film 17 at 8 oz/acre. The Isomate-C Plus was applied by hand prior to Biofix at a rate of 200 dpa. Monitoring consisted of the delta-style trap baited with SuperLures or DA kairomone lures. Fruit injury was evaluated at the end of each generation by examining 780 fruits per replicate. A supplemental insecticide, Guthion, was applied to four of the blocks (two sprayable and two Isomate) in July in response to capture of moths in traps.

Scentry fiber pheromone system: Scentry Biologicals contracted with Blue Line Manufacturing to modify the prototype fiber applicator for 2003. The modified applicator consisted of three primary changes: 1) the mast was redesigned to telescope the spray head so that it could be easily adjusted to different tree canopy heights; 2) the spray head incorporated an integrated auger/mixer that simultaneously mixed and lifted the fiber/BioTac from the bucket to the spray head; and 3) the actual spray head "aero fan" was eliminated and only centrifugal force from a spinning cone was used to deliver the fiber/BioTac to the canopy.

2003 Fiber applicator calibration: To achieve rates of 100 g and 200 g of fibers per acre delivered, the tractor was driven at 2-3 mph. To achieve the 100-g per acre rate the applicator was driven down every other row, and to achieve the 200-g per acre rate it was driven down every row.

Scentry CM fibers, Moxee: Scentry CM fibers were applied to two 5-acre blocks in the first CM generation at rates of 100 and 200 g per acre. An adjacent 5-acre block used as comparison was treated with Isomate-C Plus at 200 dpa. The first fiber application was made at "full bloom" timing, April 21. The Isomate-C Plus was applied prior to Biofix. Monitoring in this and all subsequent CM fiber test orchards consisted of delta-style traps baited with Pherotech SuperLure, Trécé 1x red septa or Trécé DA kairomone lures. Fruit injury was evaluated at the end of the first generation by examining 15 fruits per tree from 52 trees in each block.

In the second generation only the 200-g fiber rate was applied to one of the 5-acre blocks treated with fibers only, and the other 5-acre block was treated with fibers mixed with a permethrin insecticide, Pounce® 3.2 EC at a rate of 8 fl oz per acre. Scentry fiber application for the second generation was made on July 8-9. Fruit injury was evaluated prior to harvest by examining 15 fruits per tree from 52 trees in each block.

Scentry CM fibers, Royal Slope: Scentry CM fibers were applied to two 5-acre blocks at 100 or 200 g per acre. An adjacent 10-acre block was treated with Isomate-C Plus at 260 dpa. The "full bloom" timing application of fibers was made on April 25. The Isomate dispensers were placed prior to Biofix. Monitoring was as described above.

In the second generation, the 10 acres were divided into four 2.5-acre blocks. Two of these blocks received a 200 g of fiber only while the other two received the same rate of fibers that also included the insecticide Pounce® 3.2EC at 8 fl oz per acre. At the end of both generations a fruit injury assessment was conducted by examining 15 fruits per tree from 52 trees for each treatment. Fibers on fruit were also assessed at this time.

Scentry CM fibers, Quincy: In the first CM generation, Scentry CM fibers were applied to two adjacent 5-acre blocks at rates of 100 and 200 g per acre. An adjacent 2-acre block without mating disruption was used as a comparison. Both fiber blocks were monitored as described above. The 2-acre block without a pheromone treatment was monitored the same except that the Pherotech SuperLure was not used. The fibers were applied at "full bloom" timing, April 25, and repeated on May 22. Fruit injury was evaluated at the end of the first generation by examining 15 fruits per tree from 51 trees as well as 10 thinned fruits from the ground.

In the second generation rates were 200 g of fibers only or 200 g of fibers plus the insecticide Pounce® 3.2EC at a rate of 8 fl oz per acre. Fruit injury was evaluated prior to harvest by examining 15 fruits per tree from 45 trees. Fibers on fruit were also assessed at this time.

Fiber bioassay: Permethrin was mixed with BioTac at the prescribed rate per acre then mixed with fibers. The fibers were then placed on foliage and allowed to age under field conditions. At 7-day intervals through day 35 fibers were collected and stored at 4ºC until used in the bioassay. OBLR moths were picked up with a vacuum and allowed to briefly touch a fiber with its feet. Each moth was then placed in a container with liquid food and observed a 2, 24, 48 and 74 hours and mortality recorded. Twenty-five moths were tested against each fiber age.

Scentry OBLR fibers, Mattawa: Scentry OBLR fibers were applied to two adjacent 5-acre blocks at rates of 100 and 200 g per acre. An adjacent 5-acre untreated (pheromone) block was used as comparison. All blocks were monitored with the large delta-style traps baited with Trécé 1x or Trécé .05x (low-load) lures or a redesigned delta-style trap (developed by Dr. Vince Jones, 2002) that housed a live OBLR female. Six live female traps were used per treatment (block) for a total of 18 "live" traps. Pheromone traps were checked weekly and live baited traps were checked every 2 days in the first generation. Females in live traps were collected and evaluated for mating status. Larval and fruit assessments were attempted at the end of the first generation, but sites were sprayed out and fruit thinned before the evaluation could be made. The fiber application was made on May 21.

In the second generation fiber rates were 200 g per acre (fiber only) and 200 g per acre plus permethrin 3.2EC (8 fl oz per acre). Fibers were applied on July 24 and August 21. Monitoring remained the same except that female traps were checked twice per week. Prior to and during harvest 300 fruits were examined for fibers.

Scentry OBLR fibers, Desert Aire: Fibers were applied to four 5-acre blocks with treatments of 100 and 200 g replicated twice in the first generation. Fiber rates increased to 200 gin the second generation. Two blocks received fibers only while the other two received fibers plus the insecticide Asana® XL (4.8 fl oz) or permethrin (8 fl oz per acre). A 2-acre untreated block was used as a comparison. All blocks were monitored as described above (OBLR Mattawa). At the end of the first generation each treatment was evaluated for larval population by scanning 54 trees per treatment for 30 seconds per tree. Fiber treatments were applied May 20 (first generation) and July 22 and August 21 (second generation). Prior to harvest an evaluation was conducted for fibers on fruit by examining 300 fruits per treatment.

IPM Technologies' OBLR LastCall: IPM Technologies' attract & kill formulation, OBLR LastCall, was applied to orchards near Quincy, Winchester and Mattawa. Each 15-acre site was divided into three 5-acre sections with a treatment applied to each. Two rates of LastCall OBLR were applied, 600 and 1200 drops per acre, with the adjacent 5-acre block left untreated. At Quincy, applications of LastCall were made on June 2, July 1 and August 5; Winchester, May 28 and July 1; and at Mattawa, May 27. Treated and untreated areas were monitored using delta-style traps baited with Trécé 1x or Trécé .05x lures or a live female. Pheromone traps were checked weekly, and female traps were checked every 3-4 days. Females were collected and dissected to determine mating status. At the end of the first generation, larval populations were sampled at Quincy, Winchester and Mattawa by examining 20 shoots on 52 trees per treatment. Due to low populations, Winchester and Mattawa sites were eliminated from the trial in the second generation.

In the second generation treatments at Quincy remained the same, and a new site at Mattawa was added. At the Mattawa location three different rates of LastCall, 0 (untreated), 300, 600 and 1200 drops per acre, were applied to 1-acre blocks replicated four times. Monitoring in the second generation consisted of delta-style traps baited with Trécé 1x or Trécé .05x lures. LastCall applications were made on July 2 and August 11.

IPM Technologies' LastCall PLR, Wenatchee: LastCall PLR formulation was applied to one orchard near Wenatchee in the second generation only. Treatments of 0 (untreated), 300, 600 and 1200 drops per acre were applied to 1-acre plots replicated two times. All areas were monitored using the delta-style trap baited with Trécé 1x or Trécé 0.1x lures. LastCall applications were made on August 8.

Results and discussion:

Suterra CM sprayable pheromone, Wapato: This was a high-pressure site with multiple supplemental insecticides applied in both CM generations. There was no significance in moth activity as measured by traps between the sprayable or hand-applied treatments in the first generation. In the second generation the hand-applied treatments showed reductions in pheromone trap catch with the BioLure 10x- 61%, Bubble Lure- 48%, BioLure 1x- 56%, and DA- 15% compared to the sprayable plots. There was an increase in DA trap catch in the hand-applied treatments of 57% and sprayable of 78% compared to the first generation DA catch. There was no difference in fruit injury between treatments in either generation.

Suterra CM sprayable pheromone, Pateros: This was a very low-pressure site with low moth activity in the first flight. Fruit injury was less than 1% in both sprayable and Isomate treated blocks at the end of the first generation. Moth activity increased slightly during the second flight, but there was no difference between treatments. Fruit injury increased to 4% in one sprayable pheromone treated block in the second generation but was confined to borders. The Isomate treatment fruit injury remained unchanged in the second generation.

Suterra CM sprayable pheromone, Pateros: This orchard had a history of moderate to high CM pressure and multiple sprays. The sprayable pheromone was applied at 7 g ai per acre with Nu-Film 17 every 7-10 days. In the first generation, moth captures (SuperLure lure-baited traps) in the sprayable was 35% of that in the Isomate treatment. However, the Isomate treatment had an 80% reduction of moth capture in the 1x lure-baited traps compared to the sprayable treatment. In the second generation the Isomate treatment had a significantly reduced moth catch compared to the sprayable treatment, 88% in 1x lure-baited traps and 100% in SuperLure-baited traps. Fruit injury was very low after first and second generations and showed no difference between treatments.

Scentry fiber pheromone system: The modified fiber applicator worked well to position the applications so that the fibers were delivered into the canopy. The fiber/BioTac mixture was augured into a spinning cone, which forced the fibers coated with a film of BioTac into the canopy. During the summer when the temperature reached 80°F the thinner BioTac (25/100 weight) would liquefy, which made the applicator unable to auger the fiber mixture into the spinning cone. To resolve this problem, the BioTac remained on ice until ready to apply. This was cumbersome. as the mixture was in 5-gal buckets. The final application of OBLR fibers was made using a BioTac 100/300 weight (50:50 mix), which eliminated having to ice the product.

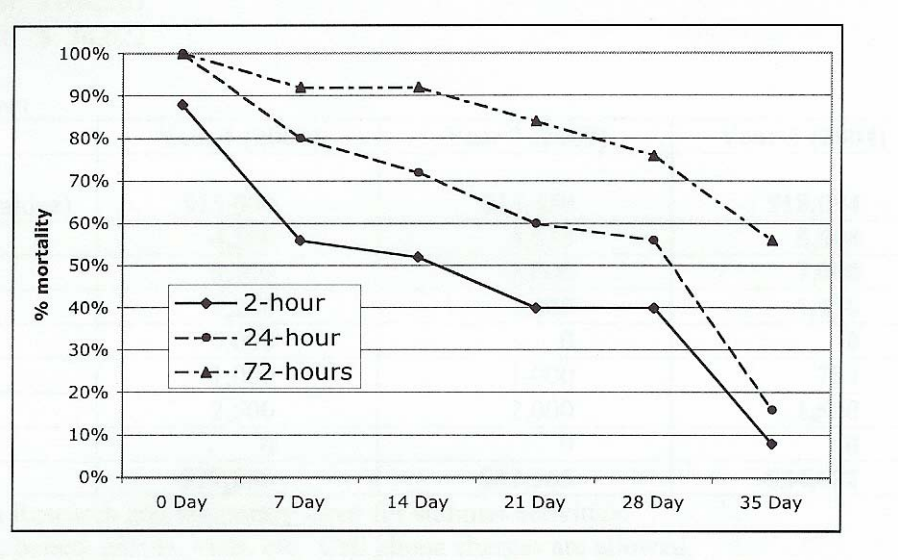
Scentry fibers:

***Codling moth*:** Results were variable between treatments and locations. At the **Quincy** site both fiber treatments in the first generation reduced moth activity in the 1x lure-baited traps by 60% (200 g per acre) and 79% (100 g per acre). The pressure from CM was higher in the block with the 200 g per acre fiber rate, accounting for the lower level of moth suppression. Damage at the end of the first generation was lower in the fiber treatments, 0.0% and 0.1% compared to the untreated (no pheromone) 0.4%. In the second generation the fiber treatments (fiber only and fiber+permethrin) reduced moth catch in 1x traps by between 95-98% compared to the untreated block. Fruit injury was higher in the untreated block, 4.6%, than in the fiber-only, 0.3%, or fiber+permethrin blocks, 1.6%. Again, the CM pressure in the fiber+permethrin was higher than in the fiber-only block.

At the **Royal Slope** site the arrangement of blocks confounded the test in the first generation. The 100 g per acre plot was positioned so that it had high CM pressure and the comparison block, Isomate-C Plus, had very low pressure. In the second generation the Isomate block was again not a good comparison due to pressure, but the fiber treatments provided some interesting information. In the fiber+permethrin and fiber-only treatments exposed to high CM pressure, the fiber+permethrin had lower moth capture, 9 vs. 32 moths per trap, and less fruit injury at harvest, 1.7% vs. 7.2%, than the fiber-only treatment. Where these treatments were exposed to lower CM pressure there was no difference.

Fiber bioassay: There was high mortality of moths exposed to new fibers coated with BioTac+permethrin (below). The percent mortality declined with age of fiber but increased as the

time after exposure was extended. It is not clear whether those moths that were exposed but did not die would be able to fly or mate.



Obliquebanded leafroller: At the Desert Aire location the100 g per acre rate reduced moth capture by 37% relative to the untreated control while the 200 g per acre rate reduced moth catch by 86% in the first generation. In the second generation the fiber treatments (fiber only and fiber+insecticide) reduced moth catch by about 90% relative to the untreated control with no difference between them. The impact of fiber treatments on larval populations was difficult to assess because of confounding insecticide applications applied by the grower.

At Mattawa the fiber treatments reduced moth capture by 87-94% compared to the untreated block in the first generation. In the second generation pheromone trap catch was greatly reduced, probably because two applications were made, reducing moth catch by 99-100% compared to the untreated block.

Prior to harvest an evaluation of fibers on fruit was conducted to get an idea of what might be seen in the bin or at the warehouse. The evaluation did show that, depending on canopy and fruit size, 3-10.3% of 300 fruits per treatment had fibers.

OBLR LastCall: At Quincy, Winchester and Mattawa all rates of LastCall (600 and 1200 drops per acre) reduced moth capture in traps by 85-100% in the first generation relative to the control, but there was no difference between LastCall treatments.

When three rates of LastCall OBLR, 300, 600 and 1200 drops per acre, were applied to replicated 1-acre plots there was a significant difference in moth catch between the highest and lowest rate. The highest rate suppressed moth catch by 95% and the lowest rate by 85%.

PLR LastCall: Only one site was located this year for PLR and not until the second generation. This site was on Stemilt Hill and had received one spray of Success at the end of July. The site was large enough to replicate treatments of 0, 300, 600 and 1200 drops per acre twice. There was no rate effect between treatments. Pheromone trap catch was reduced in all blocks by 83-100% compared to the control.

Budget:

Project title:	Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm.
PI:	Jay F. Brunner
Project duration:	2002-2004 (3 years)

Project total (3 years): $104,267
Current year request: $ 34,622

Current year breakdown

Item	Year 1 (2002)	Year 2 (2003)	**Year 3 (2004)**
Salaries [1] (0.5 Betsy Stutzman, formerly Valdez)	$15,000	$15,450	**$18,694**
Benefits (30%)	4,500	4,635	**5,608**
Wages [1]	8,000	8,000	**7,000**
Benefits (%)	1,280	1,280	**1,120**
Equipment [4]	5,000	0	**0**
Supplies [2]	1,000	1,000	**700**
Travel [3]	2,500	2,000	**1,500**
Miscellaneous	0	0	**0**
Total	$37,280	$32,365	**$34,622**

[1] 50% of Associate in Research and temporary labor for summer activities.
[2] Pays for traps, lures, baited, gloves, vials, etc. Cell phone charges are allowed.
[3] One vehicle for 6 months plus fuel and maintenance.
[4] Fans, framing and materials to construct a field wind tunnel for behavioral studies and evaluation of mating disruption products.

CONTINUING PROJECT REPORT **YEAR 2/3**

Project Title: Evaluation of a codling moth larval aggregation pheromone as an IPM tool

PI: Gary Judd

Organization: Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97 South, Summerland, B.C. CANADA V0H 1Z0

Co-PI and affiliation: Gerhard Gries, Department of Biology, Simon Fraser University, Burnaby, B.C. CANADA V5A 1S6

Objectives:

1. Develop a larval trap incorporating the larval aggregation pheromone for use as a tool to reduce and monitor overwintering populations of codling moth in orchards.
2. Assess use of larval traps and bait stations to detect infestation of harvest bins and to passively disinfest them of overwintering codling moth larvae.
3. Evaluate use of the larval aggregation pheromone as a tool to improve collection of mass-reared diapausing codling larvae to facilitate sterile insect release and biocontrol.
4. Use the aggregation pheromone to examine the impact of larval aggregation on mating success of codling moth and its potential influence on the efficacy of pheromone-based mating disruption.

Significant Findings – 2003

- Y-tube, pitfall and tree assays confirmed that larval pheromone arrests rather than attracts larvae which will dictate how this pheromone might be used
- Showed that male and female cocoon-spinning 5th-instar larvae produce the same pheromone blend; male and female larvae seeking cocooning sites respond to male and female aggregations equally, therefore everyone is attracted to everyone; cocoon-spinning 5th-instar larvae destined for diapause (2nd generation) or not (1st generation), respond equally to larval aggregation pheromone; and diapausing larvae removed from their overwintering cocoons also respond to pheromone from undisturbed diapause larvae when re-spinning their cocoons
- Optimal doses of an 8-component pheromone in pitfall bioassays was 100 cocoon-spinning larval hour equivalents (CSHLE) and in tree assays it was 1000 CSLHE
- Aldehydes were among the most important pheromone components and A-1 and A-2 when presented at 1,000 and 10,000 CSLHE in pitfall and tree assays, respectively, were shown to arrest larvae when combined 100:1 with all other compounds
- Among various pheromone release devices tested waxed cardboard strips were shown to have the best pheromone release rate in laboratory assays but still too fast for an effective field trap design

Methods:

Capture and Extraction of Larval Volatiles

Codling Moth larvae in diet trays were shipped from the Okanagan-Kootenay Sterile Insect Release Facility in Osoyoos, British Columbia, to Simon Fraser University as needed. Trays containing ca. 1,000 larvae were kept in a glass aquarium (60 × 31 × 31 cm) and stored at 15°C under a 16:8 L:D photo-regime. Fifth-instar larvae were removed from the diet as needed. Volatiles from cocoon-spinning larvae were collected from samples of 300, 5th-instar larvae. Larvae were placed in a cylindrical Pyrex glass chamber (ca. 15.5 cm ID × 20 cm in height) and an empty glass chamber served as the control. A water aspirator sucked charcoal-filtered air at a rate of ~2L/min through each

chamber and through a glass column (14 cm × 1.3 mm OD) containing Porapak Q (50-80 mesh, Waters Associates, Inc., Milford, Massachusetts 01757) trapping medium. After 72 h, filters were desorbed with 3 mL of a pentane and ether mixture (95:5) volume: volume. Extracts were concentrated under a stream of nitrogen until 1 µL was equivalent to ca. 8 cocoon-spinning larval h equivalents (8 CSLHE, i.e. volatiles released from 8 cocoon-spinning codling moth larvae during 1 h). Separate aerations from male and female larvae were made to determine if the pheromone profiles were the same for both sexes.

Daily and hourly release of pheromone from various substrates and devices (filter paper, cardboard paper, waxed cardboard paper, cotton wicks, waxed cotton wicks, rubber septa and glass capillary tubes) were measured following the same procedure used for capturing volatiles from insects.

Volatile Analysis

Aliquots of 15 CSLHE of Porapak Q-captured volatiles were subjected to analysis by coupled gas chromatographic-electroantennographic detection (GC-EAD) using the antenna of a pre-pupal specialist parasitoid of codling moth, *Mastrus ridibundus*. A Hewlett-Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25 or 0.32 mm ID) coated with DB-5, DB-210, or DB-23 (J&W Scientific, Folsom, CA) was employed. Full scan electron impact (EI) mass spectra of EAD active compounds was obtained by GC-mass spectrometry (MS) using a Varian Saturn II Ion Trap GC-MS and a HP 5985B GC-MS respectively, each fitted with the DB-210 or DB-5 column referred to above.

Pitfall Olfactometer Bioassays

In Petri dish olfactometers (Fig. 1) potential larval pupation sites (corrugated cardboard strips) were placed in each of two 4 mL vials. Individually and mixtures of natural pheromone components, their synthetic equivalents, and various larval aggregations were tested as paired comparisons by loading cardboard strips with test stimuli. For each replicate, one 5th-instar larva was placed in the centre of the olfactometer and its pupation site recorded 18-24 h later. A minimum of 30 larvae were tested in each experiment. Perforated Eppendorf tubes were placed in each vial to prevent contact of the larva with the test stimuli. All experiments were conducted at 21-26°C in complete dark.

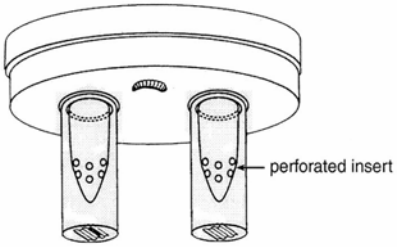


Figure 1. Larval pitfall bioassay

Field Tree Bioassays

To test pupation site preferences in the field, maple trees (*Acer* sp.) were scraped of potential pupation sites and banded with an open-fluted corrugated cardboard band. Treatment stimuli were randomly assigned to one half of the trunk band and control stimuli to the other half. Each band was divided into 6 equal 2.5 cm wide cardboard strips, 3 such strips were on the treatment side and 3 strips on the control side. Each centre strip housed the test stimulus. The bands were affixed to the trunk with metal wire, 45 cm above the ground. For each replicate, 20, 5th-instar male only, female only, or male and female larvae were placed on a modified milk carton collar placed at the base of the first lateral branch crotch. Experiments were started at 2200 h PST and the number of larvae cocooning at treatment or control pupation sites was recorded 10-12 h later. The experiments were conducted during summer of 2003 at Simon Fraser University, Burnaby, British Columbia.

Results and Discussion:

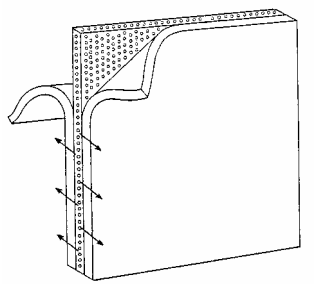


Figure 2. Larval trap

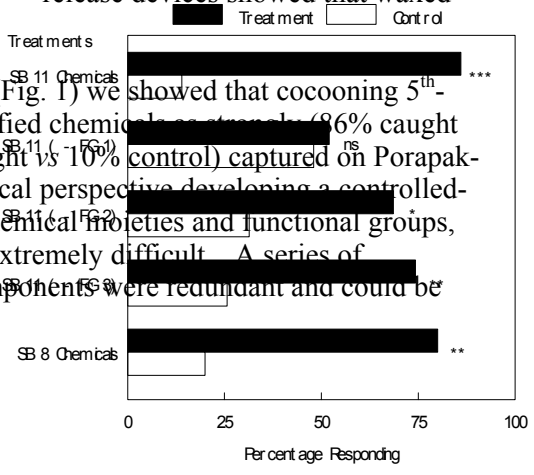
Pheromone Formulation and Trap Development: Among the dozen or more release devices and substrates evaluated for potential use in traps to capture cocooning 5th-instar larvae, waxed cardboard was the only material that provided adequate release of pheromone to see extended behavioural activity. Combined with the physical properties of corrugated cardboard, this combination provided a good starting point as we had predicted from previous research. However, the relatively high volatility of the pheromone components resulted in a loss of activity within 1-2 days, clearly insufficient for development of traps suitable for use in bins and trees, where we expect activity may be needed for several days to perhaps weeks, respectively. We have rethought our trap design and have decided we need several features to make it practical: (1) delivery of adequate amounts of pheromone over an extended period is key, (2) to be effective it must also possess critical physical properties like corrugations, (3) self-adhesive properties for easy attachment to bins and trees would be advantageous, (4) a low profile probably flat shape to make it unobtrusive in bins, and (5) robust enough to withstand sprinkler operation and other weathering forces in orchards. What we have come up with is a corrugated cardboard structure that can be cut to various sizes, sandwiched between a peel and stick adhesive backing and a laminate membrane, pheromone release system. We are in discussions with PheroTech Inc. and Hercon to develop a commercial prototype similar to that shown in Fig. 2. Provided a suitable number of traps can be constructed they will be tested thoroughly in 2004.

Table 1. Comparative analysis of head-space volatiles from 5th-instar, male and female codling moth larvae

Pheromone component	Percentage of each compound relative to most abundant component	
	Males	**Females**
A	19.8	16.7
B	8.3	7.2
C	6.4	7.9
D	19.4	16.8
E	15.5	19.2
F	75.4	81.3
G	5.0	4.4
H	98.0	92.9
I	55.1	47.2
J	29.0	18.0
K	8.4	11.1

Volatile Analysis: Our original discovery of the larval aggregation pheromone was based upon chemical analyses from collections of head-space volatiles from mixed–sex samples of 5th-instar larvae from an artificial diet. Our separate aerations of male and female larvae revealed the same 11 chemicals, in similar relative percentages, are produced by each sex (Table 1). This result alone suggested that both male and female larvae produce the same pheromone but this needed to be tested in behavioural assays. Aerations of the diet indicate it is not the source of the chemicals, ruling out a rearing artifact. Aerations from various release devices showed that waxed cardboard gave the most consistent and longest release rate.

Pitfall Olfactometer Bioassays: Using the pitfall bioassay (Fig. 1) we showed that cocooning 5th-instar larvae responded to a synthetic blend (SB) of 11 identified chemicals strongly (86% caught *vs* 14% control) as they did to the natural volatiles (90% caught *vs* 10% control) captured on Porapak-Q from cocoon spinning larvae (180 CSLHE). From a practical perspective developing a controlled-release system that can emit 11 compounds, with different chemical moieties and functional groups, in the appropriate ratio, for several days or weeks would be extremely difficult. A series of experiments showed that at least some of the pheromone components were redundant and could be



removed without losing much behavioural activity. Systematically removing, aldehydes (FG-1), ketones (FG-2), and monoterpenes (FG-3) showed that removal of aldehydes (FG-1) significantly reduced response of larvae to the synthetic blend relative to the whole blend (Fig. 3). (*E*)-2-octenal and (*E*)-2-nonenal were among the most important aldehyde compounds.
After several experiments it was determined that a minimum blend of 8 compounds was necessary to give near maximal activity (Fig. 3). Dose response experiments in pitfall assays revealed there was a slightly nonlinear response to increasing concentrations of this 8-component blend with an optimum at 100 CSLHE (Fig. 4).

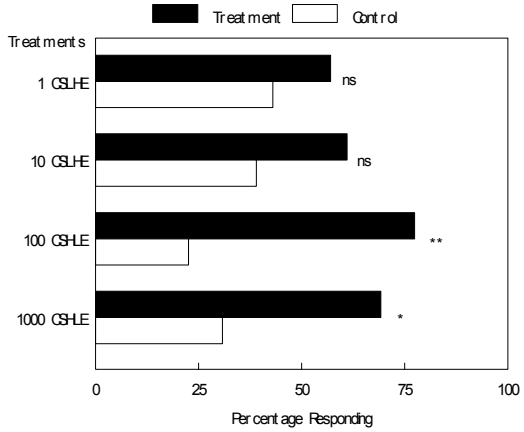


Figure 3. Larval response to an 11-component synthetic blend (SB) minus various functional groups of compounds. **(signifcance levels indicated by: * = P ≤ 0.05; ** = P ≤ 0.01; *** = P≤ 0.001; ns = P > 0.05)**

Figure SB ble **(signifcance levels indicated by: * = P ≤ 0.05; ** = P ≤ 0.01; ns = P > 0.05)**

Male and female cocooning 5th-instar larvae responded equally to aggregations of male or female larvae, and aggregations of either were equally attractive when compared head to head (Table 2). This aggregation response was also evident among overwintering, diapausing 5th-instar larvae that were removed from overwintering hibernaculae and must spin new cocoons to pupate. The presence of pheromone was retained through winter because larvae that had cocooned in summer and in diapause were attractive aggregation sites for these disturbed larvae (Table 2).

Tree Bioassays. Relative to laboratory pitfall assays, the activity of pheromone was reduced under field conditions, requiring a 10 fold increase in concentration to give the same results. In the field, bands baited with an 8-component synthetic blend, at 1000 CSLHE were as attractive as bands with 25 cocooned larvae (Fig. 5).

Table. 2. Response of male and female 5th-instar codling moth larvae to different types of larval aggregations

Treatment choices	**Responding insects**	
	Males	Females
Male larval aggregation	70.0 %	72.9 %
Empty control	30.0 %	27.1 %
Female larval aggregation	82.2 %	71.4 %
Empty control	17.8 %	29.6 %
Male larval aggregation	54.0 %	50.0 %
Female larval aggregation	46.0 %	50.0 %
Mixed sex diapause larvae	75.8 %	No test
Empty control	24.2 %	

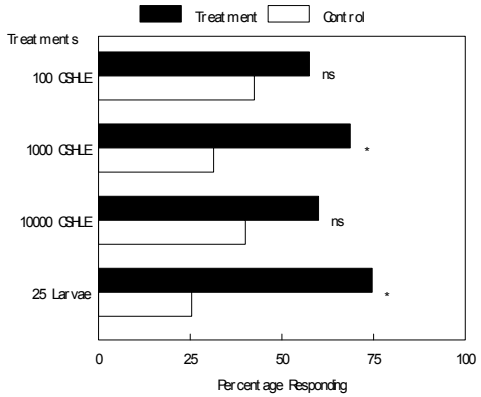


Figure 5. Larval response to 8-component SB at increasing doses in tree bioassays. **(signifcance levels for paired comparisons indicated by: * = P ≤ 0.05; ns = P > 0.05)**

Project title: Evaluation of a codling moth larval aggregation pheromone as an IPM tool
PI: Gary Judd
Project duration: 2003-2005
Current year: 2004
Project total (3 years): $59,580
Current year request: $20,980

REVISED BUDGET with current year highlighted

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)	Total
Salaries[a]	4,000	**0**	0	4,000
Benefits (20%)	800	**0**	0	800
Wages[b]	8,000	**16,000**	16,000	40,000
Benefits (4%)	320	**480**	480	1280
Equipment	0	**0**	0	0
Supplies	3,000	**3,000**	3,000	9,000
Travel[c]	1,000	**1,000**	1,000	3,000
Miscellaneous	500	**500**	500	1,500
Total[d]	17,620	**20,980**[e]	20,980	59,580

[a]Salary for chemists to do pheromone synthesis in year 1 only
[b]Wages for graduate student and summer student assistants for years 2 and 3 of the project have been revised because of changes in US/ Canada currency exchange rates and a lack of Matching Funds from AAFC, see point (d) below
[c]Travel for graduate student and help from Simon Fraser University in Vancouver to Summerland field sites during field season
[d]Application for 50:50 matching funding from AAFC-MII Program could not be submitted by the necessary deadlines because of delays on the part of PARC's Commercialization Officer in drafting a Collaborative Research Agreement with WTFRC. As a result the funds available to conduct this project were reduced by 50%. A revised budget has been submitted for the consideration of the Commission
[e]Owing to difficulties moving money between collaborating organizations all monies should be sent to Simon Fraser University in order to expedite payment to students

CONTINUING PROJECT REPORT **YEAR 2/3**

Project Title: Field testing of multi-component host plant kairomones for the codling moth.

PI: Peter J. Landolt
Organization: USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA

Coooperator(s): Jay Brunner, WSU, Wenatchee

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.

Significant findings:
1. Several chemicals and blends of chemicals reported by other laboratories to be attractive to codling moth did not result in the capture of moths in traps. The exception was the pear ester, discovered by Doug Light, USDA, Albany, CA and demonstrated to be attractive to CM. Three compounds appear to be weakly co-attractive, but are not attractive alone.

2. Codling moth males and females respond to the odor of apple fruit, in apple orchards. Numerous codling moths were captured in traps baited with apple fruit. Both infested and ripe fruit were effective.

3. Volatile collections made from fruit that were attractive in the field (#2 above) provided a list of compounds identified that may be involved in CM host finding, and the pear ester was not present in any of these samples.

Methods for 2004:
1. Testing of blends.

New two-component blends. A subset of 6 of the 13 host plant chemicals tested in 2003 will be used in an evaluation of compounds as co-attractants of ocimene, linalool, and E,E-alpha farnesene. The selection of those chemicals to be tested is necessary because of the cost of the large number of combinations of chemicals if we used all 13, and is based on ongoing GC-EAD evaluations of the relative sensitivity of the CM antenna to each of those compounds. This experiment will involve a total of 15 treatments, with 10 replicates to be set up.

Three component blends. The compounds giving some indication of attractiveness and co-attractiveness when part of 2-component blends in 2003 will be evaluated as possible 3-component combinations in 2004. These 3-component blends (there are 4 of them) will be compared to the 3 two component blends of interest, and to the pear ester.

2. Testing of close range behavior.

A low light level video recording system will be used to document close range behavior of codling moth females approaching attractive apple fruit in a field cage. The purpose of this exercise will be to obtain ideas on the relevancy of trap design in capturing moths responding to apple odor. For example, it will be important to know if the moths track the odor very closely, or if they land on something at a short distance from the fruit. We may be using inappropriate trap designs to test host attractants. Results of these observations may be used to either design a trap for testing host attractants, or to change the trap currently in use (Pherocon wing trap) for evaluating those chemicals.

3. Laboratory support of field experiments.

We are currently involved in a re-evaluation of codling moth antennal sensitivity to apple odor compounds. The objective of this present effort is to determine which compounds in apple odor from attractive apples elicit the greatest response at the lowest concentration (a measure of sensitivity). Results of this work may change or add to the choices of chemicals evaluated in the coming season. Volatile collections were made during August and have been stored for winter use. While we are currently using laboratory-reared codling moth, we have several hundred feral or wild codling moth obtained by tree banding that are in cold storage. These will be used in late winter/early spring to determine if there are differences between wild and laboratory codling moth in their sensitivity to apple odorants. This work with GC-EAD differs from previous work funded by WTFRC in that it now includes consideration of moth response to ripe fruit odors in early season apple orchards, in addition to infested fruit odors.

Results and Discussion:

Testing of possible 2 component kairomone blends. Trapping experiments in apple orchards with two component blends of possible host attractants indicated small but significant improvements in numbers of female codling moths captured. This now includes E,E-alpha farnesene, ocimene and linalool lures added to traps with pear ester lures. Evaluation of a range of ratios of pear ester plus E,E-alpha farnesene indicated a modest but significant effect of adding the farnesene up to a one to one load ratio on rubber septa, with no further improvement in numbers of codling moths captured with larger amounts of farnesene. An evaluation of pear ester and farnesene alone versus the 2 chemicals together showed an increase in males captured, but not females, not entirely consistent with results of previous experiments.

Table 1. Possible kairomones tested for attractiveness to codling moth. Compounds were tested individually, then were tested in combination with pear ester.

Pear ester	Hexyl hexanoate
caryophyllene	Germacrene
humulene	Linalool
ocimene	Methyl salicyate
E,E-alpha farnesene	2-methyl-butyl acetate
Beta farnesene	Farnesol
Z-3-hexenyl hexanoate	

Pear ester testing. To gain background information on codling moth response to pear ester in apple orchards, as the basis for comparing complex blends, three experiments were conducted. The first was a comparison of doses of pear ester on rubber septa. Greatest numbers of codling moths captured were in traps baited with a 10 mg load of pear ester in a rubber septa (Figure 1). The second test was a comparison of ratios of E,E-alpha farnesene to pear ester. There was a significant regression of codling moths captured with increasing ratio of E,E-alpha farnesene to pear ester, as the ratio went from 0.03:1 up to 1:1, for both males and females. The third test was a comparison of doses of the two-component blend of pear ester and E,E-alpha farnesene. Greatest numbers of male codling moths were captured in traps baited with the pear ester at 3 mg and E,E-alpha farnesene at 3 mg, while greatest numbers of females were in traps baited with pear ester at 10 mg and E,E-alpha farnesene at 10 mg (Fig. 2).

Table 2. Codling moths captured in Pherocon wing traps baited with pear ester, or both pear ester and E,E-alpha farnesene in rubber septa.

	Pear ester dose in mg					
Sex	0.03	0.1	0.3	1	3	10
male	0.7 ± 0.2	1.6 ± 0.4	3.9 ± 1.4	5.7 ± 2.1	5.7 ± 1.4	6.8 ± 1.9
female	0.7 ± 0.2	1.3 ± 0.3	3.0 ± 1.1	3.7 ± 1.4	5.0 ± 1.3	5.6 ± 1.3
	Pear ester dose and E,E-alpha farnesene dose					
Sex	0.03	0.1	0.3	1	3	10
male	0.5 ± 0.1	1.3 ± 0.4	2.6 ± 1.1	3.2 ± 0.8	4.7 ± 1.0	4.3 + 1.0
female	0.1 ± 0.1	0.4 ± 0.1	0.9 ± 0.4	1.1 ± 0.4	1.5 + 0.4	1.7 + 0.5

Testing of fruit. Experiments in apple orchards demonstrated attraction of both male and female codling moth to apple fruit. Fruit were placed in the buckets of UniTraps and hung in branches of apple trees in an orchard with apple fruit. Codling moths were captured in traps baited with immature apples (field picked) that were clean or infested with codling moth (Table 1), and ripe apples (purchased) that were clean and immature apple fruit infested in the laboratory with larval codling moth Table 2). This work confirms results of prior laboratory tests that demonstrated codling moth attraction to apple fruit and the odor of apple fruit in a flight tunnel.

Table 3. Mean numbers of male and female codling moth captured In UniTraps baited with 3 immature Red Delicious apples; either apples infested with codling moth larvae for 3 days in the laboratory, or apples not infested (clean). Yakima, Washington, July 2003.

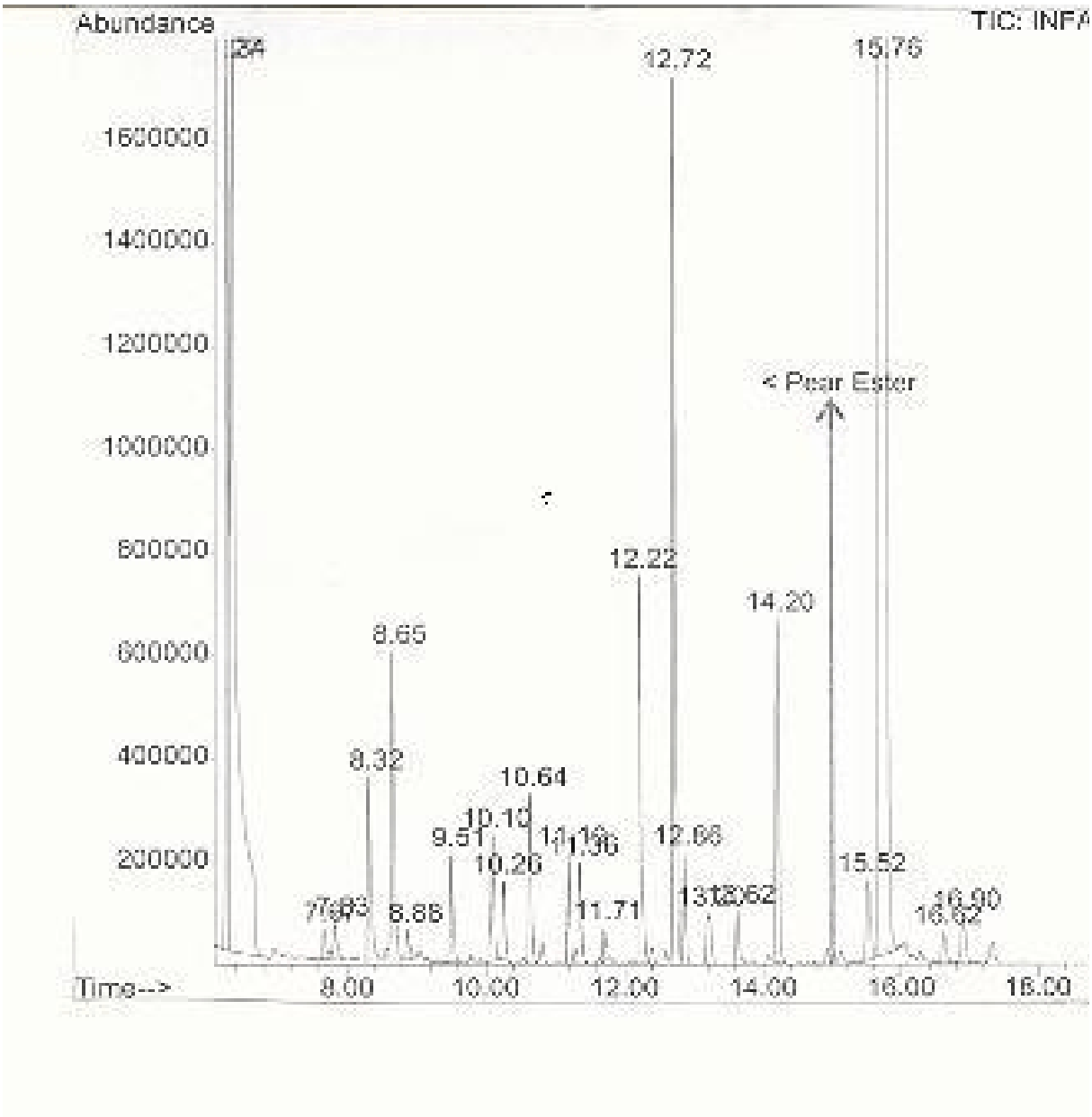
CM sex	no apples	infested apples	clean apples
males	0.3 ± 0.1	6.5 ± 1.9	6.8 ± 1.7
females	0.1 ± 0.1	3.1 ± 1.4	4.0 ± 1.6

Table. 4. Mean numbers of male and female codling moth captured in UniTraps baited with 1 ripe Braeburn (New Zealand) apple, or one immature Red Delicious apple that was infested with codling moth larvae for 3 days in the laboratory. Yakima, WA August 2003.

CM sex	none	infested immature	un-infested ripe
males	0.2 ± 0.1	4.0 ± 0.9	3.5 ± 1.3
females	0.2 ± 0.1	2.7 ± 0.6	3.5 ± 1.4

Role of pear ester in codling moth attraction to apple fruit. Although pear ester has been found in small amounts in the volatiles emitted by some apple fruit, we have in past years been unable to correlate its presence with the attractiveness of apple fruit to codling moth in a flight tunnel. Volatile collections were made and SPME sampling done using apples demonstrated to be attractive to codling moth in the field (see above), followed by analysis by GC-MS. The pear ester was not detected in any of these samples from apples attractive when placed in traps in the field. This finding reinforces the conclusion that codling moth attraction to apple, demonstrated now by multiple laboratories using olfactometers, flight tunnels, greenhouse trapping, and in-field trapping, is a response by the moth to a different chemical (not the pear ester) or a blend of chemicals that does not include the pear ester.

Figure1.Chromatogram of volatile collection made from immature Red Delicious apple infested with codling moth larva. Note absence of pear ester, indicated by a line on the chromatogram at an expected retention time of 15 minutes and 10 seconds.



Budget:
Project Title: Field testing multi-component host plant kairomones for the codling moth.
PI: Peter J. Landolt
Project Duration: 2003-2005.
Current Year 2004.
Project total (3 years): $53,200
Current year request: $17,600

Year	2003	2004	2005
	$17,500	$17,600	$18,100
Current year breakdown			
	Year 1	**Year 2**	Year 3
	$14,300	**$14,900**	$15,400
	2,500	**2,000**	2,000
	700	**700**	700
	$17,600	**$17,600**	$18,100

Salaries for 2004 are for 1/4 time GS-7 chemist and ½ time student assistant for trap checking. Materials and supplies include traps, chemicals for lures, solvents and gases for chemical analysis and purification. Travel costs are for driving to and from field sites.

CONTINUING PROJECT REPORT **YEAR 2/2**
WTFRC Project # AE-03-328 **Organization Project # 5352 22000 015 13T**

Project Title: Identification of extra-orchard host plants and habitats for key natural enemies of pome fruit pests suitable for manipulation or conservation

PI: Eugene Miliczky
Organization: USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951
CO-PI: David Horton, USDA-ARS, Wapato, WA

Objectives: 2003: 1) Determine if native psyllid species found on extra-orchard host plants are hosts for the important pear psylla parasitoid, Trechnites insidiosus, or other species of parasitoids. 2) Monitor the occurrence of plant-feeding arthropods (leafrollers, psyllids, etc.) and beneficial arthropods (those that might also attack pear psylla) on alder and begin to assess the potential of Clubiona spiders (frequent occupants of alder leafrolls) as predators of leafrollers. 3) Obtain a 2nd year's data on the occurrence of western flower thrips (WFT) and its predators on selected extra-orchard host plants whose flowering periods span the growing season. In orchards with exposure to extra-orchard habitat along one edge, determine if the density of WFT on apple blossoms during flowering declines as distance from the orchard / extra-orchard habitat border increases. 4) Survey plants in extra-orchard habitats for caterpillars exhibiting leafrolling behavior. Rear caterpillars to obtain adult moths and parasitoids, looking for parasitism by Colpoclypeus florus and other species that might attack pest leafrollers in orchards. Concentrate efforts early in the season (prior to about mid-May) and later in the season (September and October), times not well sampled during 2002.

2004: 1) Continue studies on native psyllids and their parasitoids with emphasis on a psyllid frequently found on Salix exigua (coyote willow), a plant often found near orchards. The psyllid has >1 generation per year and is often parasitized by an unidentified eulophid wasp at low to moderate levels. Objectives are to obtain enough information in 2004 to allow formal screening in 2005 of parasitoids against pear psylla. 2) Conclude studies on alder leafrollers, the alder psyllid and their parasitoids. Conclude assessment of Clubiona spiders as predators of pandemis and oblique-banded leafroller. 3) Obtain a second year's data on western flower thrips density on apple blossoms as affected by distance from adjacent, extra-orchard habitat. Sample at shorter intervals between the border and 150' into the orchard (0', 25', 50', 75', 100', 125' and 150') to more clearly define the relationship between distance from extra-orchard habitat and WFT density. Conduct a study to determine how closely the "blossom flick" method of sampling WFT in the field reflects actual thrips densities in the blossoms. 4) Conclude the survey of leafrollers on extra-orchard host plants, again concentrating on the early and later parts of the season. Begin to test suitability of native leafrollers as hosts for Colpoclypeus florus in preparation for thorough screening in 2005.

Significant findings:

- Coyote willow often grows near orchards in wet areas and along canals. It hosts a multiple generation native psyllid that often has significant levels of parasitism.
- Several other native psyllids were found to have detectable levels of parasitism although Trechnites has so far not been reared from them.
- Red alder is often heavily infested with leafrolling caterpillars. Five or 6 species of parasitic wasps and a fly have been reared from the leafrollers, but so far not Colpoclypeus florus.
- Leafrolls on red alder are utilized by a wide variety of plant-feeding and predaceous arthropods, the latter including species found in orchards such as the predatory bugs Anthocoris antevolens and Deraeocoris (both excellent predators of pear psylla), certain ladybeetles, and green and brown lacewings.
- Clubiona spiders are the most common spiders in alder leafrolls and may prove to be effective predators of pest leafroller larvae in orchards.

- Density of western flower thrips in apple blossoms in 4 orchards with extra-orchard habitat exposure along 1 edge was highest in the border trees and decreased as distance from the extra-orchard habitat increased.
- Leafrolling type caterpillars were found on about 50 species of extra-orchard host plants. Parasitoids were reared from most species, although not Colpoclypeus florus as yet.

Methods (for 2004 season):

Native Psyllids: Monitor populations of coyote willow psyllid throughout the season at 2 Tieton sites to determine number of generations and level of parasitism. Locate additional stands of this willow, especially near pear orchards, and collect parasitized nymphs and rear parasitoids. Collect parasitized psyllid nymphs on other extra-orchard host plants and rear parasitoids. Host plants will include other willow species, golden current, alder, bitter cherry, bitterbrush and yarrow. In 2005, formal screening of parasitoids against pear psylla will begin.

Alder studies: Continue assessment of Clubiona spiders as predators of PLR and OBLR. This will include small cage tests in orchards. Confirm that apple and pear are not suitable hosts for alder leafrollers. Examine samples of alder leafrolls from several sites for the presence of plant-feeding and beneficial arthropods, emphasizing species known to occur commonly in orchards. Collection of samples will begin in May.

Western flower thrips: Sampling of WFT for density versus distance studies will be as in 2003 except sampling intervals will be shorter and sampling will not extend as far into the orchard. We will attempt to better define the distance at which WFT densities drop off most markedly, allowing us to define the size of the "edge" effect for growers. Samples will be taken at 25' intervals between 0' and 150'. Bloom stages sampled and thrips extraction procedures will be as in 2003. Thrips damage to apples will be monitored at the same distances at which density samples are collected to determine if a correlation exists between thrips density and damage. This study will again be in collaboration with Steve Cockfield and Elizabeth Beers. To determine how accurately the "blossom flick" method for WFT sampling reflects actual WFT numbers in the blossoms, blossoms will be "flicked" in the field and the thrips counted. Blossoms will then be taken to the lab and subjected to detergent extraction to remove any additional thrips and the 2 results compared.

Leafroller survey on extra-orchard host plants: Extra-orchard host plants will be examined for leafrolling type caterpillars at a variety of locations as in previous years. Samples of infested foliage will be brought to the laboratory and the caterpillars reared, as will any parasitoids. We will be looking for evidence of parasitism by known PLR / OBLR parasitoids and will again emphasize sampling during the early and latter parts of the season. Develop methods for testing suitability of native leafrollers as hosts for C. florus in preparation for full screening study to begin in 2005.

Results and Discussion:

Native psyllids and their parasitoids: Psyllid species were examined on several native host plants and curly dock, an introduced weed. Psyllid infestation levels were moderate to high at all sample locations except the Zillah site, where they were low. The following table summarizes 2003 data on host plants, number of psyllid generations and psyllid parasitoids and predators.

Host plant	Location	# of Generations	Parasitoids reared?	Predators*
Coyote willow	Tieton (S)	>1	Yes	D
Coyote willow	Tieton (D)	>1	Yes	D, A
Coyote willow	Zillah	>1	Yes	
Scouler's willow	Tieton (FC)	1	Yes	D, A
Willow (unidentified)	Little Naches	1?	Yes	
Willow (unidentified)	Yakima Cnyn	1?	Yes	
Willow (unidentified)	Yakima Cnyn	1?	Yes	
Curly Dock	Tieton (S)	>1	Yes	D, A
Red Alder	Cascade Park	1	Yes	D, A
Red Alder	Little Naches	1	No	D, A
Bitter Cherry	Little Naches	1?	No	

Bitter Cherry	Peshastin	1?	Yes	
Golden Currant	Tieton (S)	1	No	

*D=Deraecoris, A=Anthocoris: both known, important predators in pear orchards

Coyote willow and its associated psyllid are of interest because this willow is frequently found near orchards in locally wet areas and along irrigation canals. It occurred near 4 of our 18 study orchards from 2001 (and probably at 3 others) and the psyllid as well was present at all 4 sites. Samples of this psyllid were obtained near 3 orchards in 2003 where infestation levels were low to quite high. Parasitized psyllid nymphs were obtained at all 3 sites. Most parasitoids were a species of small wasp in the family Eulophidae. Adults of the overwintering generation of this psyllid were still abundant and adults were still emerging as late as 28 October, 2003 at the Tieton (D) site. Activity at the Tieton (S) site had fallen off a few weeks earlier. Predators of pear psylla, (Deraeocoris and Anthocoris) were locally abundant, attacking the willow psyllid.

Arthropods associated with leafrolls on red alder: Red alder is a pioneering species on moist soils in the Pacific Northwest that is common on the east slopes of the Cascades. Stands of red alder are often heavily colonized by leafrolling caterpillars of at least 2 species. One belongs to the Tortricidae, the group that includes the Pandemis and Oblique-banded leafrollers. The second is a slender, reddish moth in the Gelechiidae, a large family many of whose members are also leaf rollers. In addition to caterpillars, alder leafrolls are utilized by a wide range of other arthropods including plant-feeding and beneficial species. Some of the latter are species also found in orchards where they play a role in orchard biocontrol. To give an idea of the number and range of arthropods associated with these leafrolls, the following table presents data from samples of leafrolls collected on 28 August 2003 at 3 locations along State Road 410 (the highway to Chinook Pass), west of the city of Naches.

Type of Arthropod	**Site 1 - Mile 84**	**Site 2 - Mile 79**	**Site 3 – Mile 75**
# Leafrolls examined	55	238	229
# Leafrolls with leafroller larvae	13	62	23
Total # of leafroller parasitoids	2	21	35
Total # of *Clubiona* spiders	5	23	62[1]
Total # of other spiders	2	3	5
Total # of *Anthocoris* bugs	9	46	0
Total # of brown lacewings	2	7	4
Total # of green lacewings	0	0	5
Total # of lady beetles	0	2	1
# LR's with "red" mites (predaceous?)	1	13	7
# LR's with phytophagous mites	19	94	5
# LR's with thrips[2]	18	71	11
# LR's with aphids	3	3	24
# LR's with alder psylla	1	6	0
# LR's with stink bugs – plantfeeder	0	0	21
# LR's with Lygaeid bugs – plantfeeder	0	0	78
# LR's with springtails	1	15	2

[1]In addition to the spiders themselves at least 50% of the leafrolls contained silken, Clubiona spin-ups, many of which contained cast skins. Thus the leafrolls are also frequently used by Clubiona as a protected location in which to moult.

[2]Most thrips were phytophagous types but predatory thrips were also present in the leafrolls.

Various other leafroll occupants found at these and other sample locations included the predatory bugs Deraeocoris, Orius, and a species of stinkbug, other types of caterpillars, sawfly larvae and barklice. Certain leafroll associates may be common at one site but rare or absent at another, the lygaeid bug and Anthocoris for example (see table). This presence/absence phenomenon may be related to site differences or the phenology of the bug. The alder psyllid, for instance, has one generation per year and was largely absent by the time of the 28 August sample.

Our interest in alder leafrolls increased upon discovering the frequent presence in leafrolls of spiders in the genus Clubiona (family Clubionidae), commonly known as sac spiders. Clubiona is

related to Cheiracanthium and members of the 2 genera are similar in color and morphology as well as behavior, both being hunting spiders (non-web spinners) and primarily active at night. Previous work on Cheiracanthium has shown it to be an effective predator of Pandemis and oblique-banded leafrollers under laboratory and field conditions. The frequent association of Clubiona with alder leafrolls led to the possibility that these spiders might be preying on the leafrollers, among other things. In preliminary tests using alder leafrollers established on alder cuttings and Pandemis leafrollers established on pear and apple seedlings, Clubiona preyed on leafrollers in all situations. The apple seedling/Pandemis test compared Clubiona and Cheiracanthium mildei. As indicated in the following table Clubiona compared favorably with C. mildei although the predation rate of Clubiona was not as fast as that of C. mildei. Spiders and PLR larvae used in this test were of similar size.

Spider	# of Pandemis available	# of Pandemis consumed
Cheiracanthium #1	3	3
Cheiracanthium #2	4	4
Clubiona #1	3	2
Clubiona #2	4	3

Western flower thrips and its predators: Four orchards in Yakima Co. with exposure to extra-orchard habitat along 1 edge were sampled for WFT at 4 stages during bloom (pink, open king bloom, 100% open bloom and 100% petal fall). Samples were taken at 4 distances from the orchard / extra-orchard habitat border. This work was done in collaboration with Steve Cockfield, who conducted a similar study in the Wenatchee area. Data for the 4 Yakima Co. orchards are summarized in the following table. Numbers of adult WFT are presented. Immature WFT and other species were few.

Orchard	Distance	Pink	Open King	Full bloom	100% Petal-fall	Totals
C	Border	1	6	9	6	22
	100’	0	0	4	1	5
	200’	0	0	4	2	6
	300’	0	0	3	1	4
H	Border	0	3	20	13	36
	100’	0	2	9	8	19
	200’	1	2	8	8	19
	300’	0	0	10	9	19
S	Border	1	9	67	83	160
	100’	2	4	44	52	102
	200’	3	4	40	38	85
	300’	0	3	23	30	56
D	Border	4	14	35	53	106
	100’	3	9	23	38	73
	200’	3	6	14	29	52
	300’	2	7	15	21	45

Thrips numbers were variable among the 4 orchards. The 2 orchards with the lowest thrips numbers were the first to come into bloom but also had the least diverse extra-orchard habitats in terms of the number of plant species in bloom at the same time as apple. Nevertheless, a similar trend in thrips numbers was seen at all locations: numbers in the blossoms decreased as distance from extra-orchard habitat increased, suggesting that thrips may be moving into orchards from the extra-orchard habitat. The most marked decrease in thrips numbers was between the orchard border and 100’ from extra-orchard habitat.

As a follow-up to 2002’s extensive sampling of extra-orchard host plants for occurrence of WFT and its predators, several of the more important WFT host plants were sampled again in 2003.

At 2 orchards where the extra-orchard flora was particularly diverse, a sequence of plants was sampled whose flowering periods spanned the season from April to September. From earliest to latest these were: balsamroot, bitterbrush, western horsemint, yarrow, tall buckwheat and green rabbitbrush. All species supported moderate to high numbers of WFT. WFT predators, particularly Orius and small, immature spiders in the genera Sassacus, Xysticus and Misumenops, were present on all species, sometimes in high numbers. Small, immature Xysticus (a crab spider), for example, were abundant on mint, yarrow and tall buckwheat, early in its lengthy flowering period. X. cunctator, the most common member of its genus in Yakima County orchards, was the most abundant species of Xysticus on these plants. Orius was found on all species and was particularly abundant on tall buckwheat during its flowering period which extended from late June to mid-October. WFT, Orius and spiders were still present in fair numbers on bitterbrush as late as 28 October, 2003. Two of the last plants to flower in the fall, gray rabbitbrush and big sage, were sampled near 3 orchards in the vicinity of the USDA's Yakima Agricultural Research Lab. WFT, Orius, and a crab spider (Misumenops lepidus, the most common member of its genus in Yakima County orchards) were particularly common on gray rabbitbrush, less so on big sage.

Leafroller and leafroller parasitoid survey on extra-orchard host plants: The survey of extra-orchard host plants for leafrolling caterpillars began on 2 April (compared to 23 May 2002) and extended into September. More than 800 specimens were collected from approximately 50 species of plants (herbs, shrubs and trees). On plants colonized by leafrollers infestation levels varied widely. Some species were heavily infested while on others infestation levels were low. This is reflected in the number of specimens collected on different plant species which ranged from a single collection on each of 11 species to a maximum of 118 specimens on lupine (more than 1 species of which was sampled). Still other plant species appear rarely, if ever, to host leafrollers. On some plants, leafrollers were present for a limited period of time and then essentially disappeared, perhaps indicative of a single generation per year. An example was common snowberry, on which a leafroller was common for 2 – 3 weeks in the spring but was then absent for the rest of the season. Infestation level also varied from site to site on the same species. This was particularly notable on wild rose in the Yakima Canyon, where 3 stands of the plant were searched but only 1 had a moderate leafroller infestation.

Parasitoids were reared from many leafroller species. Most were parasitic wasps, representing a number of families, as well as a few flies. We have not so far obtained Colpoclypeus florus. A probable population of the strawberry leafroller, Ancylis comptana, was found infesting a species of strawberry near milepost 79 on Highway 410 at an elevation between 3200' and 3600'. The following table presents data for several of the plant species on which leafrollers were found with regard to the number of moths and parasitoids reared during 2003.

Plant Species	Total specimens[1]	# leafrollers reared	# Parasitoids reared
Currant (2 species)	48	13	5
Strawberry	46	7	1
Snowberry	56	18	3
Wild rose	74	35	9
Desert parsley (5 species)	42	36	3
Grey rabbitbrush	23	6	2
Chokecherry	79	17	8
Red-osier dogwood	45	31	7
Willow (> 1 species)	60	23	10
Lupine (> 1 species)	118	43	17
Ocean spray	7	1	3
Wild buckwheat (4 species)	68	44	5
Red alder	37	19	4
Red birch	8	3	1
Cottonwood	41	13	14
Licorice root	15	5	0
Vine Maple	7	2	2
Hawthorn	6	2	1

[1]Total specimens does not equal # of leafrollers reared plus # of parasitoids reared because some specimens died during rearing and some specimens are still being reared.

Budget

Project title: Identification of extra-orchard host plants and habitats for key natural enemies of pome fruit pests suitable for manipulation or conservation

PI: Eugene Miliczky

Project Duration: 2003-2004

Current Year: 2004

Project Total (2 years): $66,700

Current year request: $35,000

Year	Year 1 (2003)	**Year 2 (2004)**
Total	31,700	**35,000**[*]

Current year breakdown

Item	Year 1 (2003)	**Year 2 (2004)**
Salaries[1]	23,407	**25,790**
Benefits (35.7%)[2]	8,293	**9,210**
Total	31,700	**35,000**

[1]Post-doc, Eugene Miliczky, 0.51 FTE (12 mos.)

[2]Benefits, Eugene Miliczky, 0.51 FTE (12 mos)

[*]Funding request for 2004 is higher than anticipated originally ($31,700) due to reduction in funding provided by IFAFS. The request to WTFRC covers slightly over ½ the salary and benefits for E. Miliczky. We have submitted a portion of this proposal (flower thrips work) to the Washington State Commission on Pesticide Registration to meet the shortfall caused by the reduced IFAFS dollars; the request to WSCPR is $16,000.

CONTINUING PROJECT REPORT **YEAR 2/3**

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 3 and expand 4 gardens (=rose plantings) in Yakima and establish 4 gardens in Wenatchee. (Completed)**…….....
Most gardens were planted by collaborators under our supervision. These include 2 gardens in Zillah, 3 gardens in Parker Heights near ARS lab, 4 expanded gardens at Parker near Union Gap (Washington Fruit), 2 gardens in Mattawa/Beverly area; 5 gardens at Moxee Research Farm; 40 small gardens at Sportfisher Orchard near Vernita; replanted and expanded garden at Wenatchee Valley College and 2 Gardens at Stemilt. We felt it was more efficient use of our efforts to use gardens planted by interested growers than those we originally planned to plant ourselves.

2) **Measure parasitism of and damage by leafrollers at different distances along transects from rose plantings into apple orchards. (2003-5)** ……
Parasitism was measured at Parker orchard (now with 1 original garden and 3 new gardens) along transects into the orchard from garden(s). Measurements along transects was not completed at other sites. This was due to the greater number of gardens requiring monitoring and the greater amount of travel associated with them. Hence we reverted to a monitoring site near and another distant from the gardens at most sites. Some disruption of parasitoid activity was associated with pesticide (Success) applications in some blocks and this also reduced completeness of data collection. We will continue monitoring in 2004-5 and more complete transect information will be collected at gardens that are suitable (no pesticide disruption, garden sufficiently developed) in 2004.

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. (2003-5)**……..
Abundance of and host and parasites were collected only in the overwintering period. Summer efforts were devoted to planting gardens and assessing parasitism in orchards. As garden planting is substantially complete we will focus more on garden fauna through spring and summer of coming years. However, we did make a large effort collecting rose hips from both gardens and natural rose patches to assess the abundance and danger posed by an unknown *Graphilita* feeding in the hips. Subsequently, we show this worm is unlikely to be a pest (see report on diagnostics).

4) **Conduct field-day demonstrations for establishing and maintaining rose plantings and widely disseminate information from project to grower community (2003-2005)**…….
We did not attempt this effort at all in 2003.

Significant findings:

- **Two to three year- old rose/strawberry gardens continued to support large numbers of the strawberry leafroller.**

- **Parasitism by *C. florus* and overall parasitism was higher both adjacent to gardens and deeper in orchard near established gardens compared to previous years.**

- **Orchards near new gardens differed a lot in parasitism rates in spring and fall.**

- **OBLR was equally susceptible to *C. florus* attack as *Pandemis* in field exposures.**

Methods:

In spring 2004, rose gardens planted in 2003 will be re-infested with *C. florus*-parasitized *Ancylis* by transplanting strawberries from Wapato nursery garden. This includes 2 gardens at Wenatchee, multiple gardens at Vernita, gardens at Zillah and several gardens associated with the EPA and WCPR funded projects.

In May 2004, we will assess parasitism of leafrollers in orchards by collecting mid-late stage larvae at 4 sites within the orchard along transect(s) extending away from each garden. If leafroller densities are too low to support these collections we will provide lab-reared 4th instar OBLR that will be deployed on orchard trees and retrieved 2-3 weeks later. In mid-August a second round of parasitism assessments in orchards will be made as above except, if sentinels larvae are used, they will only be deployed for 1-2 weeks. We will examine 300 fruit at each of 6 sites to measure fruit damage in early September: 2 sites close to each garden, 2 sites at intermediate distances from each garden, and 2 sites at the maximum distance from the gardens. Fruit damage assessment will only be conducted at orchards have reduced pesticide use.

In spring and summer, biweekly samples will be collected from gardens to assess phenology of-, parasitism rate of-, and species parasitizing-, *Ancylis* in roses and strawberries. In mid-winter, the duff layer below the roses will be sampled (6 - 0.5 m^2 hoop samples/garden) providing over wintering densities and parasitism of *Ancylis* in gardens. All of this will be repeated in 2005.

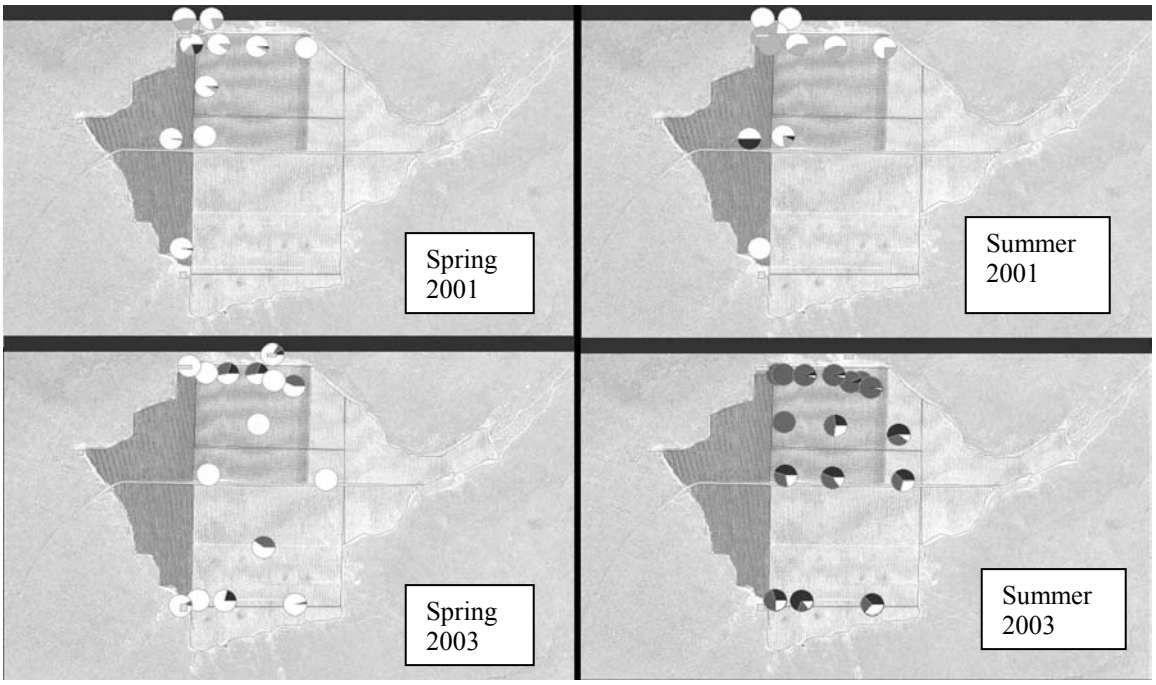
Efforts to get the word out to grower community will be expanded in 2004. We have nearly completed a website and it will go on line in late January 2004 following evaluation of 2003 data (http://www.yarl.prosser.wsu.edu/Scientists/Unruh/Unruh_Roses_BioControl.htm). We still plan to host local field days that demonstrate the how to plant a garden and what to look for in the garden to know it is working for you.

Results and Discussion:

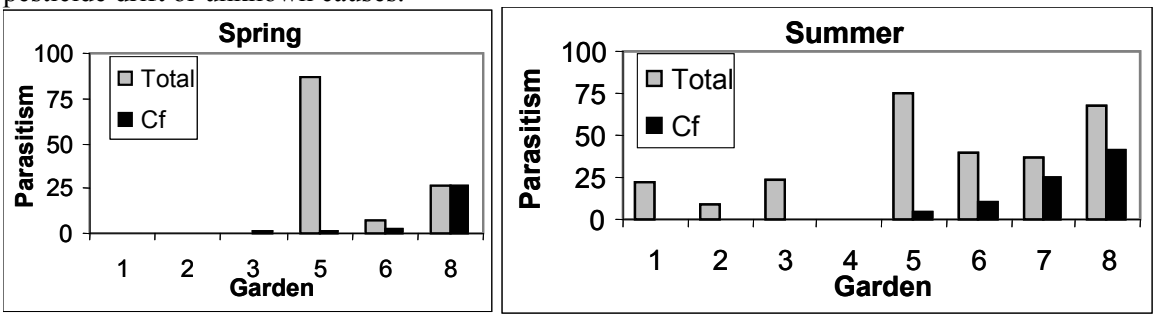
Two to three year- old rose/strawberry gardens continued to support large numbers of the strawberry leafroller. As in seasons before there is garden to garden variation but no efforts have been made to enhance insect abundance in these gardens. Our data support the hypothesis that the gardens as planted, represent a stable habitat modification. A comparison of *Ancylis* abundance and parasitism by *C. florus* in the gardens across years and sites will be presented on the poster as the data for 2003-2004 winter is being collected now.

Parasitism was higher both adjacent to gardens and deeper in orchard in 2003 compared to 2001 (and 2002). Figures 1 shows how parasitism penetrates deeply into 100 acre orchard adjacent to one of the original gardens (planted in 2000) and with the addition of three new gardens (2002).

Figure 1. Parasitism by *C. florus* are shown as grey pie slices, that by tachinid flies as black pie slices, and unparasitized as white. Orchard depicted is about 100 acres and lighter colors are nonbearing apples and cherries. Darker orchard at left is 10 year old galas which received pesticides. Note that *C. florus* first was found in these orchards in the spring of 2001 following planting of garden in summer of 2000. It was not recorded in this area at all in 1999 or 2000. Also note how summer parasitism in the orchard has improved since 2001.



Orchards near newly planted gardens showed a diversity of parasitism rates both in spring and fall. A few orchards already showed evidence of Colpoclypeus florus in spring and summer. Figures 2a and 2b show parasitism by *C. florus* and also show total parasitism at orchards with new gardens. Parasitism was assessed at 10 gardens each season but no recovery was made at some sites due to pesticide drift or unknown causes.



OBLR was equally susceptible to *C. florus* attack as was *Pandemis* in field exposures. Persistent questions as to the ability and propensity of C. florus to attack OBLR demanded some evaluation in the field. In our sentinel exposures in May and June of 2003 we used half OBLR and half *Pandemis* leafrollers of similar size. We found them equally susceptible and parasitized at high and statistically equal rates by *C. florus* (OBLR was actually parasitized numerically more often but there was no statistically significant difference; data not shown). In July and August, exposures were exclusively OBLR and the results in Figure 2 speak for themselves.

Finally, many new gardens were planted and views of these gardens at planting, and in fall will be posted on the website in January 2004 and available for viewing

Budget:

TITLE:	Biological control of leafrollers through habitat modification
PI:	Tom Unruh
Project Duration:	3 years (2003-2005)
Project total	**$151,100**
Current year request	**2004: $51,700**

In addition to support from WTFRC, we received a grants from WCPR to support gardens and their monitoring in Okanagon and from EPA region 10 for garden studies in Milton Freewater and the Dalles OR. We also received funds to support this and other work on leafrollers from the IFAFS "areawide 2 award. We failed to receive a WSARE grant submitted last year; it has been resubmitted for 2004. All told almost $100,000 per year comes to the Unruh lab to support this work on testing gardens and testing effects of pesticides on beneficials.

Year-Item	2003	**2004**	2005
Salaries	38,000	**39,000** [1]	40,000 [1]
Benefits	11,400	**11,700**	12,000
Supplies	3,000	**1,000**	1,000
Total	52,400	**51,700**	53,000

[1]Salary represents 1.7 GS-5 Technician equivalents

CONTINUING PROJECT REPORT **YEAR 3/3**

TITLE: Effects of New Insecticides on Natural enemies: Acute toxicity and sublethal effects

CO-PIS Tom Unruh, USDA-ARS Yakima
Dave Horton, USDA-ARS Yakima
Dr. E. Beers, WSU, Wenatchee
Richard Hilton, OSU, Medford
Helmut Reidl, OSU, Hood River
Dr. Nick Mills, U.C., Berkeley

COLLABORATORS Vince Jones, WSU, Wenatchee
John Stark, WSU, Puyallup

OBJECTIVES (2002-3):

1. Test acute toxicity of the next 5 new insecticides to 9 arthropods using standard topical sprays
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 (New objective since 2002)

Significant Findings:

- Sublethal bioassay procedures have been finalized for 7 test species and data collected on both acute and sublethal effects for each species. These seven are: *Forficula auricularia, Chrysoperla carnea*, *Galenodromus occidentallis, Colpoclypeus florus, Deraeocoris brevis, Mastrus ridibundus* and *Anthocorus nemoralis*.

- Acute toxicity of 6 to 11 insecticides (depending on test species) was measured in replicated bioassays and there were significant differences among species in in their response profile to the insecticides.

- Sublethal bioassays were done for several low to modest toxicity insecticides with each test species and there were significant sublethal effects and obvious differences in response among beneficial species to these insecticides

- *Galendromus occidentalis*: Showed virtually no acute toxicity responses and only modest sublethal effects to Assail, Secure, Envidor and possibly Esteem.

- *Chrysoperla carnea*: Actara, Assail and Provado caused high mortality of larvae or adults but had no effect on egg hatch. Intrepid, Esteem and Success were not acutely toxic to any stage tested but had sub-lethal effects as did Provado and Actara. Intrepid reduced adult fecundity and egg hatch; Esteem prolonged development of the last larval stage. Success reduced adult fecundity.

- *Mastrus ridibundus*. This large parasitic wasp was highly susceptible to Provado Actara and Success in acute toxicity tests. It proved susceptible to the remaining insecticides in sublethal assays (i.e., Actara, Intrepid, Esteem, Success and Assail).

- ***Deraeocoris brevis****:* Assail, Agrimek and Dannitol were acutely toxic to nymphs or adults. Of these Assail had no sublethal effects, and Agrimek extended both nymph and subsequent adult development time and lowered adult fecundity and egg hatch. Success-treated (full field rate) adults laid fewer and less viable eggs. Also, egg hatch in the subsequent generation was lower. Intrepid had no sublethal effects on adults, but treating nymphs at the full rate

increased development time of 4th instars, and lowered fecundity in the subsequent generation.

- *Colpoclypeus florus*: Actara, Assail, Provado, Pyramite and Success proved acutely toxic, and both Assail andSuccess killed more that 50% at a tenth of field rates. Esteem, Azadirect and Pyramite exposure reduced female fertility in the first clutch of eggs laid but this effect disappeared in the second clutch for both Esteem and Pyramite, but not for Azadirect.
- *Anthocoris nemoralis*: Actara, Assail and Provado were acutely toxic at field rate as was Provado and Assail at 10% of field rate. At 10% of field rate, two-week measures of fecundity were decreased by Actara, Success, Intrepid and Esteem.
- *Forficula auricularia:* Provado, Guthion, and Imidan all showed acute toxicity to immatures. Success showed acute toxicity to adults after 15 days. Sublethal bioassays required 7 months from treatment to assessment and still suffer from high control mortality. Earwigs showed a sublethal response to only one insecticide: Intrepid-treated females laid fewer egg masses of smaller size, producing fewer young overall.

Methods:
Bioassay methods are idiosyncratic to the species being tested and are available in detail by request. Acute toxicities are based on topical application of formulated insecticides at field rates or 10% field rates with water controls. All applications employ a potter spray tower. The acute bioassays entail exposing the test insects to topical application and subsequently allowing them to recover, or not, in a clean environment. In the sublethal assays a topical exposure is applied to the test insects but they are then forced to behave in an environment and non-prey food sprayed with the same insecticide. Bioassays deviate by species requirements at this point.

Results and Discussion
Results are summarized in the significant findings section above and in the summary tables (1 and 2) presented below. Some of the surprises are how very different each species response profile is to the insecticides. Also, the largest arthropod and the smalles (earwigs and predatory mites) represent the 2 most tolerant species tested, probably for very different reasons. With the exception of the mite, Provado, Actara, Success, and usually Assail, proved acutely toxic. Esteem was the most likely insecticide to produce sublethal effects.

Table 1. Acute toxicity summaries for 2002 and 2003 combined

Acute Toxicity **Green -- trivial** **Yellow – caution** **Red -- beware**	Earwig *(Forficula)*; Hilton	Lace-wing s *(Chrysoperla)*; **Larvae/Adults** Mills	Predator mite (*Galendromus*); Beers	*Colpoclypeus florus*; Unruh	*Anthocoris nemoralis*; Horton	*Deraeocoris brevis*; Riedl	*Mastrus ridibundus*; Mills
Provado 1.6F	4/1	3 / 3	0	3	4	----	4
Actara 25WDG	--/--	4 / 4	0	3	3	----	4
Intrepid 2F	0/0	0 / --	0	0	0	0/0/0	0
Esteem 0.86EC	0/0	0 / --	0	0	0	0/1/0	0
Success 2SC	--/4	0 / --	0	4	0	0/0/0	4
Assail 70WP	--/1	1 / 3	1	4	4	0/3/4	0
Aza-Direct 0.0987				0			
Acramite			0				
Secure			0				
Pyramite 60W				3			
Agri-Mek 0.15EC	1/0					3/4/4/	
Guthion 50W	4/--						
Imidan 70W	4/--					2/0/0	
Dannitol	--/0					4/4/4	
Dimiln	0/0						
Mitac	--/0						

0=no effect, 1=up to 25% mortalityt, 2 = 25-50% mortality; 3= 50-75% mortality; 4 =75-100% mortality=

Table 2. Sublethal effects of various insecticides on 7 insects tested.

Sublethal effects **Green – none** **Yellow – some** **"—" not tested**	Earwig *(Forficula)*; Hilton	Lace-wing s *(Chrysoperla)*; **Larvae/Adults** Mills	Predator mite (*Galendromus*); Beers	*Colpoclypeus florus*; Unruh	*Anthocoris nemoralis*; Horton	*Deraeocoris brevis*; Riedl	*Mastrus ridibundus*; Mills
Provado 1.6F	0	2/ 0	0	---	--	---	--
Actara 25WDG	---	2 / --	1	---	1	---	2
Intrepid 2F	1	0 / --	0	0	1	1/0	1
Esteem 0.86EC	0	1 / --	1	1	1	---	1
Success 2SC	---	0 / 2	0	0	1	0/2	2
Assail 70WP	0	2 / 0	0	---	---	0/0	2
Aza-Direct 0.0987				1			
Acramite			0				
Secure			1				
Envidor			1				
Pyramite 60W				1			
Agri-Mek 0.15EC	0					0/2	
Guthion 50W							
Imidan 70W							
Dannitol	0						
Dimiln	0						
Mitac	0						

0=no effect, 1=minor effect, 2=large effect (dramatically affects life history) – did not survive well enough to test

Proposed schedule of accomplishments:

Bioassay development is complete. We plan to complete all objective of the proposal in the final year, with year 3 emphasizing the effect of sublethal exposures to key materials, acute and sublethal testing for some materials not examined (specific to each species). Also, several sites will be testing the predicted effect of insecticide sprays on field populations of predators or parasitoids.

BUDGET:

TITLE:	Effects of New Insecticides on Natural enemies: Acute toxicity and sublethal effects
CO-PIS	Tom Unruh
Proposed Project Duration:	**3 years**
Project total:	**$150,000**
Current year request	2003: $50,000

Budget:

Item	2002[1]	2003[1]	**2004[1]**
Salaries	45,000	45,000	**45,000**
benefits	5,000	5,000	**5,000**
IFAFS Matching	86,000	86,000	**0**
Total (WRFRC)	50,000	50,000	**50,000**

[1] $10,000 per location; and funds should be sent to 5 locations as in 2003.

$15,000 charged to Pear commodity

CONTINUING PROJECT PROPOSAL **YEAR 3/3**

TITLE: Development of Genetic Markers to Identify Problematic Pests in Deciduous Fruits Intercepted at Foreign Quarantine Inspection Stations

PI: Tom Unruh. USDA-ARS-YARL. 5230 Konnowac Pass Rd.Wapato, WA 98951. Phone 509-454-6563; FAX: 509-454-5646; e-mail: unruh@yarl.ars.usda.gov

CO-PI Nina M. Bárcenas. Postdoctoral Research Associate. Washington State University

Collaborator Lisa G. Neven, USDA-ARS-YARL, Wapato.

OBJECTIVES (2004):

- Complete discovery and development of molecular protocol to discriminate between apple maggot (*Rhagoletis pomonella*) and snowberry maggot (*R. zephyria*) captured in traps for accurate monitoring and quarantine actions.
- Continue collections of spider mites from different locations, design primers and test reliability and robustness of molecular protocols to discriminate among mites of quarantine importance, mainly Pacific spider mite (*Tetranychus pacificus*) and McDaniel spider mite (*T. mcdanieli*).
- Acquire samples of world lepidoptera attacking apple, pear, and cherry, sequence mtDNA and develop protocols to identify them (emphasis on exotic fruit boring species).

Significant Findings:

- More rapid and simple DNA extraction protocols were developed to facilitate the rapid preparation of alcohol preserved larvae (2 h vs. 6-12 h with standard protocols).
- DNA diagnostics for the lepidopteran fruit boring complex [codling moth (CM), oriental fruit moth (OFM), lesser apple worm (LAW) and cherry fruit worm (CFW)] has been completed and the technology validated by and transferred to Mexico.
- DNA sequencing indicate that *Grapholita* spp. larvae found in rose-hips (similar to LAW as larvae and to CFW as an adult) is a new, undescribed species (rose-hip worm).
- Sequences for the COI gene of the mtDNA were collected, aligned, and analyzed for the Pacific Northwest spider mites and a hot spot has been detected for primer design.
- Although mtDNA sequences of *Rhagoletis pomonella* and *R. zephyria* are almost identical, extensive screening has pointed to COI, CytB and 16s rDNA genes as potential markers for DNA diagnostics by PCR-RFLP analysis.

Methods for 2003:

1. Each new group of species of interest requires literature and Gene Bank searches to capitalize previous DNA sequencing work. We also must acquire specimens, extract/release DNA, amplify part(s) of genome using universal primers, sequence amplification products, align library of sequences, discover diagnostic sites, design and test diagnostic primers/protocols.
2. To expand our diagnostic array to key world pests of temperate tree fruits requires requesting specimens from scientists around the globe. Acquisition of sequence for these species is simplified by our ability to amplify DNA from museum specimens.
3. Real time PCR optimization requires testing a series of parameter including temperature profiles, buffer components, substrate concentrations, and DNA extraction techniques, using standard molecular biology methods. Each diagnostic primer needs to be tested against multiple populations of the target and non-target species.

Results and Discussion (Based on 2003 objectives in bold):

1. Complete RT-PCR protocol optimization for apple attacking Lepidoptera of the USA and seek its acceptance by pest ID services of Mexico, Canada, Japan, and Taiwan.

a) DNA sequences at the diagnostic mitochondrial COI region were complemented for the fruit boring complex: codling moth (CM), oriental fruit moth (OFM), lesser apple worm (LAW), and cherry fruitworm (CFW). All except LAW are highly conserved at the diagnostic region.
b) Species specific primers were tested and improved, with the final version shown in Table 1. LAW primers are robust in spite of population variation.
c) Real time PCR protocols (results in 2 hours after DNA extraction) (Figure 1) and traditional PCR protocols (results in 12 hours after DNA extraction; Figure 2) were developed.
d) Species specific primers were tested with populations as diverse as possible and we found them to be highly robust (Table 2).
e) The molecular technique was validated by Enrique Vega (taxonomist), and Dr. Juan P. Martínez (Molecular Biologist and Director of the Laboratorio de Diagnóstico Fitosanitario, SAGARPA, México). They also received training to use the real time PCR protocol. However, since this equipment is not yet available at the Mexican Laboratory, the protocol was adapted for traditional PCR so they can start using it right away.

1.1. Discovery of new species of *Grapholita* in WA.

A moth from Indiana classified by taxonomists as CFW had a distinct sequence from two other CFW moths from Michigan and Washington (28 out of 420 base pair differences as opposed to 2/420 between WA and MI), suggesting a different race or even a different species. This "CFW" proved identical in DNA sequences to two moths collected in rose-hips in ecological studies in Washington using rose as a host habitat for leafroller parasitoids. The rose-hip form looks like LAW as a larva (but has 33/420 base pair differences from LAW) and the adult resembles CFW (but has 28/420 differences from CFW). We think that this group (see Figure 3) represents an undescribed species of "rose-hip-worm". We will make more collections and do more sequencing next year to clarify this issue. We find no evidence that this rose form is a pest but it may be mistaken for CFW in traps.

2 Acquire samples of remaining world lepidoptera attacking apple, pear, and cherry, sequence mtDNA and develop protocols to identify them (emphasis on fruit boring species)

No further collections or sequencing have been made of tortricids, except for filbertworm (*Cydia latiferreana*), which was suspected to be found in a pear shipment to Mexico. Sequences are unique and distinctive, so primers can be easily designed if necessary.

3 Complete collections of mite pests of tree fruits, collect DNA sequences, identify diagnostic primers, and develop pilot protocols for identification.

a. Our work has focused in literature and gene bank sequences. A 390 bp fragment of COI has been sequenced by others for 33 populations of *T. urticae* from around the world, *T. mcdanieli*, *T. pacificus*, *Paninychus ulmi* and *Eotetranychus carpini*, all known to occur in the Pacific Northwest. As with the internal fruit feeding moths, there is a hot spot in CO1 (base pairs 2310-2336) that looks promising (Table 3), especially because it is conserved in *T. urticae* (only one bp polymorphic among 33 mites populations from around the world).
b. We are designing species-specific primers and finding restriction sites, but need more populations of mites to ensure differences are conserved within the other species of mites to validate our primers or RFLP methods. We currently have *T. urticae* and *P. ulmi* from WA, *T. pacificus* and *T. turkestani* from CA, and *T. mcdanieli* from Vancouver, WA.

4 Apple maggot and Snowberry fruit flies diagnostics

This problem was not included in 2003 objectives but was undertaken independently after consultations with Mike Klaus, WSDA and Jim Archer. Since first detected in 1979 in Portland, OR, the apple maggot, *Rhagoletis pomonella*, has spread and infested apples in many parts of the Pacific Northwest. It is now considered established in the 17 Western Washington counties and in Klickitat, Skamania, Spokane and recently Kittitas (Ellensburg) counties in Central and Eastern Washington, raising concern that it is expanding its range into the main apple production areas. Apple maggot flies caught near apple orchards pose a quarantine problem for export to California and other countries and may require frequent OP application. Intensive monitoring is performed with sticky traps but *R. pomonella* is morphologically near identical to the snowberry maggot *Ragholetis zephyria*, a native species that feeds on snowberry, not on apples. Current ID methods use male genitalia and female ovipositor, both continuous traits, with some overlap. Our objective was to find molecular markers that distinguish between the apple maggot and the closely related Snowberry fly. Jim Smith of MSU is collaborating with us.

DNA sequencing efforts toward understanding the evolution of the *Rhagoletis pomonella* species group have shown that *R. pomonella* and *R. zephyria* have identical or almost identical haplotypes for most mitochondrial DNA genes analyzed (Table 4). The differences detected in COI, Cyt B and 16s rDNA will be further explored as potential diagnostic restriction sites or regions on which to design species specific primers.

BUDGET

TITLE: Development of Genetic Markers to Identify Problematic Pests in Deciduous Fruits Intercepted at Foreign Quarantine Inspection Stations

PI: Tom Unruh

Project Duration: 3 years (2002-2004)

Project total: **$92,200**

Current year request 2004: $**28,000**

Item	**2002**	**2003**	**2004**
Salaries	20,000	27,000	**21,500** [1]
Benefits	6,000	8,200	**6,500**
Supplies	2,000	1,000	**0**
Total	28,000	36,200	**28,000**

[1] 40% support for Dr. Bárcenas, research associate with WSU.

Table 1. Species specific primers for four species of internal fruit feeders: Cpo, *Cydia pomonella*; Gmo,*Grapholita molesta*; Gpr,*Grapholita prunivora*; and Gpa, *Grapholita packardi*.

Primer code	DNA sequence 5´- 3´	Base # in *D. yakuba*	Number of differences between homologous sequences by species			
			Cpo	Gmo	Gpr	Gpa
Cpo-C1-J-2063	AGCTCTTTTACTTCTTTTATCATT	2040	**0**	6	4-6	2
Cpo-C1-N-2136	ATAGGATCACCTCCACCA	2153	**0**	3	3	2
Gmo-CI-J-2062	CAGCTCTTTTATTACTACTTTCAC	2039	6	**0**	6-8	4
Gmo-C1-N-2136	AATAGGATCTCCTCCTCCT	2154	3	**0**	2	2
Gpr-C1-J-2062	CAGCTCTCCTACTTTTACTATCAC	2039	6	6	**0-2**	8
Gpr-C1-N-2136	AATAGGATCTCCACCACCT	2154	3	2	**0**	1
Gpa-C1-J-2067	CTTTTATTACTTTTATCATTACCC	2044	3	5	7-9	**0**
Gpa-C1-N-2138	ATATAGAATAGGATCTCCACCAC	2163	3-4	3	1	**0**

Species	Origin
Cydia pomonella	Yakima, WA (lab colony)
	Sebastopol, CA
	Wooster, OH
	Layrac, France
	Anapa, South Russia
	Guzeripl, Caucasus
	Mezmaj Caucasus
	Targabetei,, Kazakstan (NE)
	Ketman, Kazakstan (SE)
	Oxsai, Kazakstan (South)
Grapholita molesta	CA (Fresno lab colony)
	Bridgeton, NJ
	East Lansing, MI
Grapholita prunivora	Yakima, WA (lab colony)
	Mehoopany, PA
	Tacoma, WA
	Coles Co., IL
	Québec, Canada
Grapholita packardi	Lace Co., MI
	Seattle, WA
	Houston, TX

Table 2. Populations tested against species specific primers; field collected unless stated otherwise.

Figure 1. Example of Real Time PCR output: Codling moth DNA with CM (blue), OFM (gray), LAW (orange) and CFW (green) primers. A. Dynamics of amplification of target DNA. B. Melting profile of PCR product which is size and sequence sensitive.

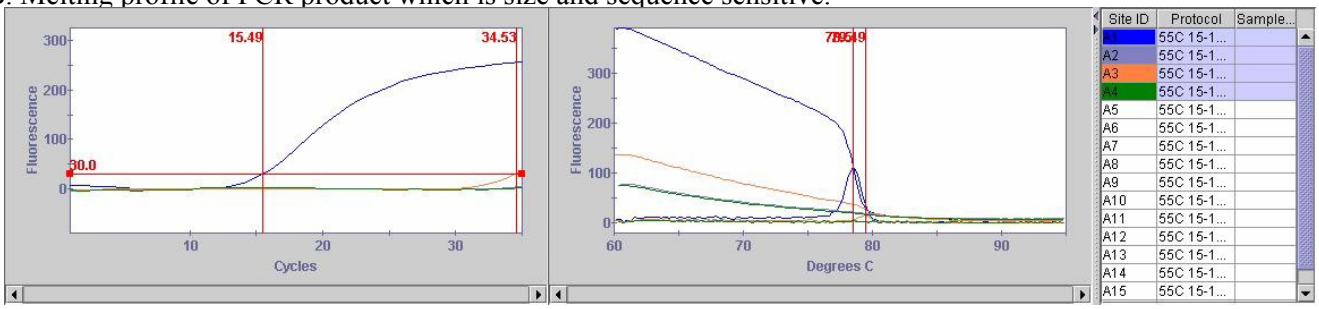


Figure 2. Traditional PCR product separated in agarose gel. Lanes in gel represent (left to right):

1. Negative control (no DNA with universal primers).
2. Positive Control (CM DNA with universal primers). (3 and 12: 100 bp ladder)

4-7, CM DNA with CM (4+), OFM (5-), LAW (6-) and CFW (7 -) primers;
8-11 OFM DNA with CM (8 -), OFM (9+), LAW (10-) and CFW (11-) primers;
13-16 LAW DNA with CM (12-), OFM (13-), LAW (14+) and CFW (15-) primers;
17-20 CFW DNA with CM (16-), OFM (17-), LAW (19-) and CFW (20+) primers.

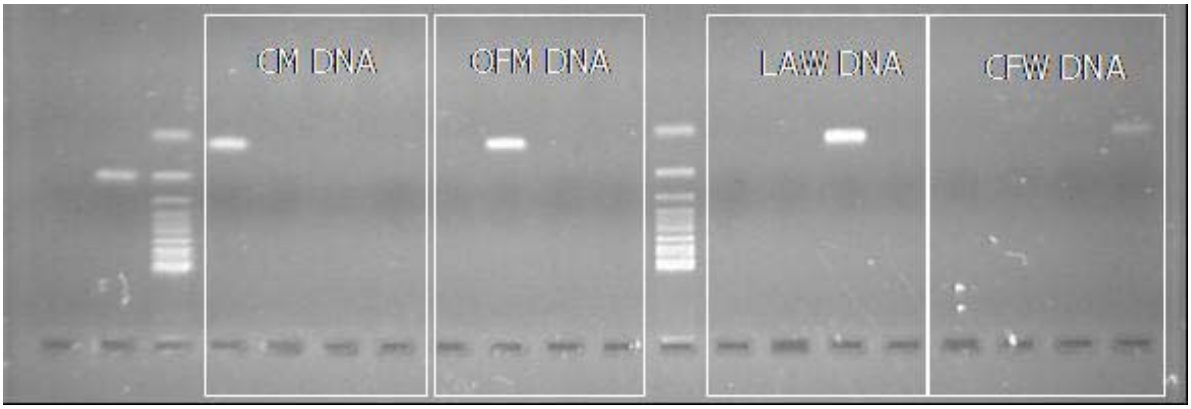


Figure 3. Neighbor-joining tree depicting DNA sequence differences among *Grapholita* and *Cydia* of export concern. Clusters of populations in the same species are highly supported by reliability tests (bootstrap). Note the discrete group with CFW from IN(diana) and two populations of R(ose)H(ip)W(orm).

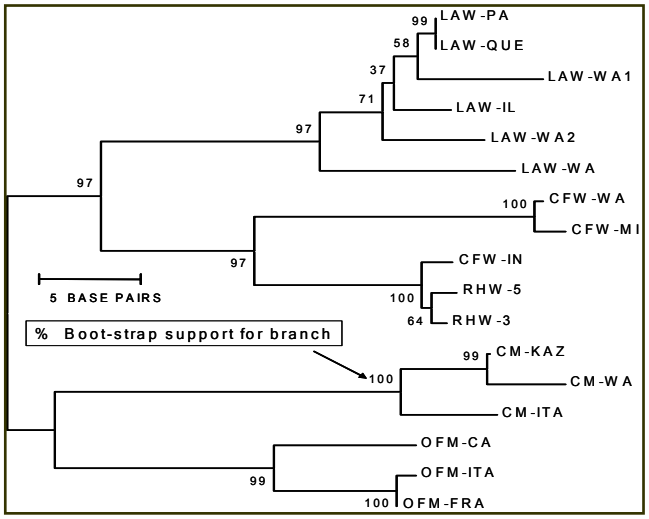


Table 3. DNA sequencing in *Rhagoletis pomonella* (Rpo) and *R. zephyria* (Rze), showing number of sequences collected and their source. Also shown are differences observed between these 2 very close species.

Mitochrondrial (mt) or nuclear (n) DNA	Gene (region)	Fragment Size	# Rpo	# Rze	# base - pair diff.	Reference
Mt	COI (1718-2191)	501	3 (25)[1]	6 (20)[1]	3	herein
Mt	COI (2183-2329)	192	2	2	0	herein
Mt	COI/COII (2792-3722)	946	35	10	0	Smith et al. 2002
Mt	CytB (10612-11367)	780	2	2	1	herein
Mt	12s rDNA (14233-14594)	405	2	2	0	herein
Mt	16s rDNA (12920-13760)	848	2	2	1	McPheron and Han, 1997
N	G6PDH	460	1	1	5	Soto-Adames, 1994

1 The 3 COI haplotypes were characterized by restriction analysis in 25 and 20 insects.

Table 4. DNA sequence of spider mites (sm) at the diagnostic region. The right column shows the number of base pair differences between the diagnostic region for two spotted sm (32 populations of *T. urticae)* compared to McDaniel sm (*T. mcdanieli*), Pacific sm (*T. pacificus*), the European red mite (*Panonychus ulmi*), and the yellow sm (*Eotetranychus carpini)* a recently established exotic mite found in Oregon. Note 3 differences between the Pacific sm and McDaniel sm (shaded & underlined).

Population	base pair differences from *T. urticae*:	x
Tu (27 pops)	: CTATAATATCAATTGGTTTATTAGGATTTATTGTTTGAGCACAT:	0
Tu (5 pop)	: ....C.....:	1
Tmc (1 pop)	: .....A.C.T.....T...:	4
Tpaci (1 pop)	: .....C.T.....C..C:	4
Pulmi (2 pops)	: .....T.....C...C.....G.A.....T...:	6
Ecarp (1 pop)	: .....T.....A...C.T..T.....G.....:	6

CONTINUING PROJECT REPORT **YEAR 3/3**
WTFRC PROJECT # TR-02-235

Project Title: Reduction of Pesticide Inputs, Worker Exposure, and Drift Through Alternative Sprayer Technology
PI: Allan S. Felsot (Washington State University, Dept. of Entomology; afelsot@tricity.wsu.edu)
Organization: Washington State University, 2710 University Drive, Richland, WA 99352; Voice 509-372-7365; Fax 509-372-7460
Co-PI(s) : Vince Hebert (WSU, Dept. of Entomology; vhebert@tricity.wsu.edu)
Cooperator(s): Tom Auvil (Washington Tree Fruit Research Commission)
Jay Brunner & Mike Doerr (Washington State University, Dept. of Entomology)
Linda Finch (USDA-Wapato)

Objectives:

The goal of our research is to help growers reduce the cost of pesticide applications while simultaneously maintaining efficacy and reducing worker exposure and off-target drift. This project is designed to help meet the goals of the 'technology roadmap' to reduce production costs while enhancing fruit quality and sustaining a quality environment.

Previously, we hypothesized that new sprayer technology can allow reduced application rates in reduced volumes of water. We have shown using a combination of neonate codling moth (CM) larval bioassays and leaf residue analyses that Proptec and airblast sprayers probably work comparably. Furthermore, we have shown that 0.5X application rates of Guthion cause significant larval mortality in bioassays of field-collected leaves. This year we have observed that alternate row spraying patterns (see Figure 1) may also be effective in controlling CM larvae. Furthermore, we have evidence from bioassays that suggest that prior applications of Intrepid and/or Sevin may enhance bioactivity of subsequent applications of Assail or Guthion. This year we plan experiments to test the hypothesis that alternate row (i.e., every other row) and skip row (i.e., leaving an unsprayed row between two sprayed rows) can be efficacious in suppressing CM larvae. The second hypothesis that we will test is that Intrepid, by virtue of its unique mode of action, may enhance activity of subsequent insecticide sprays and therefore make more feasible the reduction in application rates. These two hypotheses will be tested under the following objectives that have been modified from the previous year.

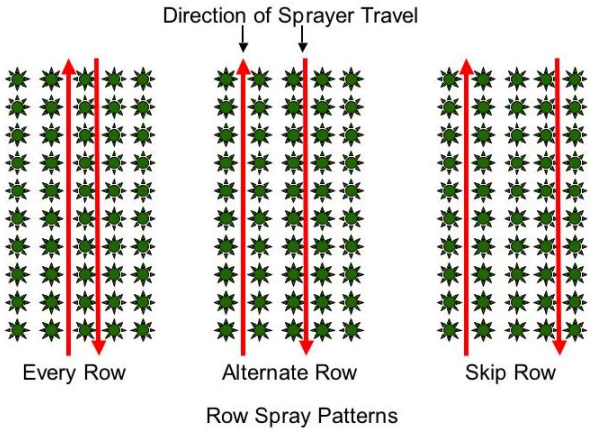
1. Determine the bioactivity of residues deposited by airblast, Proptec tower, and Accutech sprayers operating with reduced carrier volumes and using reduced application rates (0.25X, 0.5X, and 1X rates).
2. Determine the bioactivity of residues deposited by airblast and Proptec tower sprayers using alternative row spraying plans (i.e., every row sprays contrasted with alternate row and skip row sprays).
3. Determine whether bioactivity of cover spray insecticides are additive to or synergized by early applications of either Sevin or Intrepid.
4. Determine the residues deposited from reduced rates of application and from alternative row spraying plans.
5. Characterize within canopy and out of orchard drift deposition and determine the validity of the industry developed AgDrift model in predicting deposited residues.

Significant Findings (Crop Year 2003):

- The LC50 for Assail was statistically estimated to be about three times lower than for Guthion, but the Assail LC95 was estimated to be about two times higher. This result suggested that the label-recommended Assail application rate of 0.15 lb AI/acre may be lower than needed for prolonged efficacy.

- Laboratory bioassays showed that temperature under which the insecticide-exposed larvae were held influenced the magnitude of the LC50, suggesting that larvae active during early morning or late evening in the first part of the summer may be less susceptible than larvae exposed during the day or later in the summer.
- Exposure of neonate CM larvae to field-treated leaves for 3-5 hours resulted in significant mortality. After 24 hours of exposure, 80-100% of larvae generally died in nearly all treatments regardless of rate of application, type of sprayer, and row spray pattern. (see Figures 2, 3)
- Average Assail residues up to 36 days after application with the airblast sprayer exceeded the LC50. At 20 days after application, average Assail residues exceeded the LC95. Residues tended to be lower after Proptec sprays, but all were still above the LC50 after 36 days.
- Guthion residues one day after application exceeded the LC95 estimated for neonate CM regardless of the row spay pattern (see Figure 4). In other words, residues that drifted onto an unsprayed row were sufficient to cause 100% mortality of larvae. The biological activity persisted for 30 days after application (see Figure 2).
- Biological activity against CM larvae on apples was measured using a larval entry hole assay. Persistence of biological activity (as measured by prevention of an entry hole) of deposited residues on apples was very short in comparison to persistence of bioactivity on leaves.
- Fluorescent tracer dye photographs showed uneven coverage on apples, with large areas of apple surfaces lacking spray residues.

Figure 1. Row spray patterns tested during crop year 2003. In alternate row treatments, leaf and apple samples were collected on the leeward side of the row. For the skip row treatment, samples were collected from the untreated row only.



Methods (Plans for Crop Year 2004):

Objective 1 (Effect of Sprayer Type and Application Rate on Bioactivity of Residues)

A cooperating commercial farm near Quincy will be chosen for continued testing of the Proptec sprayer and replicated subplots delineated for sprayer and application rate treatments. The Rears Pakblast sprayer will continue to be used for comparison. In addition, cooperation will be sought from the WSU-Prosser facility to use the Accutech sprayer to study the efficacy of reduced application rates. The Accutech will likely be studied at a cooperating farm close to Prosser.

Application rates will include 0.25X, 0.5X, and 1X treatments of Guthion and Imidan, and 0.5X, 1X, and 1.5X treatments of Assail. Based on estimations of the LC95 for Assail, we believe the recommended Assail rates may too low for optimum efficacy. Thus, a comparison of a slightly higher rate is needed to determine whether Assail bioactivity is more persistent than at the lower rates. To determine the effects of the comparatively cooler temperatures during late May and June and the much hotter temperatures in July and August on persistence of bioactivity, two spray applications will be made. However, enough subplots will be delineated so that each only receives one spray. However, one Assail treatment will include two cover sprays a month apart.

From each treatment, apples and surrounding leaves will be collected within a day after application and on a seven-day schedule for 35 days and returned to the lab for bioassay with neonate

CM larvae. Leaf disks (5 cm^2 diameter) will be punched and assayed. Larvae will be placed on apples that will be examined within 48 hours for the presence of entry holes.

Objective 2 (Bioactivity of Residues Deposited Following Alternate Row Spraying Patterns)

Because bioactivity seemed adequate when alternate row and skip row spraying patterns were used (see Figure 1), we will repeat this experiment. In addition to collecting leaves for bioassay, we plan to place mesh sleeve cages over sprayed branches and add either neonate larvae and/or late developmental stage eggs in known quantities to each cage. The larvae or eggs will be placed on leaves surrounding the apple to simulate probable oviposition sites of natural populations. Leaves and apples will be examined for larvae after several days. Apples will be collected and examined for presence or absence of entry holes as evidence of insecticide efficacy. Based on some preliminary experiments during crop year 2003, we have confidence that this method of in-field bioassay will be a feasible adjunct to the leaf disk bioassay we presently use. During this past year, branches with attached apples were collected and returned to the lab where they were placed in nutrient solutions. Larvae were placed on leaves at varying distances from the apple, and after several days, we were able to detect entry holes in the apples.

Objective 3 (Potential for Additivity or Synergism in Biological Activity of Cover Sprays following Early Season Sevin or Intrepid Applications)

In the laboratory, leaf disks will be treated with mixtures of Guthion or Assail and Sevin or Intrepid. A typical dose-response curve with single insecticide treatments and combinations of insecticides will be used to determine if bioactivity is additive or synergistic. During the second field application trial, we will use mixtures of Intrepid or Sevin at label recommended rates with Guthion or Assail at full or reduced rates to determine if interactions can occur under field conditions.

Objective 4 (Coverage and Magnitude of the Residues Following Low Rate Applications and Alternative Row Spraying Patterns)

Leaf punches and composited whole apples will be collected and analyzed within one day after application to determine initial deposition of residues from the various treatments previously described under Objectives 1 and 2. Samples will then be analyzed 30 days later to determine if residues were above or below the LC50 and LC95 estimated from laboratory dose-response bioassays.

Objective 5 (Characterize within Canopy and Out of Orchard Drift and Determination of the Validity of the AgDrift Model in Predicting Residue Deposition)

Past observations showed significant within canopy drift of residues across rows at levels that were biologically active against CM larvae. These observations coupled with an off-target drift study earlier in the season at the Klingele orchard near Whitstran, WA suggested that drifting residues will be difficult to manage. However, we still do not understand how a well-managed low volume application by the Proptec sprayer will affect out of the orchard movement. Thus, a separate experiment will be set up in an orchard where movement beyond the end tree row can be easily studied. In this study, drift within the canopy will be studied by placing silica gel cards in the canopy and on the ground (both inside and outside of the orchard). The second row from the outside will be sprayed with insecticide and fluorescent tracer, and deposition will be examined on the unsprayed canopy and on the ground both inside and outside of the orchard. The results will be compared to predictions made with the spray drift model, AgDrift.

Results and Discussion:

All field experiments were conducted at the H&M orchard west of Quincy. A block of about 5000 trees was divided into subplots, each 10 trees by five rows, and assigned to treatments that were replicated four times. Main treatments included sprayer types (Rears Pakblast sprayer and Proptec Tower), application rates (0.25X, 0.5X, 1X of label recommendation), and row spray pattern (every row, alternate rows, skip rows) (Figure 1).

The insecticides tested included Guthion, Assail, Imidan (phosmet) and Intrepid (methoxyfenozide). Intrepid and Imidan were only sprayed once (June 10 and July 23, respectively), and Assail and Guthion were sprayed twice. For the second spray, subplots not previously sprayed were newly sprayed. One group of subplots treated with Assail in June was retreated with Assail in July. A single row was treated with adjacent rows left as no-spray buffers. After the start of the experiment we learned that, H&M Orchard had made a cover spray of Intrepid in mid May.

At various times after application, apples and surrounding leaves were collected randomly from each of six trees per replicated subplot. Leaves and apples were returned to the WSU Food and Environmental Quality Lab in Richland where they were subjected to bioassay with CM neonate larvae and chemical analysis for residues of Guthion, Assail, and Imidan.

Innate Bioactivity of Assail and Guthion

Toxicological responses (LC50; LC95) of CM neonate larvae to Assail (acetamiprid) were estimated on leaf surfaces using the leaf disk bioassay procedure developed during 2002. Although the LC50 for Assail (0.007 $\mu g/cm^2$) was about three times lower than for Guthion (azinphos-methyl) (0.019 $\mu g/cm^2$), the LC95 was actually higher owing to a flatter response curve (0.166 $\mu g/cm^2$ for Assail compared to 0.095 $\mu g/cm^2$ for Guthion). Nevertheless, the recommended application rate for Assail is nearly 7-fold lower than for Guthion (0.15 lb AI/acre vs. 1 lb AI/acre), suggesting that Assail may be more effective in the field over a longer period of time if the recommended application rate was raised.

Persistence of Insecticidal Bioactivity on Field-Collected Leaves

Excessive morbidity and mortality of CM larvae occurred during the 24-hour bioassay exposure period and was likely caused by persisting residues of Intrepid and/or Sevin that had been applied by H&M orchard in mid-May (Figures 2, 3). However, lower mortality on untreated leaves collected from a private residence revealed clear differences in mortality caused by the prior Intrepid (or Sevin) application and the insecticide treatments in this study. Furthermore, significant larval mortality occurred on Guthion and Assail treated leaves but not on the orchard leaves within 3-5 hours after exposure (Figures 2, 3). Reduced rates of Guthion (0.25X and 0.5X) caused 100% mortality of neonate CM after 24 hours of exposure.

Reduced rates of Assail (0.5X) were comparatively less effective. The high level of larval mortality exposed to both Assail and Guthion residues 30 days after application of reduced rates suggested that an additive or synergistic interaction may have occurred between the persisting Intrepid (or Sevin) residues and the insecticide residues resulting from the June and July applications (Figure 2, 3). Based on lab bioassays of field-sprayed leaves, reduced rates of Guthion seem to be just as efficacious as full label rates. Further studies of Assail are needed to validate the effect of lower than labeled rates.

Deposition and Persistence of Insecticide Residues in Relation to LC50/LC95

Initially deposited residues of Guthion were above the LC50 and LC95 in all treatments that examined different row spray patterns (Figures 1, 4). Thus, significant within canopy movement of spray occurs among rows, and spraying Guthion on only alternate rows has the potential to reduce use of insecticide without sacrificing efficacy. Residues of Guthion seemed to be biologically persistent for 30 days after application. Residues of Assail, on the other hand, were greater than the LC50 for 36 days following application, but after 20 days they were less than the LC95 for all treatments

except the 1X airblast spray treatment (data not shown). Residue measurements seem to predict reasonably well bioactivity when the LC50/LC95 has been determined on a leaf area basis (µg/cm^2).

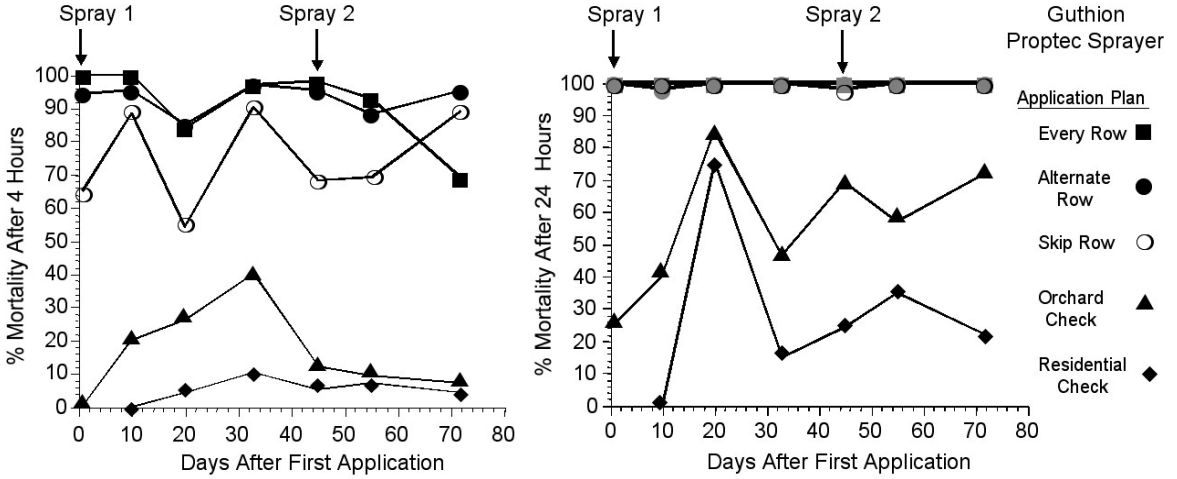


Figure 2. Bioactivity (% mortality of neonate CM) of Guthion residues on field-collected leaves sprayed with a Proptec sprayer using three row-spraying patterns. Bioactivity was measured after 4 h (left side) and after 24 h (right side). The second spray was made to previously untreated subplots.

Figure 3. Persistence of Assail bioactivity (% mortality of CM neonates) on field-sprayed foliage (Proptec sprayer, 30 gal/acre). All subplots designated "1 spray" were sprayed either once on June 10, 2003 or once on July 23, 2003 with either 0.15 lb AI/a (1X) or 0.075 lb AI/a (0.5X). The designated "2 spray" treatments (black symbols) represented subplots receiving two sprays of Assail. Mortality was determined after 4 and 24 hours.

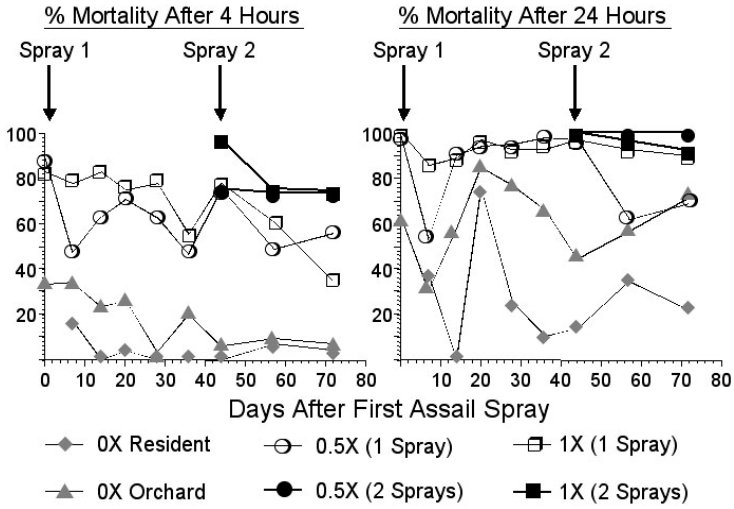
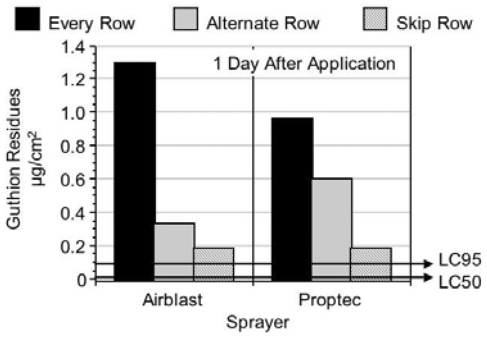


Figure 4. Initial residues of Guthion (µg/cm^2) following alternative row spraying patterns in relationship to the estimated LC50 and LC95. The airblast sprayer delivered 100 gal/acre of finished spray, and the Proptec delivered 30 gal/acre. Application rate was 1 lb AI/acre.



Budget:
Project Title: Reduction of Pesticide Inputs, Worker Exposure, and Drift Through Alternative Sprayer Technology
PI: Allan Felsot
Project Duration: 2002–2004 (3 years)
Current Year: 2003
Project Total (3 years): $161,807
Current Year Request: $55,106

Year	Year 1 (2002)	Year 2 (2003	Year 3 (2004)
Total	$52,448	$54,253	$55,106

Current Year Breakdown

Item	**Year 1 (2002)**	**Year 2 (2003)**	**Year 3 (2004)**
Salary (1.0 FTE)	17,730	21,072	**35,115**
Benefits (29%)	4,787	5,900	**10,183**
Wages [1/]	6,880	6,880	**1,550**
Benefits (16%)	1,101	1,101	**248**
Equipment	4,500	0	**0**
Supplies [2/]	16,550	18,403	**7,010**
Travel [3/]	900	897	**1,000**
Total	$52,448	$54,253	**$55,106**

Explanation of budget request differential from original proposal: The original proposal (submitted for FY2002) projected a 2004 budget request of $49,786. In FY2003, the budget request projected for the third year was $55,332. This year's request is slightly less than last year's projections. The change in initially proposed budget is due to the salary line. During this past year, WSU budget cuts forced the elimination of a state-funded graduate research assistantship assigned to the PI. Presently, the PI does not have a state-funded technical assistant (nor graduate student). Thus, it has become necessary to allocate funding for a long-time "soft" money funded Research Aide who is well trained with the GLP standards required for operation at the FEQL and has experience with residue analyses.

[1/] The wages are for a temporary summer technical assistant to assist part time with processing of leaves for bioassay and to clean glassware associated with the residue analyses.
[2/] Supplies are partly used for glass containers to store leaf punch disks and Petri dishes and filter paper for the bioassays. Most of the funding however is used to support residue analyses of both leaves and apples. Based on past experience, we can conduct less residue analyses in the past but use strategic time intervals that would give the same information as when residues were analyzed on a weekly basis. Thus, we should achieve a cost reduction in analytical supply needs from previous years.
[3/] **In the past, the designated research plots have been located nearly 250 miles round trip distance from the Food and Environmental Quality Lab in the Tri-Cities. Samples are collected every 7-10 days after each spray treatment, and two days are usually required per sampling interval. Thus, the requested funding is strictly used to defray mileage costs associated with trips to the field plots.**

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #AE-03-331A **WSU Project #13C-3643-4387**

Project title: Sensor-webs: developing sensors for automated monitoring of the orchard environment for improving insect and disease management.

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee, WA; (509) 663-8181 ext. 238; jfb@wsu.edu

Co-PIs and affiliations: Vince Jones, Associate Entomologist, and Chang-Lin Xiao, Assistant Plant Pathologist, WSU Tree Fruit Research and Extension Center, Wenatchee, WA
Francis Pierce, Director, Center for Precision Agricultural Systems, WSU Prosser IAREC, Prosser, WA
Gary Grove, Plant Pathologist, WSU Prosser IAREC, Prosser, WA.

Objectives:
1. Develop sensor-web technology that will allow us to deploy wireless sensor-webs within an orchard to measure temperature, leaf wetness and automated insect traps.
2. Develop and validate the use of automated insect traps focusing on codling moth and leafrollers.
3. Evaluate the weather data and trap data to determine if higher density of weather data and trap data improves IPM programs.
4. Compare micro- and mesoclimatic modeling of apple, cherry and nectarine/peach powdery mildew epidemics.

Significant findings:
1. The development of a 900 MHz spread spectrum frequency hopping radio with data logging capabilities was completed. It provides a low cost, high power device required in the concept of the AgWeatherNet. Assuming FCC approval, the radio will be ready for field testing in 2004.
2. An automated trap following the design of a Multipher trap was developed and tested. The trap was large and was not as efficient as the comparative trap without the automated sensor.
3. The sensor used in the automated trap did record moths that entered and died. However, the sensor also recorded a large amount of count data not associated with leafroller moths or any other insects entering the trap.
4. An AgWeatherNet backbone was established in five cherry orchards in the lower Yakima valley. In addition, a sensor web was established in a 5-acre cherry orchard at IAREC.
5. Data from the AgWeatherNet sensors provided information that was useful in understanding and potentially predicting cherry powdery mildew disease potential.

Methods:
Objective 1: The sensor-web technology is similar to the AgWeatherNet weather stations but modified to reduce costs and to meet the needs of an orchard-specific sensor net. For example, the AgWeatherNet station must have radio equipment strong enough to reach 20 miles, power capabilities able to deal with the extremes of temperature and sunlight and transmit data in real-time. Conversely, each node in the sensor-web will have a range of about one mile, be powered by batteries that cannot be trickle-charged in the field, and be in the orchard during March-October. In the sensor net nodes we will be able to store weather or insect count data as it is received, then relay the data every 24 hours to a base station for transmission to the Internet. This approach has the potential of dramatically reducing the cost of in-orchard units while maintaining good reliability within the framework of the growing season.

Objective 2: Although the sensor-web technology will not be fully available in its final design configuration during the first year of the proposal, we will be able to test trap designs during the first year. We will evaluate several different types of traps or monitoring units for specificity for codling moth or leafrollers. We will initially test a standard delta trap, a tube-type trap (a plastic tube with two inward facing wire-screen funnels), along with other commercially available traps. In studies this past summer (2002), we found that, using the tube trap with a pheromone lure, we only caught the target moth and, just as importantly, no other insects were found. This is promising enough that the tube trap will be the basis of our design concept for an automated trap.

Automated trap designs will be compared with the current delta trap to determine efficiency and the relationship between captures in both traps. We will also be able to test the automated traps during the first year in a datalogger-web (i.e., each trap will have a datalogger that must be hand-downloaded attached to the trap to record data, not a wireless module). The sensor-web will be tested in years two and three once a final automated trap design is determined. We will place five automated traps in an orchard along with five standard delta traps during the flights of codling moth and leafrollers. The traps will be placed in pairs, separated by ≈30 meters. The number of insects collected in each trap type will be recorded at frequent intervals (1-3 days). Analysis will look for not only the total numbers caught in the two trap types but also the frequency with which a trap catches moths.

In the first year we will also determine the density of monitoring units required to adequately monitor a given orchard size for leafroller and codling moth. The number of units required for monitoring will then be determined by the number of dataloggers and traps that we have available in years two and three. We will geocode (record the exact position of) each trap and use geostatistical analysis to determine the number of traps necessary to adequately monitor the orchard. It is anticipated that we will monitor a minimum of five orchards in this fashion.

***Objective 3*:** The sensor-webs deployed for testing during the second and third years will be chosen so that we can evaluate the effect of more accurate weather data on models for powdery mildew and insects as well as trap catch. We will use apple orchards that contain susceptible varieties and apply fungicides based on recommendations from the sensor-web versus those from the closest traditional weather station. The number of sprays required and costs of the sprays will be compared. Model predictions for insect flight will also be compared to see if high-density temperature data improve predictions of the codling moth model.

***Objective 4*:** The initial year will consist of selecting suitable orchard sites, measuring mesoclimatic data and assessing disease incidence and severity at those sites. Years two and three will include the monitoring of both meso- and microclimatic data (using newly developed sensor-webs) and disease assessment.

Results and discussion:

Objective 1: The intent of this objective was to develop a different datalogger/telemetry solution from that used in AgWeatherNet that would be lower in cost and consequently would have less radio range but also lower power requirements. Lower cost datalogger/radios would allow the deployment of clusters of relatively inexpensive radio telemetry dataloggers configured with temperature, leaf wetness and electronic pest sensors. The cluster members would be designed to report back to a master that could be interfaced with an AgWeatherNet repeater providing access to the network backbone and ultimately to the Internet.

We had intended to build this new system around a commercial radio that met the desired design specifications – lower power, lower range and lower price. However, we embarked on the process of building our own radio and datalogger from the ground up. With additional support from other projects, we have completed the development of a 900 MHz spread spectrum frequency hopping

radio with data logging capabilities. This new device provides the desired low cost solution while retaining many of the features of the AgWeatherNet device. The modular radio portion of this device is being tested for FCC certification in mid-December. It must pass FCC certification before it is approved for use. The radio has high power for long-range performance with low power consumption. Up to 255 units can be deployed in a cluster per a single master unit. Data collection is very fast with the capability of polling 25 units per second. The data logging capabilities in its current configuration accommodate many of the sensors used in AgWeatherNet including the ones desired for this application. We cannot perform the range of tests we would like to do until FCC certification is approved. However, laboratory tests are very promising. The modular design of the radio makes it possible to build a variety of application technologies around it. With FCC approval, this device should be ready for field testing with the electronic pest sensors in 2004.

Objective 2: Our basic approach this year was to measure the passage of an insect through the collection tube in a pheromone trap like the one in this picture. We used two initial approaches to the design of the pest sensor. One is roughly based on capacitance in which an insect passing through the chamber either enhances or de-enhances the charge field coupling. While in principle this approach should work well, we were not able to refine this sufficiently in time for field use in 2003. We are continuing the development of this approach this winter. The second approach uses optical components consisting of infrared light emitting diodes (IRLED) and phototransistors to create a photo interrupter. Basically, this creates a beam of light which is interrupted when an insect passes through the beam which, in turn, changes the output of the device that is be read by a microcontroller. Thus, our optical sensing device consists of a microcontroller and a photo interrupter on a printed circuit board along with some other relevant components. The complete sensing device is connected to a datalogger that enables and monitors the device through firmware operating the logger. The optical insect sensor is installed on two external sides of a tube extending from the funnel into the base of the insect trap. The pheromone is placed at the top of the trap along with a kill strip. To improve the detection of the falling dead insect, the tube was crimped into a cross-sectional V shape to narrow the passage opening. The crimping was used to focus the beam and avoid multipath reflections.

The optical insect sensors were installed on five traps like the one pictured above and connected to AgWeatherNet dataloggers equipped with leaf wetness and air temp/relative humidity sensors. These units were deployed in experimental orchards during the codling moth flights in late summer. Two Automata electronic insect sensors were also evaluated. The Automata sensor senses the impact of the falling insect upon contact with the sensor installed at the base of the trap.

Traps received from the Center for Precision Agricultural Systems were placed in a non-bearing block of apples in the Beverly orchard in Mattawa on 11 August and baited with a standard OBLR lure. Five standard Multipher traps were used as controls, and five of the auto traps were modified by the Center for Precision Ag group. Traps were checked multiple times per week (usually 4x). Each time the trap was checked, the number of OBLR moths and other insects was recorded and the trap was emptied. Lures were replaced in all the traps on 3 September. Initially, a half kill strip was placed in the bottom of the traps, but on 22 August we placed a kill strip in the upper pheromone basket as well to ensure that moths were dead when they fell through the counting mechanism.

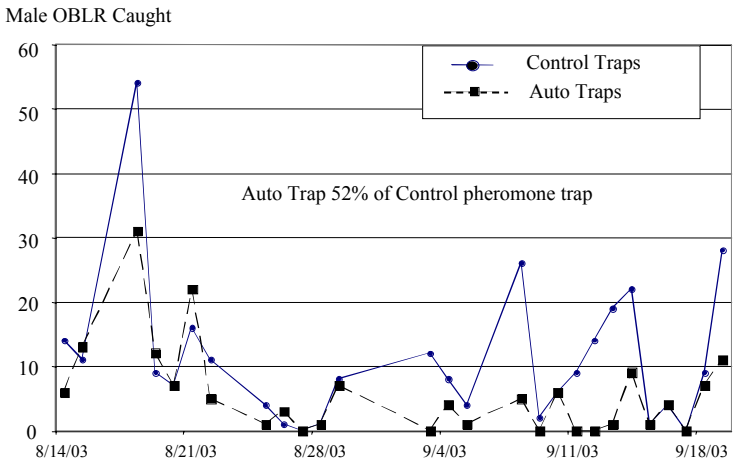


Figure 1. The average number of OBLR moths captured in control (Multipher) traps and the automated trap in Aug-Sept, 2003.

The automated trap captured about 52% of the control trap of the same design (Fig. 1). The lack of efficiency is difficult to explain at this time since the functional opening to the trap was similar. The only factor that could be responsible for the difference is the increased size or volume of the automated trap, which would dilute the effect of the kill strip and possibly allow moths to escape before being killed and thus counted as entering the trap. Another issue associated with the automated trap that will need fixing this winter is the number of "false" counts recorded. Each automated trap had a slightly different degree of these false readings that in some cases accounted for more than 80% of the data generated by a trap. These "false" counts could be the result of sensor malfunction or the sensor recording "noise" in the environment that was unrelated to moth captures.

A redesign of the automated trap will be developed this winter. The focus will be on a sensor that measures moth entrance to a trap, not counts of moths after they have been killed. One or more prototype automated traps will be evaluated in the spring of 2004 with the best design selected for additional testing in the summer.

Objective 3: Progress on this objective is slated for years two and three of the project.

Objective 4: A backbone of AgWeatherNet stations was established (at bud burst) in five cherry orchards in the Lower Yakima Valley. Orchards ranged in age from 6 to 32 years and were characterized by moderate to high historical disease pressure. All stations were equipped with sensors to measure temperature, relative humidity, rainfall, leaf wetness (above and below canopy), and soil moisture.

A small sensor web was established in a 5-acre cherry orchard at WSU Prosser IAREC. Stations equipped to measure temperature, leaf wetness (above and below canopies, soil moisture, and relative humidity were placed every 10 tree rows over an east-west axis. The stations were to be used to measure spatial and temporal variability in intra-orchard meteorological conditions and disease incidence and severity.

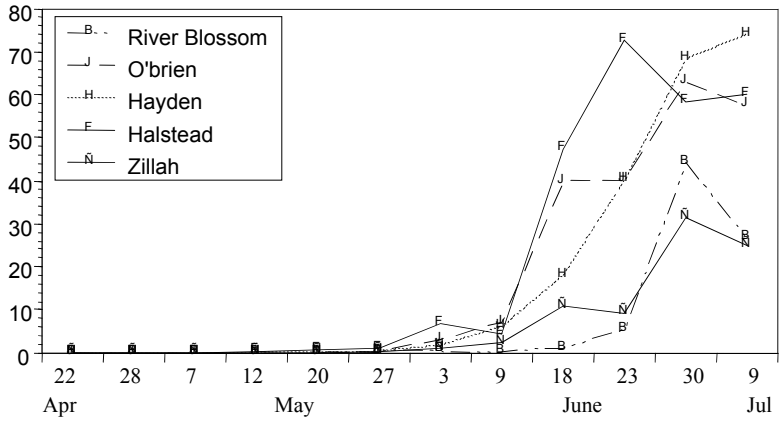


Figure 2. Powdery mildew progression in 5 south-central Washington sweet cherry orchards during spring-early summer 2003. Disease severity evaluations were obtained at 7- to 10-day intervals by determining the percentage of leaf surface area colonized by powdery mildew on 10 leaves selected at random from 10 trees in each orchard.

Disease incidence and severity were evaluated and 7- to 10-day intervals from shuck fall through midsummer. Severity at harvest ranged from 5.3 to 72.8% (Fig. 2). In the orchard with the highest pressure, severity progressed from trace levels (0.05%) to high levels over a 6-week period. Although these data are preliminary, repeated annual observations will be used for mathematical validation of cherry-specific Gubler-Thomas temperature algorithms being developed in parallel controlled-environment studies.

In specific orchards the largest increases in disease severity coincided with warming temperatures. For example, in one orchard the largest increase in severity was recorded at the conclusion of a time period where average daily temperatures exceeded 65°F for six consecutive days (Fig. 3).

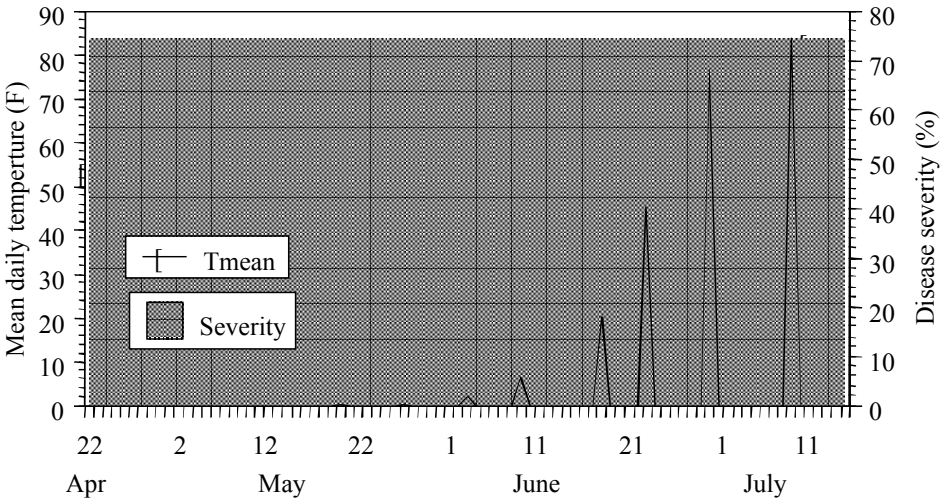


Figure 3. Mean daily temperature and disease severity values in a sweet cherry orchard near Pasco, WA, 2003. Note large increase in disease severity between 21 June and 1 July.

Budget:

Project title: Sensor-webs: developing sensors for automated monitoring of the orchard environment for improving insect and disease management
PI: Jay F. Brunner
Project duration: 3 years (2003-2005)
Current year: 2004
Project total (3 years): $109,575
Current year request: $ 39,221

Current year breakdown

Item	Year 1 (2003)	**Year 2 (2004)**	Year 3 (2005)
Salaries (0.25 to 0.1 FTE tech.)[1]	$10,692	**$11,119**	$ 4,626
Benefits (27%)	2,887	**3,002**	1,249
Wages	10,000	**10,000**	20,000
Benefits (16%)	1,600	**1,600**	3,200
Equipment			
Bug counters ± diseases[2]	5,000	**10,000**	0
Base stations (2)[3]	1,600		
Materials and supplies[4]	4,000	**1,000**	1,000
Within-state travel[5]	2,000	**2,500**	2,500
Total	$37,779	**$39,221**	$32,575

[1] Engineering Technologist Prosser associated with this project alone. Years one and two are for development and preliminary testing and year three is to support the establishment of extensive sensor-webs.
[2] Years two and three are for testing monitoring and disease management models in orchards.
[3] For prototype monitoring units year one and final units for year two.
[4] Supplies traps, lures, chips. Cell phone charges are allowed on this grant.
[5] Includes rental of a vehicle for this project as required by PIs.

FINAL REPORT

Project Title: **Optimizing Ammonia with Traps to Manage Apple Maggot in Washington**
PI: Wee Yee, Research Entomologist
Co-PI: Pete Landolt, Research Entomologist
Organization: USDA-ARS, Wapato, WA

Objectives:
2001-2002
- Determine the effectiveness of ammonia release rates from yellow panel and red sphere traps
- Determine optimal ammonia concentrations and release rates
- Compare ammonium hydroxide with conventional, commercially available ammonia lures
- Test effects of different ammonia volumes on trap effectiveness.

2003
- Further determine season-long effectiveness of ammonium hydroxide and commercial lures
- Determine effectiveness of ammonia together with apple volatile lures.
- Effectiveness of ammonia- and apple volatile-baited traps in large- and small-tree orchards.

2001-2002 Significant Findings:
- All rates of 29% ammonia release, based on release from vials with 0.05-, 0.16-, and 0.32-cm diameter holes, were equally effective in trapping apple maggots. Baited traps usually captured more flies than unbaited traps. Baited spheres were better than baited yellow rectangles, especially for males.
- Effects of traps depended on the sites – in a site with small trees, spheres were better, whereas sites with larger trees, spheres and yellow panels were equally good.
- Females generally responded to higher ammonia concentrations, males to a wider range of concentrations. Male responses were more variable.
- The optimal ammonia release for females and males seemed to be about 1-4 mg ammonia/hour, as rates higher than this did not increase captures.
- The 29% ammonium hydroxide lures significantly outlasted the commercial supercharger and Pherocon AM lures over 3-week tests.
- Increasing volumes (10-60 ml/trap) of ammonium hydroxide using multiple bottles did not increase attractiveness; only 10 ml was needed.

2003 Significant Findings:
- With yellow panels, the most effective lure based on the Washington State Department of Agriculture (WSDA) design had six 1-mm holes baited with 15 g ammonium carbonate. This lure released a mean of 4.39 mg ammonium carbonate/hour over 121 days. However, lures with 2 and 4 holes with both 10 and 15 g ammonium carbonate were nearly as good.
- With red spheres, lures with mean ammonium carbonate release rates of 2-6 mg/hour over 70-112 days were more effective than lures with butyl hexanoate and the 5-component fruit volatile blend that released 0.250-0.840 mg volatiles/hour. There was no synergistic effect of combining ammonium carbonate and fruit volatiles.
- With both yellow panels and red spheres, lures with two 1-mm holes and 20 g ammonium carbonate were more effective than lures that released about 0.450-0.600 mg component blend/hour late in the year when flies apparently seek fruit for oviposition.
- Similar results using ammonium carbonate and fruit volatiles with yellow panels were seen on large and small trees.

Methods for 2001-2002, summary:

1. Different trap types, ammonia-dispensing systems, sugar-derived baits, and ammonia concentrations were used to trap apple maggots at two disparate sites in western Washington.
2. Longevity of commercial and experimental lures was determined**.**

Methods for 2003

1. The longevity of experimental ammonium hydroxide lures were compared with WSDA lures for 3 months from mid June through mid September 2003, using similar designs and same sites as in 2002. WSDA lures were 3.7 x 2.6 cm yellow containers with 10 or 15 g ammonium carbonate and had two, four, or six 1-mm holes.
2. Ammonia lures were tested with apple volatiles to determine if there are synergistic effects by placing both on yellow panels and red spheres. Apple volatiles that have been identified for apple maggot in the eastern U.S. were tested: these were butyl hexanoate or a 5-component blend that includes butyl hexanoate and 2 other hexanoates and 2 butanoates from apples. For both trap types, treatments were 1) a control, 2) ammonia only, 3) butyl hexanoate only, 4) 5-component blend only, (5) ammonia-butyl hexanotae, and (6) ammonia-5-component blend. Experiments were conducted in Puyallup and the Vancouver areas and lasted the entire 3 months of the season.
3. The effect of tree size on use of ammonia/apple volatile cues or visual cues was tested in lots in Puyallup. Large and small trees within the same orchards or within the same general areas were trapped to determine how tree size and density affect the ability of flies to find traps. (In large, dense tree stands, flies may need to rely more on smell to find traps.) Yellow panels were baited with 20 g ammonium carbonate, the 5-component blend, or the two together, along with controls.

Results and Discussion

In 2001-2002, ammonia lures used were 15-ml polyethylene bottles filled with 10 ml of the 100% ammonium hydroxide solutions saturated in 0.75 g cotton. Bottles with 0.025, 0.05, and 0.64 cm holes or different numbers of bottles with the 0.05 cm holes were used to regulate release rate from yellow panels and red spheres. No consistent difference in female and male fly captures were seen using the three hole sizes from sticky yellow panels or sticky red spheres in Pierce and Cowlitz Counties (Fig. 1). However, there were differences in the relative numbers caught on panels and spheres in the two sites. In Pierce County, a site with small 1.5-2.5 m tall trees, significantly more flies were caught on spheres than panels, especially for males. Differences between control and baited traps were small. In Cowlitz County, a site with 5-7 m tall trees, spheres caught only slightly more flies than panels, and control versus baited trap differences were larger (Fig. 1). Traps with more than one bottle did not increase responses within trap types in Pierce and Cowlitz Co. (Fig. 2). However, as in the previous experiment, red spheres were superior for males and females in Pierce Co., whereas red spheres and yellow panels were equal in Cowlitz Co. All baited traps were superior to controls for both sexes. The combined results from these two experiments suggest that habitat type and perhaps tree size affect the numbers detected using spheres or panels by altering the visibility of the traps.

In 2001, acetic acid, ethanol, and butyl compounds together with ammonia did not enhance apple maggot captures above that of using ammonia alone. Flies appear not to use sugar fermentation cues to find sugar.

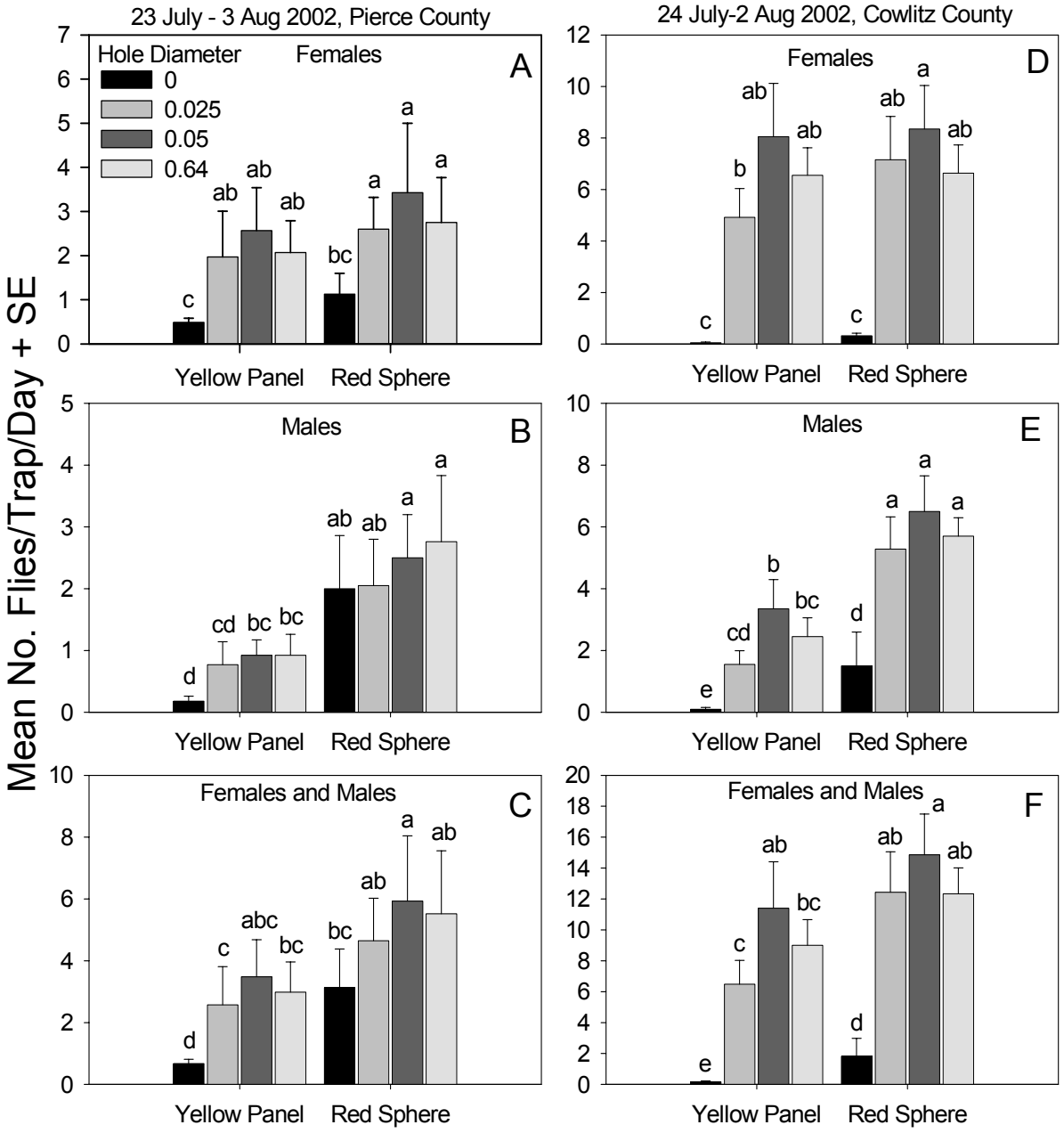


Fig. 1. Effects of trap type and hole size in ammonium hydroxide-baited bottles on fly captures in Pierce and Clark Co., WA, 2002. Means with same letters are not significantly different (*P* > 0.05).

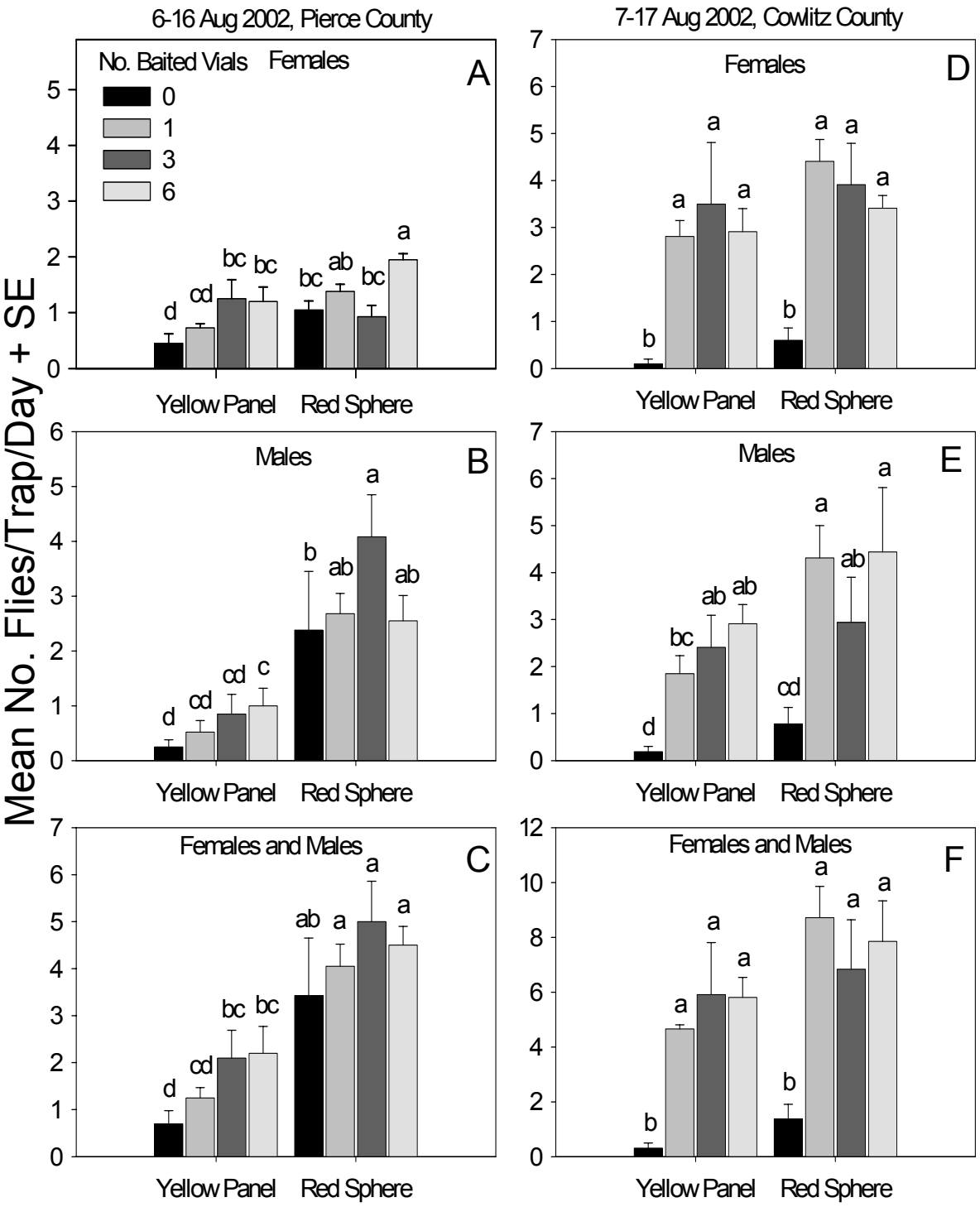


Fig. 2. Effects of trap type and numbers of bottles filled with ammonium hydroxide on fly captures in Pierce and Cowlitz Co., WA, 2002. Means with same letters are not significantly different ($P > 0.05$).

In 2001-2002, flies were also trapped using yellow panels baited with experimental ammonium hydroxide lures containing 0 and 1.9 to 29% ammonia in apple orchards in Pierce, Clark, Cowlitz, and Skamania Counties, WA. Ammonia was released from a 0.05 cm diameter hole in each bottle. Females responded to 1.9% ammonia, but were most attracted to 20-29% ammonia (Fig. 3). Males also responded to 1.9% ammonia, but were equally responsive to
to 7.3 to 29% ammonia and showed more variability in responses to ammonia than females (Fig. 4). Site differences were also detected, indicating environmental factors influence responses. The wide range of ammonia concentrations attracted females with similar egg loads. Based on these and previous results and those from laboratory determinations, flies responded maximally to ammonia released from traps at 1-4 mg/h. Higher amounts had no positive or negative effect.

The experimental lure containing 10 ml of 100% ammonium hydroxide (AH) was compared with two commercial apple maggot lures, the Pherocon AM trap with ammonium acetate + protein hydrolysate mixed in adhesive, and the supercharger (SC), containing 2.14 g ammonium carbonate (AC). All were equally effective over 4 days. However, the AH lure was superior to both commercial lures over 3 weeks (Fig. 4). A modified supercharger (MS) containing 8.46 g AC, 4 times the amount in SC, was also more effective than the Pherocon AM trap and SC, although less effective than the AH lure in one test. As before, based on field and laboratory tests, an ammonia release rate of 1-4 mg/h was necessary to effectively attract AM to traps. Ammonia was released more steadily from the AH and MS lures than from the Pherocon AM trap and SC, which lost most of their ammonia within one week (Fig. 5). Laboratory ammonia release rates from AH, Pherocon AM, SC, and MS lures at 0 and 3 weeks post exposure were 5.24 and 1.14, 10.55 and 0, 36.75 and 0.52, and 49.05 and 7.36 mg/h, respectively. Results indicate that the simple AH or MS lure used in this study are superior to some commercial lures for apple maggot detection under Washington conditions. They also demonstrate the importance of maintaining ammonia release rates over time when trapping apple maggot in a detection and management program.

In 2003, experiments were conducted in the Puyallup and Vancouver areas using WSDA ammonia lures with two, four, and six 1-mm holes and 10 or 15 g AC with yellow panels. The best lure in Puyallup was a lure with 6 holes containing 15 g AC (Table 1), which released a mean of 4.39 mg AC/hour over 121 days. In the laboratory, release rates of the 10 g lures were sustained longest with 2 holes, and shortest with 6 holes (Fig. 6). Experiments were also conducted at the two sites comparing ammonia and fruit volatile lures, butyl hexanoate and a 5-component blend of apple volatiles, with red spheres. Spheres baited with 20 g AC that released 2-6 mg/h over 70-112 days were more effective than those baited with butyl hexanoate and the 5-component blend released at 0.250-0.840 mg/h (Table 2). Because the fruit volatile release rate from these may have been inadequate, another test was conducted later in the year with yellow panels and red spheres. Holes for fruit volatile lures were increased to 4.5 mm in diameter, resulting in release rates of 0.457-0.597 mg/hour over 81 days in the laboratory (rain prevented calculations of weight losses used to calculate release rates in the field). The higher fruit volatile release rates did not increase attractiveness, and the 20 g AC lure was still more effective (Table 3). The greater effectiveness of ammonia was also seen in another test on both large and small trees (Table 4).

Several conclusions can be drawn from this project. Optimizing the use of ammonia is critical for effective apple maggot detection. Ammonia release rates have not adequately documented in past studies. Lures that release high amounts initially but small amounts later are less useful than those that maintain a sustained release. Results indicate the optimal ammonia release rate is 1-4 mg/hour. These rates can be obtained by using 10 ml ammonium hydroxide (one 0.5 mm hole) or 10 g AC (two-six mm holes) in containers. Combining these with red spheres may be more effective than with yellow panels. Fruit volatiles do not enhance catches, but the effects of higher release rates need to be studied.

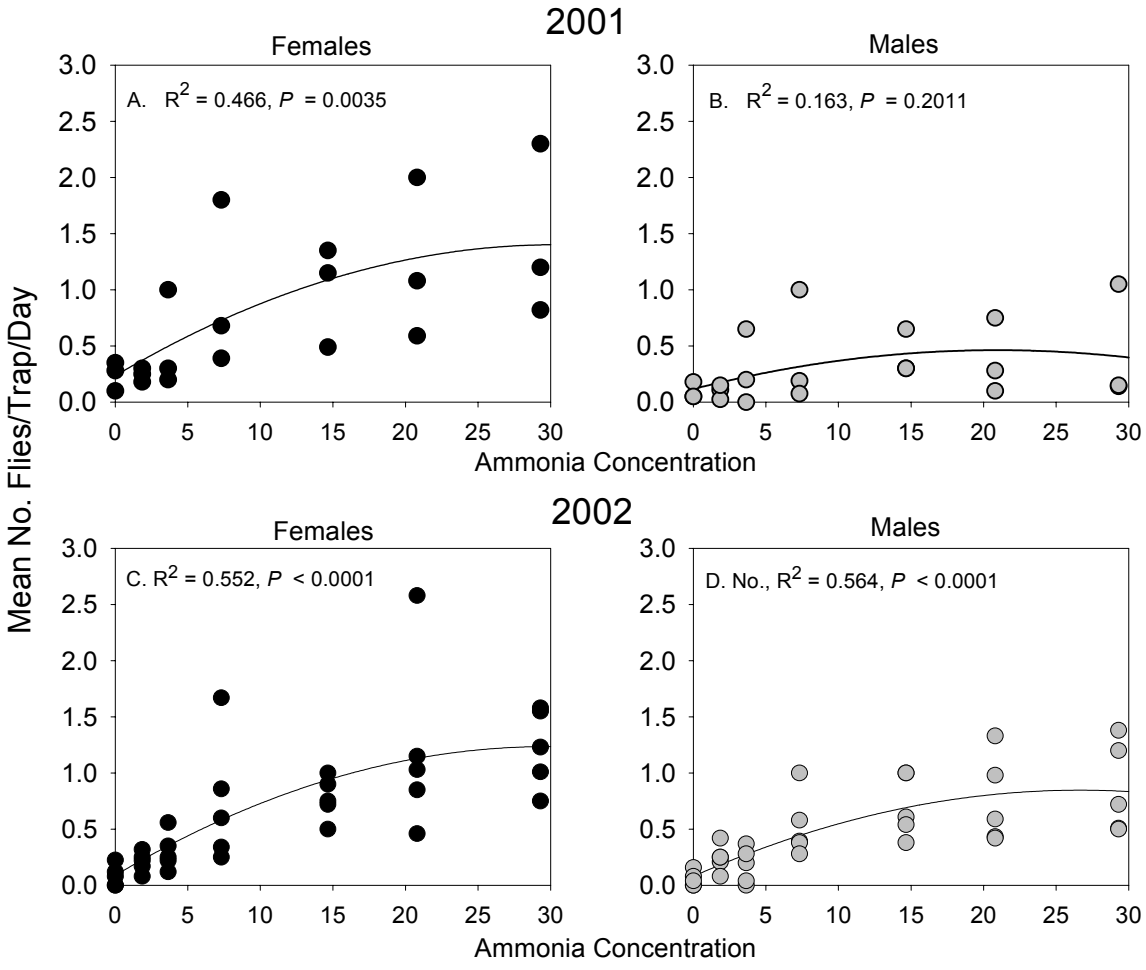


Figure 3. Relationship between ammonia concentrations and apple maggot captures in western WA, 2001-2002.

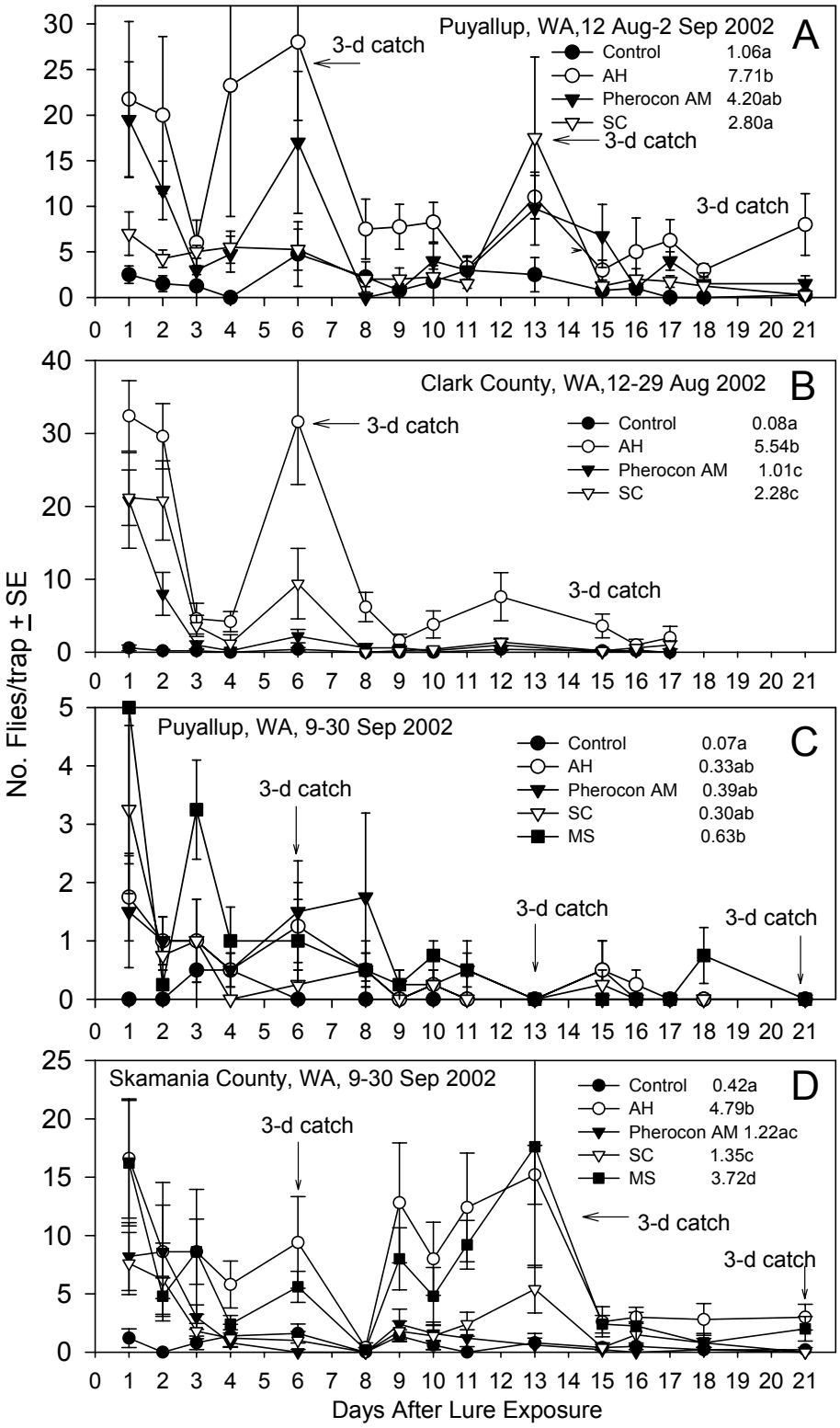


Fig. 4. Season-long captures of apple maggot using various ammonia lures, 2002.

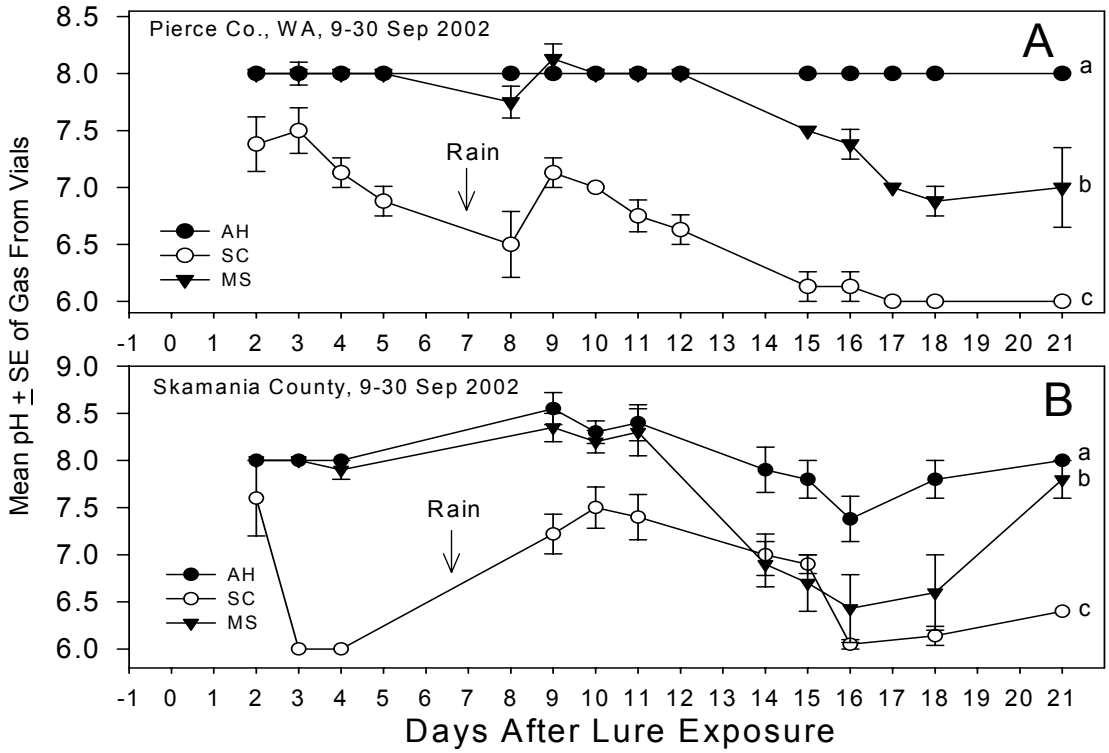


Fig. 5. Longevity of ammonium hydroxide and commercial lures as determined by pH of volatiles emitted from lures over 3 weeks in Pierce and Skamania Co., 2002. Lines followed by the same letters are not significantly different ($P > 0.05$).

Table 1. Seasonal trap captures (expressed as % of total flies within a block) ± SE of apple maggot on yellow panels using ammonia lures of different designs in Puyallup and Vancouver areas, WA, in 2003.

	Puyallup		Vancouver	
Treatment	%	Total No.	%	Total No.
Control	1.9 ± 0.2a	45	1.9 ± 1.0a	40
100% NH_4OH	15.4 ± 15.4bc	285	13.1 ± 2.9b	291
2 h 10 g AC	16.1 ± 4.1bc	451	14.4 ± 5.3b	283
4 h 10 g AC	11.2 ± 1.5b	286	17.4 ± 0.8b	357
6 h 10 g AC	9.1 ± 1.0b	233	12.6 ± 0.6b	262
2 h 15 g AC	9.8 ± 1.0b	200	11.3 ± 1.8b	237
4 h 15 g AC	14.8 ± 4.6bc	362	12.8 ± 2.4b	258
6 h 15 g AC	21.6 ± 5.6c	415	16.6 ± 0.9b	349

h = Number of 1-mm holes; g = Amount of ammonium carbonate (AC).
Means followed by same letters within columns are not significantly different ($P > 0.05$).
Four replicates of each treatment in a randomized block design.

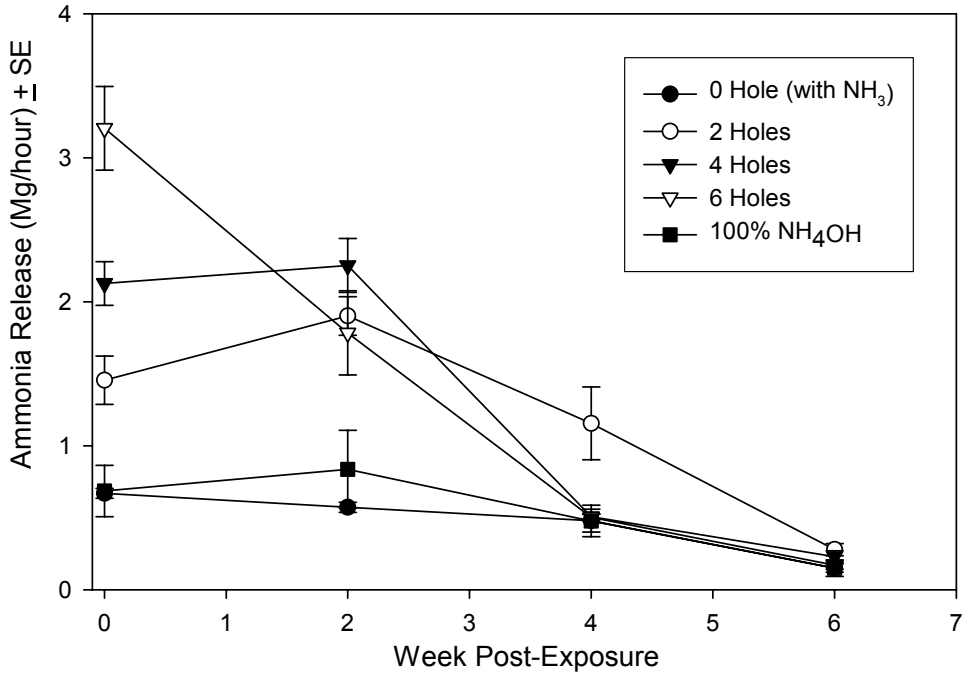


Fig. 6. Ammonia release rates of lures with 10 g of ammonia carbonate and 10 ml of 100% NH_4OH over time in the laboratory.

Table 2. Seasonal trap captures (expressed as % of total flies within a block) ± SE of apple maggot on red spheres using ammonia and fruit volatile lures in Puyallup and Vancouver areas in 2003.

	Puyallup		Vancouver	
Treatment	%	Total No.	%	Total No.
Control	3.8 ± 1.2c	85	4.4 ± 0.2f	227
10 g AC	13.2 ± 2.6ab	266	14.0 ± 1.4bc	744
20 g AC	13.4 ± 2.9ab	226	23.6 ± 2.2a	1,258
BH	10.7 ± 2.7abc	219	4.7 ± 0.6f	245
BH-10 g AC	6.3 ± 1.8bc	133	9.7 ± 1.3d	478
BH-20 g AC	11.8 ± 4.3abc	216	9.6 ± 2.0de	498
CB	14.8 ± 3.3ab	246	5.8 ± 0.8ef	288
CB-10 g AC	8.0 ± 1.9abc	167	10.4 ± 0.6cd	540
CB-20 g AC	17.8 + 4.8a	333	17.7 ± 1.3b	903

AC= ammonium carbonate; BH = butyl hexanoate; CB = 5-component blend.
AC lures, Two 1-mm holes; g = amount of AC; BH, CB lures, 10 ml, one 0.5 mm hole.
Means followed by same letters within columns are not significantly different ($P > 0.05$).
Four (Puyallup) or three (Vancouver) replicates of each treatment in a randomized block design.

Table 3. Seasonal trap captures (expressed as % of total flies within a block) of apple maggot on red spheres using ammonia and fruit volatile lures on tall and short trees in Puyallup, 2003. 5 replicates.

	Yellow Panels (29 Aug-2 Sep)		Red Spheres (3 Sep-27 Oct)	
Treatment	%	Total No.	%	Total No.
Control	4.1 ± 1.7a	50	6.0 ± 1.4a	116
20 g AC	46.8 ± 5.6b	534	38.6 ± 2.8d	733
BH	4.1 ± 1.7a	49	12.6 ± 2.9ab	228
CB	6.2 ± 0.3a	72	18.6 ± 2.0bc	360
CB-20 g AC	38.8 ± 4.2b	453	24.1 ± 3.4c	426

AC= ammonium carbonate; BH = butyl hexanoate; CB = 5-component blend.
AC lures, Two 1-mm holes; g = amount of AC; BH, CB lures, 10 ml, 0ne 4.5 mm hole.
Means followed by same letters within columns are not significantly different ($P > 0.05$).

Table 4. Seasonal trap captures (expressed as % of total flies within a block) of apple maggot on yellow panels using ammonia and fruit volatile lures on tall and short trees in Puyallup, 2003. 5 replicates.

	Large Trees (5-6 m tall)		Small Trees (2-3 m tall)	
Treatment	%	Total No.	%	Total No.
Control	2.8 ± 1.3a	16	5.3 ± 1.2a	94
20 g AC	42.9 ± 2.2 b	292	42.6 ± 3.8b	731
20 g AC/CB	46.2 ± 2.7 b	286	33.6 ± 4.1b	572
CB	8.1 + 1.1c	55	18.0 + 2.7c	303

AC= ammonium carbonate; BH = butyl hexanoate; CB = 5-component blend.
Means followed by same letters within columns are not significantly different ($P > 0.05$).

Final Report Budget:

Project title: Optimizing Ammonia with Traps to Manage Apple Maggot
PI: Wee Yee
Co-PI: Pete Landolt
Project duration: 2001-2003
Final year: 2003
Project total (3 years):$111,000

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Total	35,500	35,500	40,000

Current year breakdown:

Item	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Salaries and Benefits	28,500[1]	28,500[1]	32,500[1]
Goods and Services[2]	4,000	4,000	4,500
Travel[3]	3,000	3,000	3,000
Total	35,500	35,500	40,000

[1]Two GS-5 employees ($12.20/hour), full-time, 6-9 month appointments, and 10% benefits
[2] Sticky yellow traps, sticky spheres, vials, chemical attractants, miscellaneous supplies
[3]Travel to field sites, fuel costs for private vehicles

FINAL REPORT

Project title: Re-evaluation of Host Usage By Apple Maggot in Washington

PI: Wee L. Yee, Research Entomologist
Organization: USDA-ARS, Wapato, WA

Objectives:
2002
●Determine trap catches of apple maggots on previously unrecorded, non-apple hosts, in western Washington.
●Determine if apple maggots develop in these new hosts and the sites where this occurs.

2003
●Further determine trap catches of apple maggots on previously unrecorded, non-apple hosts, in western Washington.
●Further determine if apple maggots develop in these new hosts.
●Determine longevity and fecundity of apple maggots from different hosts.

2002 Significant Findings
●Adult apple maggots were collected on traps placed on a wide variety of previously unrecorded hosts in western Washington, including Asian pear, pear, and rose.
●Asian pear, *Pyrus serotina*, was confirmed to be a new apple maggot developmental host in Washington in 2002.
●Fly pupae were recovered from Asian pear, pear, plum, rose, and native cherries in 2002.

2003 Significant Findings
● Adults developed from pupae collected from bitter cherry, *Prunus emarginata*; this is a new host for the apple maggot.
● Adults developed from pupae from common pear, *Pyrus communis*; this is a new Washington state record; pupae were collected from pear at three disparate locations.
●Pupae were collected from Asian pear for a third straight year, confirming that this host is consistently used.
●Plums, *Prunus domestica*, yielded high numbers of pupae that will probably be apple maggots; pupae were also collected from spreading cotoneaster, *Cotoneaster divaricatus*. Rearing the adults out in the winter and spring of 2004 will confirm whether these are apple maggots – studies are still in progress.

2002-2003 Methods:
1. Sticky yellow traps were placed in Asian pear, pear, rose, cascara, plum, and other trees in Cowlitz, Clark, and Skamania Counties from July through September 2003. Cultivated and native wild cherries were also trapped. Traps were placed in apple, hawthorn, and crabapple trees, the known hosts in Washington, for comparative host use purposes. Several sites 100 km apart were surveyed to establish that infestations on non-apple hosts are not limited to just a few trees or localized sites. Flies were removed from traps every 1 to 3 weeks and identified.
2. Representative fruit from all trapped trees were collected and brought into the laboratory. Larvae dropped from the fruit into a moist vermiculite/peat moss/sand mixture. After 1-2 weeks at room temperature, the pupae were placed in a 3-4 °C refrigerator where they will be held for 5-6 months. Flies will be brought up to room temperature (25-27 °C) for emergence. After emergence, flies will be maintained on a diet of 20% yeast and 80% sugar. Longevity

and fecundity of flies from new hosts will be compared with those of flies from known hosts (apples and hawthorn). Wax domes will be used as oviposition sites to collect eggs. After all flies die, they will be positively identified as apple maggot using wing band patterns, ovipositor lengths, and male reproductive morphology (Bush 1966, Westcott 1982).

Results

Adult apple maggots were trapped on many non-apple hosts in 2002 and 2003, although not all appeared to be developmental hosts. Five of these - Asian pear, common pear, bitter cherry, spreading cotoneaster, *C. divaricatus*, and plum – appeared to be or were developmental hosts (Table 1). Many fruit fly pupae (some clearly were the western cherry fruit fly, *Rhagoletis indifferens* Curran) were collected, but only those that emerged as adults could be confirmed to be apple maggots. Asian pear (cv. '20th Century' and 'Kosui') was a developmental host in all three years from 2001-2003, and was a host at 3 of 4 sites. Common pear (cv. 'Bartlett') was a host at 4 of 7 sites and native bitter cherry (feral) was a host in 2002 at 2 sites (80 km apart) out of 13 total sites. Cotoneaster was a host at 1 of 6 sites (*C. microphilus* and *C. horizontalis* were not infested), and plum, *Prunus domestica* (cv.'Green Grange') was a host at 3 of 6 sites. In at least 2003, these hosts were less infested than the known hosts. Other fruit produced fruit fly pupae that resembled apple maggot (i.e., rose maggot, *Rhagoletis basiola* [Osten Sacken] from rose), but most pupae from other hosts failed to emerge, and thus these were unconfirmed or non-hosts (Table 1). Flies from all the newly identified hosts had similar ovipositor lengths (0.91-1.07 mm) as flies from apples (Westcott 1982). Flies that developed in new hosts were as large as flies from apples, but the longevity and fecundity of flies from these hosts have not been determined. Adult flies from the new hosts are expected to emerge, after 5-6 months storage at 3 ºC, in winter and spring 2004. Longevity and fecundity will be determined at this time.

Discussion

Knowing the host use range of apple maggot is important because it determines which hosts need to be considered as potential sources of infestation in commercial orchards. The apple maggot is a major threat to the apple industry in Washington. Currently, it is restricted to western Washington, the Spokane area, and Ellensburg and sporadic catches occur in Yakima and Tri-Cities areas. To delimit its spread and to eradicate its local populations, the Washington State Department of Agriculture runs an active apple maggot surveillance program. Traps are normally placed in apple and hawthorn trees to detect fly presence. If a fly is caught, the County Pest Control Board immediately sprays the trees with the organophosphate phosmet. The results in this study suggest that traps may need to be placed in abandoned and unsprayed Asian pear, pear, bitter cherry, and specific shrubs such as cotoneaster in areas with apple maggots on previously known hosts.

In Washington, the apple maggot reportedly "has been found only on apple, crab apple and hawthorn" (Beers et al. 1993, Brunner 1996). The results reported here clearly show the fly has a broader host range than this in Washington. This is not surprising, as in other regions of the U.S. apple maggot attacks a wide range of hosts in the rose family (e.g., Bush 1966, Shervis 1970, Prokopy and Bush 1972, Prokopy and Berlocher 1980, Tracewski et al. 1987, Allred and Jorgenson 1993). The finding of apple maggot usage of pear is important because of the large pear industry in central Washington. Should apple maggots ever become established in this region, both apples and pears may be subject to attack by this fly. This would make eradication efforts more difficult. Fortunately, at this time, infestation of pear even in high apple maggot population areas seems relatively low. Nevertheless, because apple maggots do use pear and other hosts in Washington, trapping efforts made by the state to delimit the fly's spread may need to include alternate hosts.

Table 1. Numbers of fruit fly pupae[a] and adult *Rhagoletis pomonella* reared from various fruit collected in southwestern Washington State, 2001-2003.

	Total Numbers							
		2001[b]		2002			2003	
New Hosts in WA	Pupae	Adults[c]	Fruit	Pupae	Adults[c]	Fruit	Pupae[d]	Adults[c]
Asian Pear	161	**7**	153	68	0	216	66	-------
			----	33[e]	**16**			
Common Pear	24	0	332	36	**2**	347	32	**-------**
Bitter Cherry	----	----	2,807	2	**2**	8,761	------	-------
Cotoneaster	----	----	120	1	0	3,224	5	-------
Garden Plum	----	----	30	0	0	419	316	-------
Known Hosts in WA								
Apple	575	13	44	33	0	883	459	-------
Black Hawthorn	100	6	2,095	371	65	5,194	442	-------
Ornamental Hawthorn	665	3	----	----	____	12,079	502	-------
Crab Apple	187	6	88	100	____	379	32	-------
Unconfirmed or Non-Hosts[f]								
Red Plum	----	----	28	6	0	-------	-------	-------
Italian Plum	-----	----	80	0	0	-------	-------	-------
Rose	95	0	394	5	0	2,925	52	-------
Quince	1	0	111	1	0	86	0	-------
Snowberry	237	0	-----	------	------	--------	-------	---------
Cascara	7	0	3,885	0	0	5,514	0	0
Blueberry	------	------	87	0	0	-------	-------	-------
Red Huckleberry	------	------	120	1	0	-------	-------	-------
English Laurel	------	------	------	-------	-------	1,772	0	0
Sweet/Sour Cherries	-------	------	808	0	0	1,260	0	0
Blueberries	-------	------	------	-------	-------	877	0	0
Grapes	-------	------	------	-------	-------	1,118	0	0
Skimia	-------	------	------	-------	-------	217	0	0
Yew	-------	------	------	-------	-------	115	0	0
Honeysuckle	-------	------	------	-------	-------	184	0	0
Bush Cranberries	-------	------	------	-------	-------	320	0	0
Pyracantha	-------	------	------	-------	-------	566	0	0
Holly	-------	------	------	-------	-------	265	0	0

-------, data not collected.

[a]Includes only pupae that had the appearance of *R. pomonella*, but not all emerged as adults.

[b]Fruit not counted in 2001.

[c]Emerged and confirmed as adult *R. pomonella*.

[d]In cold and in diapause; emergence expected in winter-spring 2004.

[e]Collected from soil directly beneath an Asian pear

[f]Collected near known hosts.

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Budget: Final Report

Project title: Re-evaluation of host usage by apple maggot in Washington state

PI: Wee Yee

Project Duration: 2002-2003

Final year: 2003

Project total (2 years): $10,000

Year	Year 1 (2002)	Year 2 (2003)
Total	5,000	5,000

Item	Year 1 (2002)	Year 2(2003)
Salaries and Benefits	4,500	4,800[1]
Goods and Services	500	200[2]
Total	5,000	5,000

[1]One summer GS-5 employee ($12.20/hour) at 75% time + 10% benefits for 3 months.

[2]Traps and tubs for collecting larvae.

FINAL REPORT
WTFRC Project #AE-01-54 **WSU Project #13C-3643-3366**

Project title: Developing sampling plans for leafrollers and their natural enemies

PI: Vincent P. Jones, Associate Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA
(509) 663-8181 ext. 273, vpjones@wsu.edu
Co-PI: Jay F. Brunner, Entomologist & Director, WSU-TFREC, Wenatchee, WA
Cooperators: Tom Unruh, USDA-ARS, Wapato, WA

Objectives:

1. Develop sampling plans for Pandemis leafroller (PLR) and Oblique-banded leafroller (OBLR) that minimize the number of samples per acre, maximize repeatability of the estimate and reduce the time and cost involved.

2. Improve the phenology models for PLR and OBLR so that the time for sampling parasitism of these two species can be standardized. This will improve accuracy in estimates of parasitism and reduce the number of times samples need to be taken throughout the season.

3. Develop sampling plans for parasitoids of PLR and OBLR to accurately estimate the effect of these natural enemies with minimized sampling.

Significant findings:

- Geostatistical analysis and sampling simulations show that sample densities greater than one per acre are inefficient. Optimal sampling intensity cannot be determined from our data, but is probably no more than one sample per 1.5 or 2 acres.
- *Bt* and Intrepid™ applied in the lab at sublethal doses to third or fourth instar larvae increased the developmental time up to 20% compared to untreated larvae. Intrepid™ caused about twice as many larvae to molt to the sixth instar and up to 29% of the larvae to go through a seventh instar compared to none for untreated larvae.
- Examination of PLR adult flight in 13 data sets suggest that the current WSU PLR model predicts flights too early and that application of either *Bt* or Intrepid™ causes later flights and greater deviation from the model predictions.
- Pesticide application will change virtually any model prediction of adult flight. The effect is dependent on the efficacy of the pesticide, the duration of activity and the timing with respect to stage distribution of the larvae exposed. These effects are independent of the change in developmental time caused by materials like *Bt* and Intrepid™.
- The OBLR model is also being evaluated, but analysis is not yet completed.
- Five parasitoids were found attacking PLR and six attacking OBLR. The phenology of attack and best time to sample is presented in Table 4.

Results:

Objective 1: Standard statistical theory suggests that the number of samples required to estimate the average population density is a function of the relationship between the mean population density and its variance and assumes that the area sampled has no spatial structure (that is, the samples are statistically independent). Our data clearly show that leafroller populations typically have a significant underlying spatial structure, and therefore we need to understand how that spatial structure affects sampling programs.

Geostatistics is a method that helps understand the underlying spatial structure. Our data from the past three years showed that spatial variation was significant in 54 of 68 (80%) orchards sampled and accounted for an average of 79% of the total variability in population density. The exceptions to the rule generally occurred when populations were extremely high or low. In the case of high populations, spatial structure becomes uniform and sampling is easily performed with just a few samples. In the case of low populations, the non-fit of the data is probably related to simply not finding the larvae (or damage) and having mostly zeros in the entire data set. The size of the plot sampled did not appear to vary the importance of spatial variation.

This past year, we followed two orchards at two-week intervals from the middle of April until the start of September. The orchards were sampled by examining buds from April to the end of May and shoots from that point onward. Every two weeks, ≈76 trees were sampled on a regular grid by examining bud or shoot damage. The damaged samples were brought to the lab and inspected for larvae. At the Mattawa location (OBLR), we sampled 15 buds or shoots per tree (non-bearing small trees), and at the WVC site (PLR) 25 buds or shoots per tree were sampled. To prevent removal of the damaged buds or shoots from affecting the sample estimates in the future, we offset the samples by a row or two each time we sampled. We used palm pilots coupled with GPS units to determine the actual location of each tree sampled for the geostatistical analysis.

Examination of the distribution of damage showed that the point at which spatial structure ceases to be important statistically averaged about 46 meters but varied from 17 to 50 with two unusual values of 92 and 272 meters. The point at which spatial structure ceases for larval distribution (as opposed to damage) was about 26 meters with no outliers in these plots. ***The data suggest that samples should never be collected less than ≈50 meters apart on a large field.***

Examination of the damage distribution every two weeks over the season showed variations that were useful and some not useful for IPM. Damage is a cumulative function; that is, once damage occurs it does not disappear unless the shoot or bud is removed from the tree. The damage map was primarily useful in showing the areas with the greatest damage, which could be used to help determine the

Fig. 1. Distribution of OBLR damage at the Mattawa orchard in a non-bearing block.

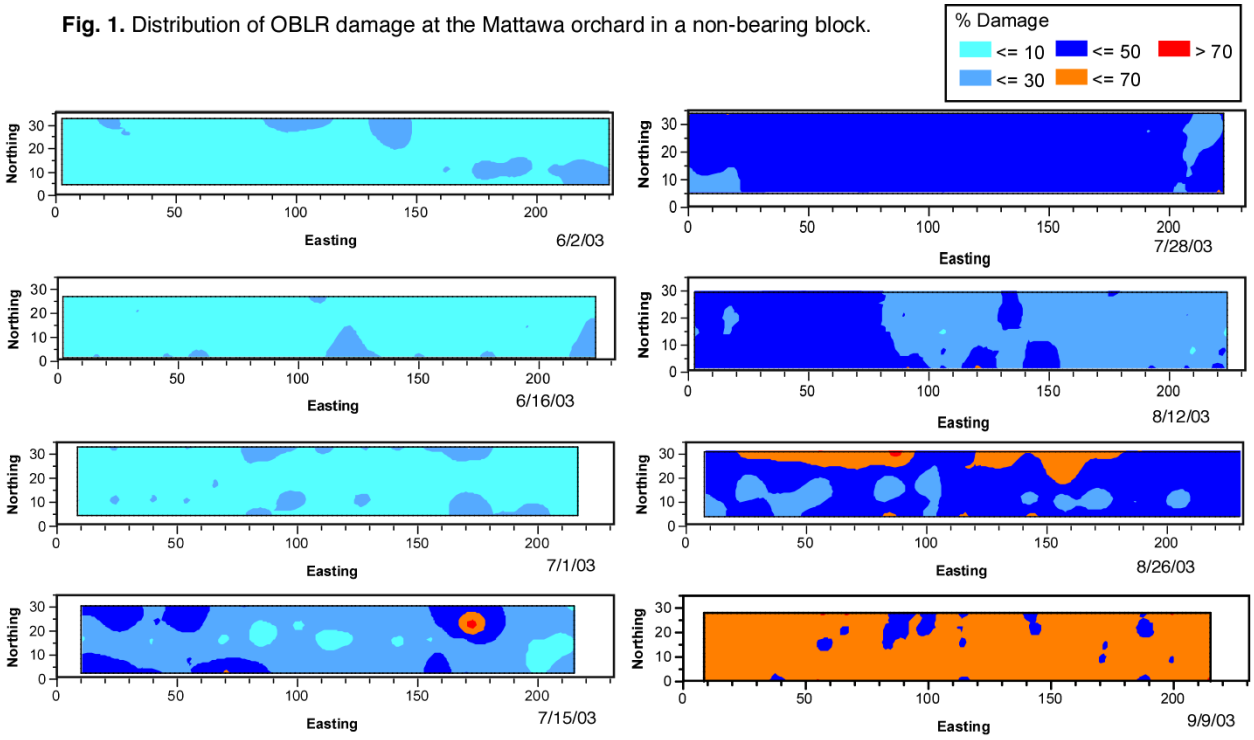
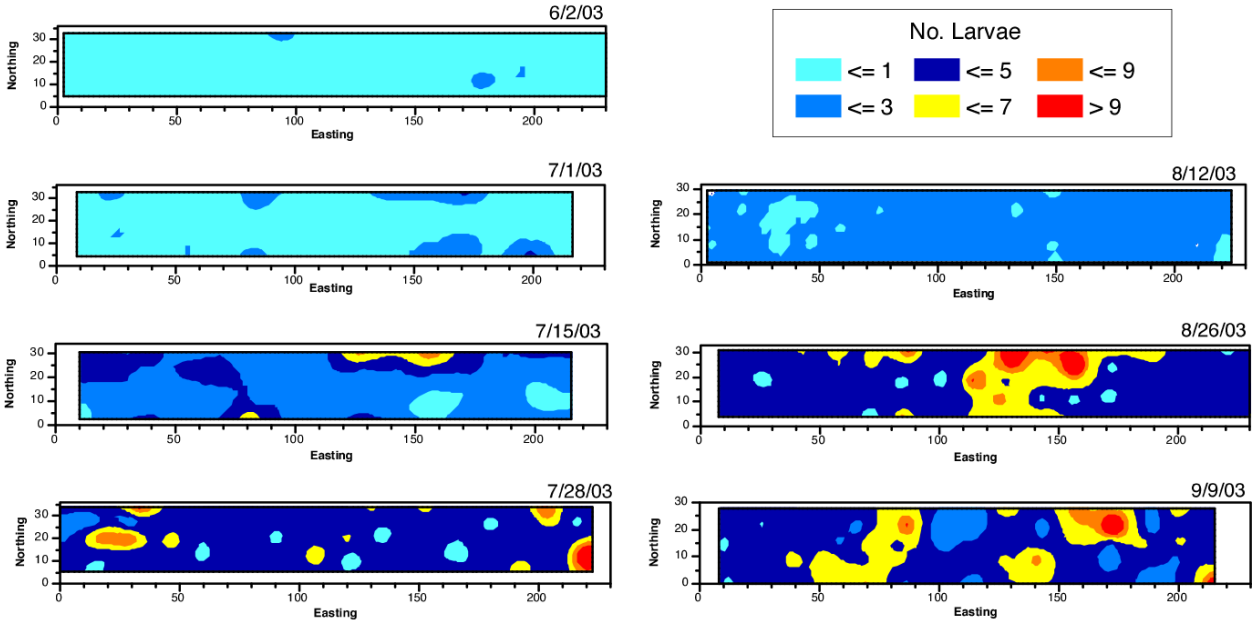


Fig. 2. Distribution of OBLR larvae at the Mattawa orchard in a non-bearing block



direction from which females were moving into the plot. Early in the season the variability in the maps was high, and later in the season they tended to be similar as damage became uniformly spread throughout the plot (Fig. 1).

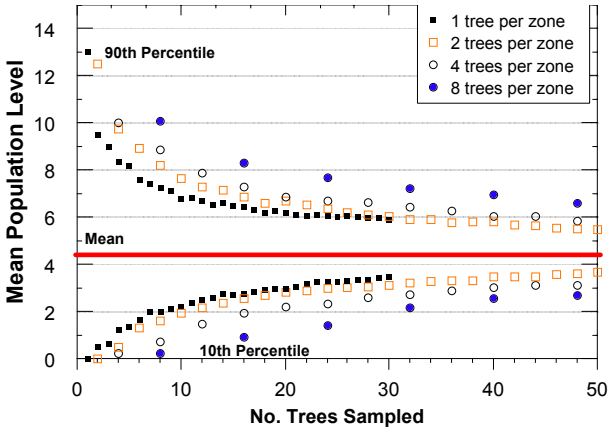
The distribution of larvae in the plots from week to week was extremely variable. This is likely caused by the phenology of the larvae – the sampling intervals (roughly two weeks) were long enough that older larvae "disappear" (complete development) and new larvae "appear" (eggs hatch) (Fig. 2). Because females emerge over a 2- to 3-week period, when a new hot spot of larvae appears it is probably an indication that a female chose that area and laid her egg masses there. Multiple hot spots of same age larvae therefore likely indicate multiple females of approximately the same age emerging and laying eggs.

The overall conclusion from the geostatistical analysis is that sampling distances can be larger, and that maps of the damage or density of larvae are likely not important for IPM at the level of a single field. If large orchards (100 acres or more) are sampled at large distances, maps may be crucial in determining overall trends that would aid IPM.

The effect of different distances between samples can be tested for several of the larger data sets taken over the past three years. Before running the simulation model, each data set was broken into a number of equally sized "zones." A simulation model was developed that randomly chooses a zone, and then samples a tree from that zone, and repeats the process until all the zones in the orchard are sampled. We then tabulate the estimate of the population level if one zone, two zones, etc. and out to the total number of zones in the orchard were sampled. The model then repeats the process 500 times so that we can calculate how the estimate of the mean population level varies when we sample only one zone, etc. The data were analyzed by plotting the 10^{th} and 90^{th} percentiles of the means (i.e., if you sort the simulated means from lowest to highest, we chose the 50^{th} and 450^{th} points on the list). Thus, the difference between the 10^{th} and 90^{th} percentiles is where 80% of the estimates lie.

We can also sample more than a single tree per zone to see how that affects the estimates so that we can determine the best way to sample. In addition, we can change the number of zones that cover a field so that we can simulate the effect of increasing the average distance between samples and determine how it affects the accuracy of the mean.

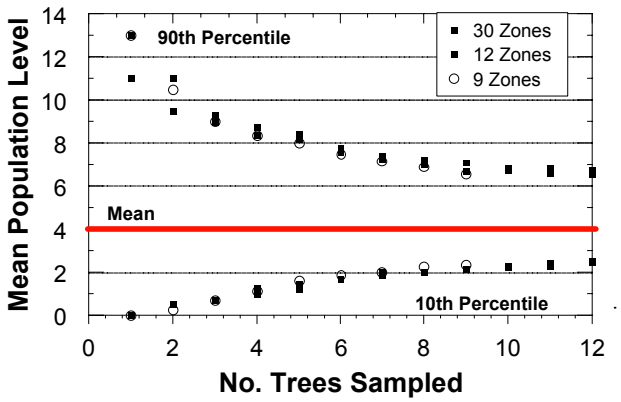
Fig. 3. Effect of sampling 1, 2, 4, or 8 trees per zone on the variability in estimate of the mean



The simulations showed that taking multiple samples per zone was generally less effective than sampling an additional zone (Fig. 3). Therefore, all the simulations discussed from here on out were run using one sample per zone.

Examining the effect of distance between samples (i.e., sample density), showed that there was no real difference between sampling 30 zones (0.25 acre/zone = 31.8 m between samples), 12 zones (0.6 acres/zone = 49.3 m between samples) or 9 zones (0.8 acres/zone = 56.9 m between samples) in terms of the variability in the estimate of the mean (Fig. 4). This supports the geostatistical analysis that suggested that we could sample further than ≈50 m apart with no loss of precision. Unfortunately, our largest orchard sample over the past three years was 17 acres, and that had extremely low leafroller levels, so we could not test zones larger than the 0.8-acre zone used in this simulation. However, densities of one sample per 1.5 acres would likely be no problem and for a 15-acre block would provide a reasonable level of precision.

Fig. 4. Variability in the estimate of the mean as a function of sample density.



Objective 2:

Studies by Knight and Cockfield (1996) suggest that if spring sprays of *Bt* are applied for leafroller control, flight of the summer generation could be delayed 2-3 weeks from the phenology model prediction. Intrepid™ (methoxyfenozide) has been reported by many consultants to cause delays according to the model, and we saw the delay in one of our blocks last year.

Lab Studies:

Our studies were performed by collecting egg masses from the TFREC OBLR colony and placing newly hatched larvae on diet. When the larvae reached either the third or fourth instar they were separated and placed into 1-oz cups containing either control diet or diet with *Bt* (1% field rate) or Intrepid™ (either 1 or 0.5% field rate). These low doses were used because we are interested in the sublethal effects of the pesticides. Incorporating higher doses into the diet was tried, but virtually 100% mortality occurred.

For the *Bt* trials, larvae were fed either one or two days on treated diet and then transferred to untreated diet for the remainder of the experiment. Larvae were checked daily for molting, and the number of instars each larva completed; time required for each molt, and mortality was recorded.

Larvae were followed until the adult stage. For the Intrepid™ studies the same procedures were used, but larvae were fed treated diet only for one day because even at the low rates used 100% mortality occurred when fed for two or more days.

The *Bt*-treated larvae required about 70-100% longer to complete the instar immediately after the one fed *Bt* (Table 1). However, the following molts occurred more quickly than in the control larvae and, overall, the time between treatment and adult emergence was only delayed by about 10-20% compared to the untreated controls. The effect of this delay is to increase the variability in prediction of the summer generation, particularly if two or more sprays of *Bt* are used during the spring generation. The delay may increase parasitism because the larvae are in susceptible stages longer. But probably only a fraction of the total population in the field is exposed to a low dose that causes the delay in development and not death, and parasitoids may deposit eggs or attack an already dying larvae, which may not support parasitoid development.

Table 1. Effect of *Bt* on developmental rate of larvae treated as either third or fourth instars at two sublethal doses.

		% of Control Time from Treated Instar Required to Complete Development			
Length of Time Fed (d)	**Instar Treated**	**4th Instar**	**5th Instar**	**Pupae**	**Moth**
1	3	177.4	150.6	107.7	106.0
2	3	170.2	142.9	123.1	113.9
1	4	–	189.5	121.2	112.9
2	4	–	197.7	137.3	120.6

The larvae fed Intrepid™ responded differently than the *Bt*-fed larvae. When Intrepid™ was ingested, larvae molted more quickly than untreated larvae, but the time required to complete development to the pupal and adult stages was about 10% longer than control larvae (Table 2). Examination of the data showed that larvae fed Intrepid™ virtually always had supernumerary molts (Table 3), whereas only 41-45% of the control larvae had more than five molts. In contrast, about 85% of third instar larvae treated with Intrepid™ went through six or more molts (nine instars for one larva), and about 65% of the larvae treated as fourth instars went through six or more molts.

Table 2. Effect of Intrepid™ on developmental rate of larvae treated as either third or fourth instars at two sublethal doses.

		% of Control Time from Treated Instar Required to Complete Development			
% Field Rate	**Instar Treated**	**4th Instar**	**5th Instar**	**Pupae**	**Moth**
1	3	54.8	84.5	116.8	111.0
1	4	–	61.2	127.4	114.4
0.5	3	55.8	83.8	115.1	112.3
0.5	4	–	61.6	120.6	110.4

Table 3. Effect of Intrepid™ on the number of molts past the fifth instar at two sublethal doses.

		% Population Molting to the Indicated Instar					
% Field Rate	Instar Treated	Control to 6th	Treated to 6th	Control to 7th	Treated to 7th	Treated to 8th	Treated to 9th
1	3	41.4	84.3	0.0	18.6	12.5	100.0
1	4	45.6	62.9	0.0	25.0	9.1	0.0
0.5	3	41.4	86.4	0.0	13.2	20.0	0.0
0.5	4	45.6	67.4	0.0	29.0	22.2	0.0

Field Phenology of PLR:

The phenology of PLR was investigated in 13 data sets collected over the period of this grant. We visited the orchards once or twice a week from mid-March to mid-September and collected larval samples. In the lab the head capsule of each larva was measured, given a unique ID number, and placed into a 1-oz cup with artificial diet and reared to the adult stage or until parasitoids emerged. This allowed us to determine the range of instars present in the field at any point in time and to determine the time of peak parasitism and stage preference of the parasitoids (see Objective 3). We also had temperature records and traps to determine adult flight curves. Spray records were available for each orchard, and we were able to pick four orchards with either *Bt* or Intrepid™ applied to help determine how those applications affected the flight curves.

For PLR, we found in our locations that the current WSU model generally predicted the flight considerably before the average flight curve for our sites not treated with *Bt* or Intrepid™ (Fig. 5). PLR flights in orchards treated with *Bt* or Intrepid™ during the spring occurred considerably later and had a broader emergence curve in the first flight and in the second flight had a similar shape, but was delayed about 100 DD compared to the orchards not treated with *Bt* or Intrepid™ (Fig. 6).

Interestingly, when the model used a biofix of the first capture of a generation, predictions were much less precise than using the 1 March start of DD accumulations.

Many of our orchards were untreated (the trees were non-bearing), which may be why our flight curves differ from those in the current WSU model. It is important to understand that the model predictions will appear to be different in the field depending on the treatment regime used, even if the pesticides (like *Bt* and Intrepid™) do not affect developmental rate. A pesticide such as Success™ that is highly efficacious will kill a portion of the larvae for a given period after application, meaning that down the road when those larvae would have

Fig. 5. First flight of PLR observed and the predictions from the current WSU model, the model developed from this grant, and when pesticides were applied.

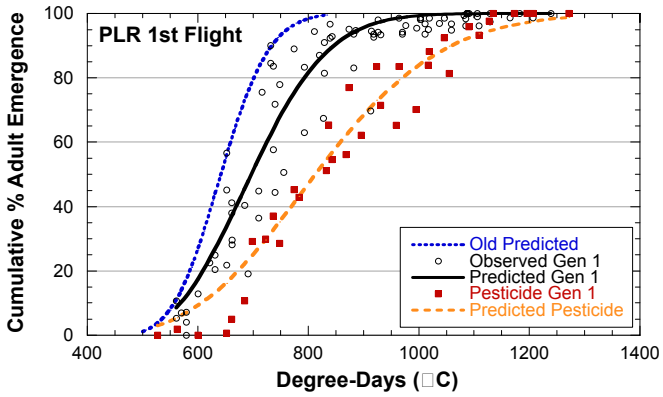
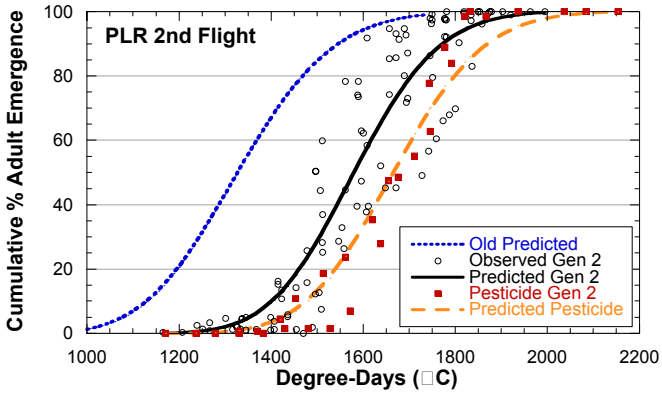


Fig. 6. Second flight of PLR observed and the predictions from the current WSU model, the model developed from this grant, and when pesticides were applied in the first generation.

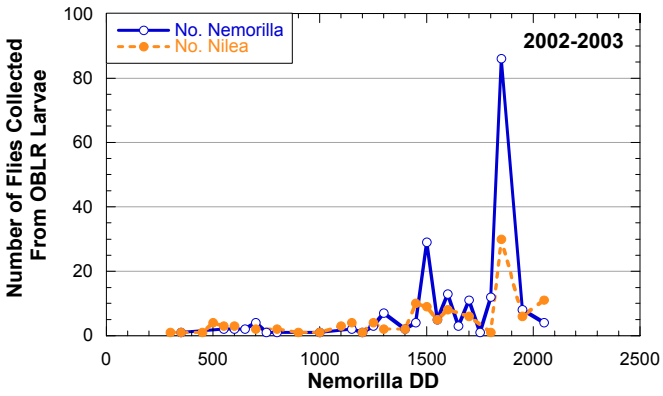


become adults the flight curve will have a severe depression compared to an area where it was not applied. The more efficacious and the greater the number of applications, the greater the difference in the flight curves between the treated and untreated areas. Thus, using the model in conjunction with the timing of pesticide applications can help you understand trapping results that occur later on. For example, suppose you apply the pesticide Success™ when the oldest larvae are in the third instar and achieve an 80% mortality rate for 5 days (at 25 DD per day = 125 DD period). This means you would expect suppression in trap catch of about 80% that would start ≈595 DD later (the time required to complete development from third instar to adult emergence) and the reduction should last about 125 DD.

Development of a model for OBLR is also underway and should be completed this winter.

Objective 3. The dominant parasitoids found in each orchard varied depending on whether OBLR or PLR was the dominant (or only) leafroller present and the location of the orchard and the treatments used for management. OBLR parasitoids were dominated by two species of tachinid flies, *Nemorilla* and *Nilea*, both of which have their greatest impact late in the summer (Fig. 7) and which occurred at virtually all the locations. Overall, these two tachinids account for an average of 50% of all the parasitoids collected from OBLR. At other locations, we also collected *Macrocentrus* (a braconid wasp dominant at four locations), *Sympiesis* (a eulophid wasp, summer generation only, two locations), and *Transonema* (an ichneumonid wasp, spring generation only). *Colpoclypeus florus* (eulophid wasp) was rarely collected at any of our sites attacking OBLR.

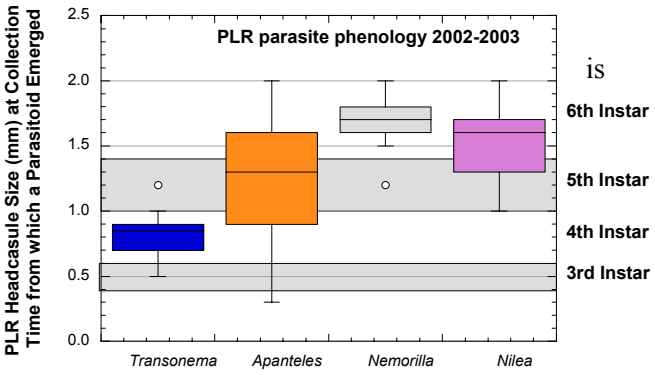
Fig. 7. Parasitism of OBLR by the tachinids Nemorilla and Nilea over all OBLR orchards sampled in 2002-2003.



PLR parasitism was dominated by *Apanteles* (a braconid wasp at five locations), *C. florus* (two locations), and *Transonema* (three locations, first generation only). *Nemorilla* and *Nilea* were dominant at two sites, but overall had a lower importance for PLR than for OBLR.

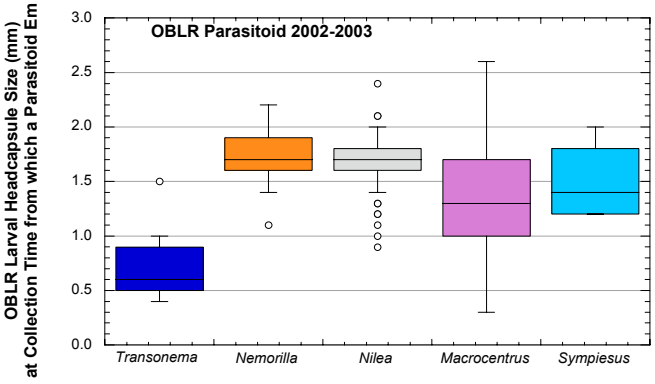
The seasonality of the PLR parasitoids was markedly different, reflecting their different stage specificity and occurrence throughout the year. For example, *Transonema* only occurred during the spring generation and typically was found attacking third or fourth instar larvae (Fig. 8) and is a larval parasitoid that emerges before the pupal stage. *Apanteles* also a larval parasitoid that was collected over a broad range of instars (in fact, 2-6) during both generations. The tachinids are internal parasitoids that were collected primarily when the population was in the fifth and sixth instars and primarily late in the summer generation. *C. florus* is not included in Fig. 8 because they consume most of the larvae and head capsules could not be measured.

Fig. 8. Size of PLR larval head capsule at sample collection time from which a parasitoid emerged is



The OBLR parasitoids also showed stage specificity and activity that was restricted to different periods. For example, *Transonema* again was only found on the smaller larvae and only during the spring generation. *Sympiesis* was only found at two sites, both within the same large orchard and only during the summer generation. It was only collected from larvae of or after the fifth instar. Tachinids were very rare during the spring generation, but became common and the dominant ones during the second generation. *Nilea* was collected from a more restricted and smaller size class of larvae than *Nemorilla* (Figs. 8) on PLR, but no real differences in size preference were seen on OBLR.

Fig. 9. Size of OBLR larval head capsule at sample collection time from which a parasitoid emerged.



To aid in determining when to sample for percentage parasitism, we have included a table showing periods of peak activity for each parasitoid and stages of LR that are attacked (Table 4). All degree-days in the table are based on the host LR.

Table 4. Best times of the season to sample parasitoids of OBLR and PLR. Times are in DD for the host insect.

Parasitoid spp.	Size Larvae Attacked	Peak (DD) Overwintering Generation	Peak (DD) Summer Generation	Notes
OBLR				
Nilea	large	500-800	1500-2200	Biggest peak at end of season. Accounts for ≈50% of all parasitism of OBLR
Nemorilla	large	550-700	1400-1900	
Macrocentrus	small-large	100-500	1000-1500	
Transonema	small-medium	100-350	–	Only in overwintering generation
Sympiesis	large	–	1300 - 1600	Only in spring generation
PLR				
Nilea	large	400-500	1150-1350	Much less impact than on OBLR
Nemorilla	large	800-950	1150-1750	
Apanteles	small-large	100-700	900-1600	Important all season long
Transonema	small	150-400	–	Only in overwintering generation
Colpoclypeus florus	medium-large	500	1300-1600	Minor impact in overwintering generation

Fig. 11. Parasitoids attacking leafrollers. Top row from left to right *Nemorilla*, *Nilea*, and *Macrocentrus*. Middle row *Transonema*, *Sympiesis*, *Apanteles*. Bottom*: C. florus*

Budget:
Project title: Developing sampling plans for leafrollers and their natural enemies
PI: Vincent P. Jones
Project duration: Three years (2001-2003)
Project total: $133,208

Item	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Salaries	$8,750	$14,560	$15,142
Benefits[1] (30%)	2,800	4,368	4,543
Wages	14,000	14,251	14,251
Benefits (16%)	2,240	2,280	2,280
Equipment	15,900	0	0
Supplies[2]	3,200	3,475	3,118
Travel[3]	2,850	2,800	2,400
Miscellaneous			
Total	$49,740	$41,734	$41,734

[1]Benefits changed from 32% in 2001 to $30% in 2002
[2]Supplies include rearing supplies, telecom charges, miscellaneous lab and field supplies
[3]Travel is for rental of a vehicle for this project for the field season, gas, and upkeep.

FINAL REPORT
WTFRC Project #AE-03-334 **WSU Project #13C-3643-6366**

Project title: Laboratory and field-testing of protein markers to determine large-scale movement patterns of pests and their natural enemies

PI: Vince Jones, Associate Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA
(509) 663-8181 ext. 273, vpjones@wsu.edu

Co-PI and affiliation: Jay Brunner, Entomologist and Director
WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Cooperators: James Hagler, USDA-ARS Western Cotton Research Lab, Phoenix, AZ
Qing Li, Department of Molecular Biology and Biosystems Engineering, University of Hawaii at Manoa, Honolulu, HI
Joan Cossentine, Agriculture and Agri-Food Canada, Summerland, BC
Tom Unruh, USDA-ARS, Yakima, WA

Objectives:
1. Determine the importance of the different methods by which an insect may acquire our marking materials. This includes direct contact, contact with a treated surface, consumption of a treated surface, or contact with a marked individual.
2. Determine if parasitoids acquire the mark by feeding on marked hosts.
3. Determine the longevity under field conditions of the different markers.
4. Determine the cross-reactivity of the antibodies to other pesticides used in the system (e.g., other chloronicotinyls to Provado antibodies).
5. Perform a complete workup of the codling moth granulosis virus antibody to determine cross-reactivity, longevity and sensitivity.

Significant findings:
- OBLR or pear psylla walking across dried egg white residues less than 3 days old strongly acquired the mark.
- Egg whites were detected from apple leaves for >45 days when applied by airblast sprayer.
- Casein (non-fat milk) was detected from apple leaves for >18 days later during a field test in the fall.
- The casein, egg whites and codling moth granulosis virus (CpGV) assays are highly specific and do not cross-react.
- The markers cannot be applied with most of the non-ionic surfactants (Silwet, Regulaid, Reguard, Tween-20, Latron B-1956, Sylguard) or Nufilm-17. They are compatible with Orchex (650 ppm), Raynox (600 ppm) or Nufilm-P (650 ppm).
- The egg white marker can be detected at less than 100 ppb, the casein marker at less than 250 ppb, and the CpGV can be detected at 1 ppm of the formulated Virosoft™.
- The type of water used to mix up the markers (10-25% solution) has a significant effect on the sensitivity of the assays, and its effect varies by which assay is being tested. Tap water is best, followed by irrigation water, with type I laboratory (deionized, distilled and filtered) water completely suppressing activity.

Objective 1.

Methods:

To test the ability of an insect to acquire a mark by walking on a dried residue, we dipped apple leaves in either a 10 or 30% solution of the marker in type I lab water and allowed them to air dry. Leaves were then used to line the inside of a half-liter container. Adult pear psylla or OBLR moths were placed in the container for two days, then removed and tested for presence of the mark. Because neither pear psylla nor adult moths feed on the leaves, the only way to acquire the mark is by direct contact with the residue.

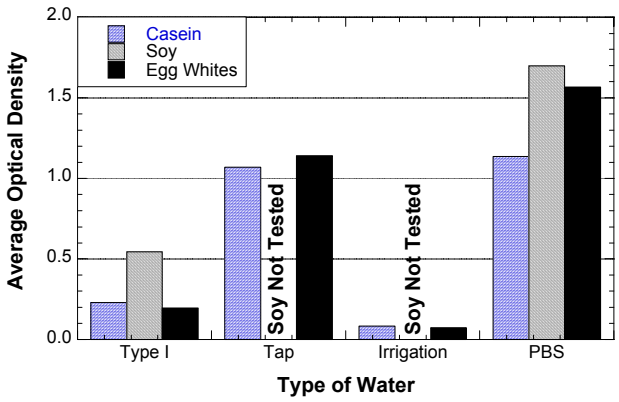
OBLR larvae were also reared on marked leaves, but because they actually ingest the marker we had to process the samples differently. In this case, we washed each larva in 1 ml of buffer, removed the buffer and stored it in a separate microcentrifuge tube to test for the external mark. A larva was washed an additional six times to remove as much of the external mark as possible, then ground up with disposable pestles, and the ELISA was run on the ground-up larva to assess it for an internal mark. We used third instar larvae and after two days removed them from the leaves and froze them. The fourth and fifth instars were allowed to continue feeding on the marked leaves and were removed only after they had attained the desired age. Thus, the older larvae had fed for a longer period on the marked leaves and should have acquired more of the mark internally.

Results:

We found all the pear psylla and OBLR moths that were held on leaves treated with either 10 or 30% solution of egg whites to be strongly marked. The buffer used to wash the external mark off larvae reared for two days on egg-treated leaves tested positive during the first wash for all instars tested, but activity in the later washes declined dramatically. The marker was still detected after the third wash for the fourth and fifth instars, but not for the third instars. Larvae of OBLR reared on egg-treated leaves also tested positive for the internal mark in the third instar (62.5%) and during the fourth instar (37.5%), but not during the fifth instar. Frass collected from the treatment containers was marked at much higher levels than the larvae. These results suggest that the fresh residue contributes strongly to marking the insects not directly contacted by the spray, at least while the residue is fresh. The internal marking is not highly effective, probably because a large amount of the protein is not incorporated into the larvae (at least not in a form that is recognizable to the antibody).

None of the psylla reared on leaves treated with 10% soy milk acquired the mark, and only one psylla was weakly positive when reared on the leaves treated with 25% soy milk. Only two OBLR larvae reared on the soy-treated leaves acquired the mark, and those were at very low levels. The wash buffer was weakly positive in only the first wash for those larvae that tested positive. Frass collected and tested showed no trace of the mark.

Fig. 1. *Effect of different types of water on the ELISA reactions for casein, soy, and egg whites.*



The low activity of the soy milk assay was puzzling until we began to analyze some of the apple leaves treated in the field to determine field longevity (Objective 3). We had treated that orchard with a concentration of soy milk >100,000 × higher than the detection limit and were barely able to detect it from the leaves collected the next day. When we tested the solution applied in the field, it also had a low response (considering the level used). Further investigation revealed that the activity of the marker was strongly affected by the type of water used to dilute the marker. We used type I laboratory water, phosphate buffer saline (PBS), tap water or irrigation water to dilute the markers for the casein and egg whites marker and only type I water for the soy assay (Fig. 1).

Diluting the markers in type I lab water or irrigation water severely reduced the sensitivity of the tests. Tap water had the least effect on sensitivity, scoring similar to the PBS buffer with the casein test and in the egg test reducing the sensitivity about one-third compared to the PBS buffer.

Because the phosphate buffer is a packet of salts (KCl and NaCl) mixed up in type I lab water, we designed a test to check if the loss of activity was because of the removal of all the ions in the type I water. We tested type I water with a serial dilution of either NaCl or $CaCl_2$ at a diagnostic concentration of each marker. We found that activity was dose-independent; that is, even a small amount of the salts would restore activity (Figs. 2, 3 A, B). The soy assay was relatively unaffected by $CaCl_2$ but responded well to NaCl. However, the 8 g/L rate caused leaf burn, and we would need to test lower levels to be sure that activity was restored and leaf damage would be minimized. We settled on $CaCl_2$ for the egg white and casein assays, primarily because it is normally used to prevent bitter pit, and the effects on the leaves were more predictable.

Tests with the casein residues showed that 25% of the moths placed on a 3-day old field-aged residue of non-fat milk (in both the 100 and 25% milk treatments) could acquire the mark but at low levels. If moths were caged on a 6-day old residue for two days, 25% of the moths acquired the mark in the 100% milk treatment but none in the 25% milk treatment.

Tests to determine if the mark can be passed by direct physical contact (i.e., between mating pairs) will be run this winter.

Fig. 2. Effect of the addition of calcium chloride and sodium chloride to soy reaction when the soy was mixed in Type I water.

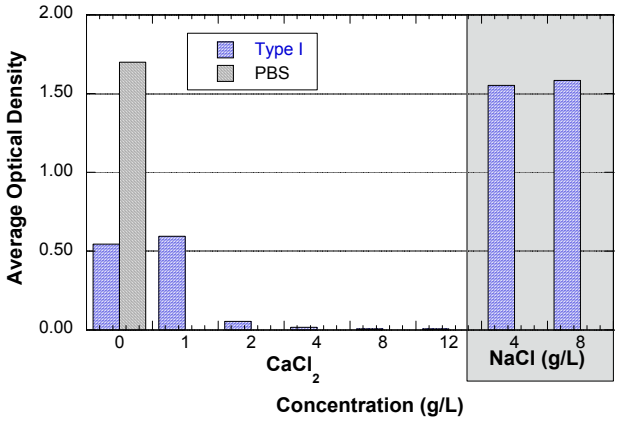
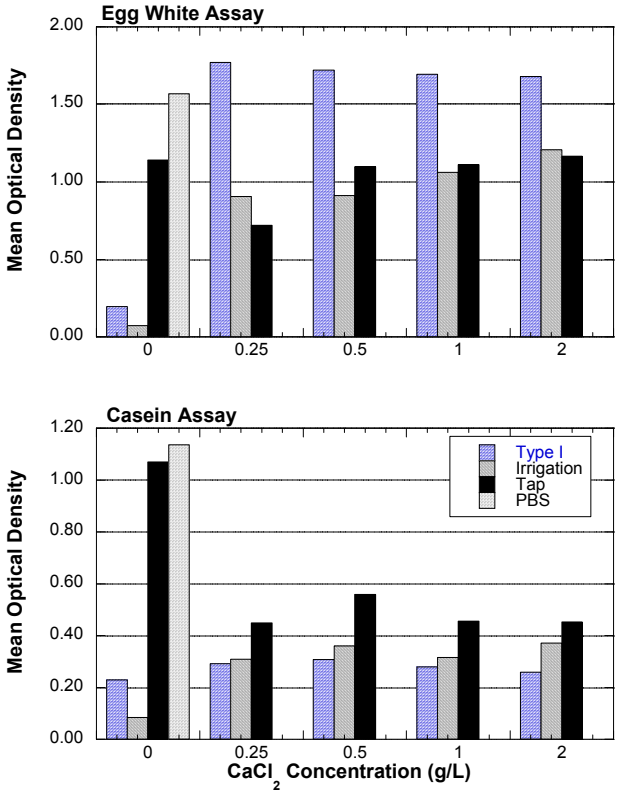


Fig. 3. Effect of adding calcium chloride to different types of water spiked with the appropriate marker.



Objective 2. These tests will be completed by early spring.

Objective 3.

Methods:

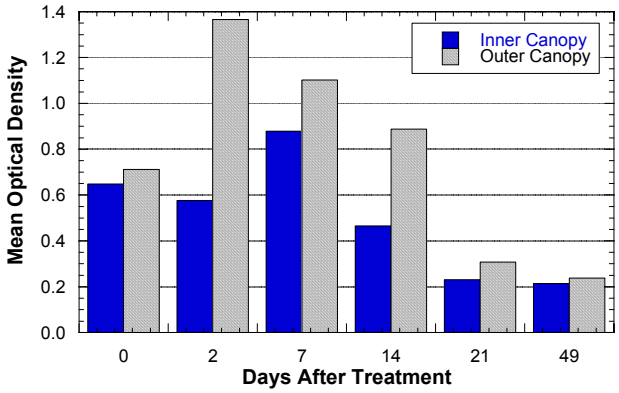
Our field trials consisted of two large-scale tests and several smaller trials. The large-scale plots were one acre each in two separate blocks at the WSU Columbia View orchard. The first trial was applied on 16 June (10% egg to one block and 10% soy milk to the other), and the second was applied on 11 Sept (10% egg to one and 15% casein to the other). For each trial, a Proptec sprayer was used to apply 30 gal/acre of the different markers. In the first trial both markers were diluted in irrigation water, and in the second trial irrigation water was used for the egg marker but 1.0 g/L $CaCl_2$ was added to the water for the casein marker. We collected 10 ml of the treatment solution before the solution was applied; this served as a test that the marker actually applied to the trees was active. Yellow sticky traps (to collect a broad range of insects) and CM and PLR pheromone traps were placed in the field near the interior of the plots. We also collected leaves separately from outside and inside the canopy to help determine longevity of the mark in the environment without having to worry about movement between areas or emergence curves that would be complicating factors with the insect samples.

Samples were processed by collecting insects directly from the traps, placing them in buffer and then using the standard ELISA protocol to determine if the mark was present. Leaves were processed by using a cork borer to remove a 7-mm diameter disc from each leaf that was placed in buffer and tested as above. The cork borer was thoroughly cleaned before and after processing each leaf to prevent cross-contamination.

Results:

The egg marker was detectable on all the leaves tested for the entire duration of the first test (21 days). The activity of the marked leaves showed an interesting pattern where the level was good initially but increased before declining slowly over the period of the test (Fig. 4). For the outer canopy leaves, the increase in activity occurred by the 2-day sample, and the inner canopy leaves showed the pattern by the 7-day sample. This may be the result of redistribution of the marker by dew over time. Before the second test was run, we collected a pre-treatment sample to be sure that none of the marker from the previous test was still present. This sample was taken 49 days after the initial treatment, and 74% of the inner canopy and 58% of the outer canopy leaves still tested positive.

Fig. 4. Longevity of egg white marker applied by proptec sprayer on inner and outer canopy apple leaves.



Marked insects from the first egg trial have been tested for two sample dates. The day after application, 24% of the insects tested were marked. At 14 days, 14% were still marked.

In the second Proptec-applied egg trial, we partially completed the testing for those sampled up to eight days. In this trial, there were enough white apple leafhopper (WALH) and noctuid moths to test them separately. We were able to detect the egg marker on 22% of the WALH the day after treatment, on 39.5% by four days, and on 35% by eight days. With the noctuids, 38.4% were marked one day after application of the marker, and by six days 23.8% were marked.

The insects used in the first Proptec experiment with soy have not been tested, primarily because none of the leaf samples showed positive. As mentioned above, this is probably related to the use of irrigation water and its severe depression on detectability of the soy ELISA assay. The egg assay worked better than expected from the lab assay (Objective 1), probably because we were using such a high rate compared to the sensitivity of the test (egg assay sensitivity down to 100 ppb, we used 100,000 ppm).

Tests of the casein marker were run in both a large-plot (CV19 in September) and in several small trials using a backpack mist blower in one case and leaf dips in two others. The leaf dips were used primarily to determine the effects of the addition of salts on longevity so no data on insects were taken (only branches were dipped). To date, we have only examined data from leaves collected up to eight days after application from the large-plot WALH trial. At eight days, 95% of the inner canopy leaves tested positive and 100% of the outer canopy leaves tested positive. Unfortunately, none of the WALH adults tested positive. However, the studies with the backpack mist blower, which were run on single-tree replicates that were surrounded by trees with high populations of (unmarked) WALH, allowed us to mark up to 40% of the population four days after treatment using the same concentration of marker (15%) but mixed in tap water.

Objective 4.

Results:

We decided to drop the imidacloprid assay because of problems with being able to determine that insects had not contacted the pesticide. The type of ELISA used for imidacloprid (an indirect competitive ELISA) works differently from the types of ELISA that we use on all the other marker candidates (an indirect ELISA). In the indirect ELISA, the greater the concentration of the marker in the initial sample, the darker the reaction at the final stage; conversely, if there is no marker present in the sample the well is clear. With the indirect competitive ELISA, the lower the concentration of the marker in the sample, the darker the sample at the final stage. Unfortunately, although we could easily detect the presence of the imidacloprid, the negative controls were highly variable, which made it extremely difficult to tell when it was absent.

We did test if imidacloprid was cross-reactive with the soy, egg white or casein antibodies (it was not) but did not test the other chloronicotinyls since we would not be using the imidacloprid assay. Tests for cross-reaction between the soy, egg white and casein antibodies showed that each was specific and did not respond to any of the other markers (e.g., egg white did not react to soy milk, casein or imidacloprid).

We also tested the effect on the assays of mixing the markers with low concentrations of a variety of agricultural adjuvants. In general, non-ionic surfactants (Tween-20™, Silwet™, Sylguard™, Regulaid™, Regard™, Latron B-1956™) almost completely inhibit detection by the indirect ELISA. Nufilm-17 also was extremely detrimental to sensitivity, but Nufilm P™ and Orchex™ horticultural oil could be used up to 650 and 1300 ppm, respectively. Raynox™ had no detrimental effect up to 600 ppm but by 1200 ppm reduced the sensitivity of the tests by ≈50%.

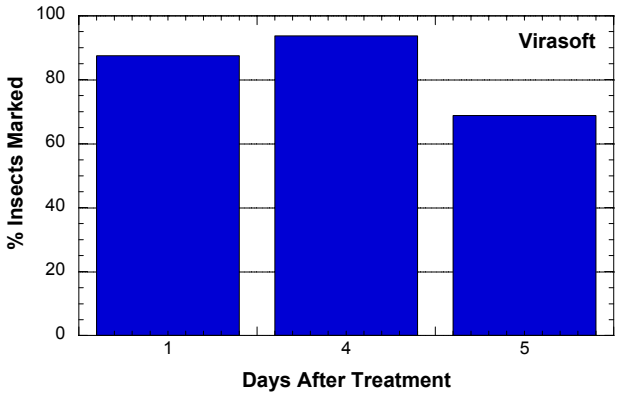
Although we did not field test the surfactants, we did mix up different concentrations, apply them to moths and observe whether the droplets would wet the moth. At virtually any concentration where wetting was observed, our studies showed that the ELISA reaction was inhibited. We also performed lab tests by spraying in a fume hood moths with markers mixed at rates that had low effects on the ELISA reaction, and we found that there was no benefit compared to just applying the markers in buffer or tap water.

Objective 5.

Results:

The antibodies for the codling moth granulosis virus were obtained from Canada. These antibodies were developed by Dr. Ken Eastwell, who is now a faculty member at WSU-Prosser, to detect CpGV-infected codling moth. We tested three formulations of the codling moth granulosis virus as markers, Virosoft™, Cyd-X™, and Carpovirusine™. Our studies showed that Carpovirusine™ was a poor antigen, probably because the UV stabilizers and the formulation inhibit the ELISA reaction. However, either Virosoft™ or Cyd-X™ could be used, and each had approximately the same activity. We were able to detect Virosoft™ mixed in buffer down to 1.0 ppm of formulated material. In the field, we only used the Virosoft™ material, primarily because the manufacturer recommends that Cyd-X™ be applied with Nufilm-17, which greatly reduced the sensitivity of the test.

Fig. 5. Percentage insects on sticky cards testing positive for CpGV in single tree plots. Formulated material (Virosoft) was applied by handgun at 3.24 fl. oz./acre.



The field tests of the CpGV antibody were run in conjunction with an efficacy trial with single-tree replicates. We followed only the Virosoft™ treatment that was applied at 3.24 fl oz/100 gal (253 ppm of formulated material) and only after the second application had been applied. The treatments were repeated at ≈10-day intervals so our tests could not follow the insects longer than that period without an additional spray being applied.

We were unable to detect the Virosoft™ on the leaves, even on leaves collected the day after treatment. However, insects collected from this trial were very strongly marked, with more than 60% being positive five days after treatment (Fig. 5). Tests are underway of whether the insects can pick up the enough Virosoft to become marked by coming into contact with recently dried residue.

Overall comments:

The way each insect acquires the mark is probably a unique combination determined by its mode of feeding (leaf chewers vs. phloem feeders vs. predators), surface of its body (scales vs. bare, the wax layers present, etc.) and its behavior (activity level, location on tree). These differences and others mean that what works for one insect with one particular marker may not work for another insect or marker and that the only way to know is actually to test those insects of interest.

We expect that much of the difference in ability to acquire the mark that is unrelated to the surface properties of the insect will come from two sources: 1) the quality of the water and 2) the method used to apply the marker to the field. The quality of the water can be overcome by using tap water (preferably) or with proper testing beforehand and the use of irrigation water with calcium chloride. The method of application is an area we need to investigate further. Our studies show that leaves are easily marked using the 30 gal/acre rate that we used with the Proptec sprayer. However, the insects collected in those tests were marked at lower rates than we expected. Conversely, even using the backpack mist blower resulted in higher numbers of marked WALH. Finally, the CpGV application (Objective 5) showed a much higher proportion of marked individuals, despite the fact that the egg and casein assays can detect much lower levels of their respective markers. It is even more important in light of the fact that the dose applied to the CpGV was only 253-fold higher than the detection level compared to the egg and casein markers that were applied at >100,000 times their respective detection levels. This suggests that the handgun application, or at least an increase in the amount per

acre when using the Proptec sprayer, would be beneficial and we will be testing this as soon as trees leaf out in the spring.

Budget:

Project title: Laboratory and field-testing of protein markers to determine large-scale movement patterns of pests and their natural enemies

PI: Vince Jones

Project duration: one year (2003)

Project total: $24,497

Category	**Year 1 (2003)**
Salaries (Associate in Research)[1]	$12,200
Benefits (30%)	3,660
Wages	1,230
Benefits (16%)	197
Equipment[2]	3,000
Materials and supplies[3]	3,810
Within-state travel[4]	400
Total	**$24,497**

[1] Associated with this project alone.

[2] Equipment required was a high quality still to produce distilled water that was used in all the assays.

[3] Supplies included ELISA supplies, telecom charges, traps, miscellaneous lab and field supplies.

[4] Included rental of a vehicle for this project.

FINAL REPORT

Project Title: Insect Responses to Induced Apple Defensive Chemistry

PI: Peter J. Landolt
Organization: USDA, ARS, Yakima Agricultural Research Laboratory

Cooperator: Roy Navarre, USDA-ARS, Prosser

Objectives:
1. Determine effects of induced defensive chemistry and leaf age on suitability of apple leaves for caterpillars.
2. Determine effects of induced defensive chemistry of apple leaves on caterpillar movement.
3. Determine effects of induced defensive chemistry on codling moth oviposition.

Significant Findings:

1. Apple leaf quality deteriorates with the season. We confirmed in multiple seasons that *L. subjuncta* larval development is prolonged and pupal weight is decreased on apple foliage as the season progresses. These data indicate the apple leaves are a relatively good food material for Lacanobia larvae early in the season but deteriorate in quality later in the season. Similar results were obtained for Pandemis leafroller larvae on apple leaves. Poor leaf quality may cause more damage to fruit by leaf-eating caterpillars.

2. Sources of variance for apple leaf quality. The quality of apple leaves as caterpillar food was not strongly tied to leaf relative age (as leaf position on a branch). Leaf quality was not reduced with methyl jasmonate treatments (to induce chemical defenses), or with prior feeding on the leaves by other caterpillars (also should induce chemical defenses). Leaf quality did vary with apple variety, with fastest development on Red Delicious leaves.

3. Potato is a superb host of Lacanobia larvae, compared to many weeds, annual crops, and foliage of tree fruits evaluated. Also, in 2003, Lacanobia larvae, mixed with cutworms and armyworms, were found defoliating areas of commercial potato plantings near Othello and near Ephrata.

4. Pandemis leafroller and Lacanobia fruitworm larvae prefer apple foliage over fruit and Lacanobia fruitworm larvae preference for foliage was greatest with Spring foliage. These findings support the hypothesis that damage to apple fruit may be exacerbated by deterioration in foliage quality.

Methods:

A. Suitability of apple foliage:

Two assay methods were used to evaluate caterpillar development on foliage. Individual neonate (newly hatched) caterpillars were held in 2 oz clear plastic cups with leaves. The leaves were added or replaced daily and old leaves and droppings were removed as needed. These were maintained until adults emerged and data were recorded on mortality, pupation, and adult emergence. Pupae were weighed to the nearest milligram.The second assay involved placing 3rd instar larvae in the cups with foliage for 7 days. Larvae were weighed on day 1 and day 7 to determine weight gained, and leaves were added daily. These assay methods were used in a series of experiments to evaluate larvae performance on apple foliage.

1. Suitability of apple foliage as Lacanobia food through the season.

Neonate larvae were placed with foliage throughout much of the season, depending on the availability of Lacanobia eggs, to get data on development time, mortality, and pupal weight. These assays were conducted over three years to accumulate replicates in May through August.

2. Effect of leaf position (as relative indicator of leaf age) on Lacanobia development.

Third instar larvae were placed with leaves that were a) 1st and 2nd from the tip, b) 3rd and 4th from the tip, c) 5th and 6th from the tip, d) 7th and 8th from the tip, and 9th and 10th from the tip. Leaves were replaced daily for 7 days to obtain data on weight gained versus treatment (leaf position).

3. Effect of methyl jasmonate treatment to apple leaves on Lacanobia development.

Application of methyl jasmonate to foliage was done with a plastic spray bottle containing a 0.5 mM/L methyl jasmonate solution in water. Leaves were wetted and foliage was held for 48 hours before use in experiments. Third instar larvae were placed with treated and untreated leaves, which were replaced daily for 7 days to determine weight gained versus methyl jasmonate treatment.

4. Effect of apple variety on Lacanobia development.

Neonate larvae were placed with foliage from Red Delicious, Golden Delicious, Gala, Fuji, and Granny Smith apples, and were maintained through to pupation in order to determine if suitability of foliage as larval food varied with variety. This experiment was conducted in August.

5. Effect of insect damage to apple leaves on Lacanobia development.

Cloth sleeves were placed over apple branches on multiple trees and 3 third instar Lacanobia larvae were placed within each of the sleeves for 48 hours to effect feeding damage to the foliage and provide time for induced biochemical responses by the tree. Foliage was then harvested for use in the assay, in comparison to foliage from another set of trees that were not so treated. Third instar larvae were placed with either treated or untreated foliage, which was replaced daily for 7 days to obtain data on weight gain versus treatment.

6. Potato foliage as food for Lacanobia larvae.

Field grown Russet Burbank potato foliage was used as food for larvae, using both the egg to adult assay and the 7 day weigh gain assay. Using the first assay design, Lacanobia larvae were fed potato foliage daily until they pupated, with data on survival, development time, and pupal weight. The second assay design was used to determine the amounts of weight gained by larvae over a 7 day period, provided potato foliage daily. This was used to compare potato foliage treated with methyl jasmonate to untreated foliage, and potato foliage to apple foliage.

B. Behavior of codling moth.

1. Larval choices in arena assay.

An arena type assay was used to evaluate larval behavior in the presence of fruit and foliage. A 16 oz clear plastic cup with a screened lid was provided a small apple fruit and a cluster of apple leaves. A 3rd instar larva was placed within the cup and observed at one hour, 3 hours, and at 24 hours to determine feeding on fruit and foliage. These assays were conducted in batches of 20 replicates. The basic experiment (comparison of Fuji fruit and foliage) was conducted using Lacanobia fruitworm larvae and Pandemis leafroller larvae, in winter with greenhouse grown apple saplings and with cold-stored Golden Delicious apples, and again in early summer using fresh cut Fuji apple foliage and fresh picked thinning sized Fuji apples. After comparing data at one hour, 3 hours, and 24 hours, it was decided that the 24 hour data was most useful, due to variance in the time it took larvae to begin feeding.

2. Adult codling moth oviposition in response to fruit in flight tunnel.

A flight tunnel was used to evaluate codling moth rates of oviposition when in the airstream downwind of pear fruit, and infested pear fruit, both in comparison to no fruit as a control. This experiment tested the hypothesis that pear fruit will produce greater amounts of kairomones in response to feeding damage by codling moth larvae (induction of chemical synthesis), as occurs with apple fruit, stimulating adult moth oviposition.

Other attempts to develop discriminating assays for the study of codling moth oviposition as a response to fruit or foliage were not successful. Moths appeared to respond primarily to physical parameters, such as light and reflectance, and to surface features such as roughness.

C. Chemical defenses of Apple Foliage.

Apple foliage posseses a variety of types of compounds thought to protect against attack by pathogens, insects and other organisms. These include tannins, terpenoid compounds, green leaf

volatiles, and protease inhibitors. We have conducted analyses of terpenoid and green leaf volatile compounds, using organic solvent extractions followed by GC-MS, and a fluorimeter to quantify changes in levels of a protein standard with a protease standard and apple leaf extract added. Protease inhibition is quantified as the degree of reduction in protease breakdown of the protein standard.

Results and Discussion:

A.

1. Suitability of apple foliage as Lacanobia food through the season.

Cumulative data over 2001-2003 indicates a consistent pattern of reduced performance of Lacanobia larvae on apple foliage as the season progresses. From late May through August larvae fed apple leaves took longer to develop, suffered higher mortality rates, and grew to a smaller size before pupation, as the season progressed.

There is potential significance of these findings in the areas of biological control, on modeling based on degree days, and on larval damage to fruit and the economic injury levels of Lacanobia populations. First, when larvae take longer to develop to pupation, they are exposed for a longer period of time in the field to predators and parasites, potentially increasing biological control. Second, the differential rates of development on apple through the season were not temperature related (this was done under controlled conditions) and development models would have to take into consideration that development rates are dependent in part on food quality, in addition to temperature. This relationship will affect the patterns of emergence of adults, and life stage phenology generally. Third, the deterioration in quality of apple foliage as larval food and increased length of time for larval development might increase risk to damage to apple fruit, as larvae search for higher quality food, and over a longer period of time than would occur on better foliage. This latter point also relates to economic injury levels of larval densities on apple trees. At most larval densities encountered, the risk is fruit damage, not significant tree defoliation. If that risk increases through the season, as from first generation larvae to second generation larvae, then the economic injury threshold of populations of Lacanobia may be lower in the second generation. This remains however to be determined.

2. Effect of leaf position (as relative indicator of leaf age) on Lacanobia development.

The amount of weight gained by larvae when fed leaves of apple did not vary with the position of the leaf on the branch. Leaf age decreases from the base of a branch to the tip, as the branch grows. The hypothesis was that leaf quality as larval food might deteriorate with leaf age, as constitutive defensive chemicals are accumulated over time in leaf tissue. Thus, older leaves at the base of a branch might have greater amounts of such chemicals, such as tannins, protease inhibitors, etc, compared to leaves at the growing tip. In this experiment, we did not have information on the exact age of each leaf however, and we do not know how much leaf age varied with position on the branch.

3. Effect of methyl jasmonate treatment to apple leaves on Lacanobia development.

The amount of weight gained by larvae of Lacanobia when fed apple leaves treated with methyl jasmonate was significantly greater than weight gained by larvae fed untreated apple leaves. The starting hypothesis was that methyl jasmonate will induce the production of increased levels of defensive chemicals in the foliage that will decrease the quality of the leaves as food for larvae, resulting in a lowered weight gain. Clearly, the larvae did not suffer reduced weight gain on treated apple leaves. There is data in the literature indicating that increased levels of defensive chemicals can include an increase in leaf nitrogen through the production of certain proteins (see C below). Perhaps increased nitrogen level in leaves improved leaf quality, while the larvae were not significantly affected by induced chemical defenses of apple (they are adapted). This experiment was repeated with Lacanobia on potato (after finding these larvae defoliating commercial potato fields). Those larvae had a dramatic reduction in growth when fed potato foliage treated with methyl jasmonate, compared to untreated potato foliage. These findings are interpreted to mean that Lacanobia larvae are not well

adapted to potato defensive chemicals (such as alkaloids) but are well adapated to apple defensive chemicals (such as tannins and protease inhibitors).

These findings are interesting but do not help us interpret the reductions in leaf quality as larval food through the season. Another interesting aspect of these results relates to the use of chemicals commercially to trigger SAR, or systemic acquired resistance. This phenomenon (SAR) involves much of the same induction of synthesis of defensive chemicals as methyl jasmonate treatments that we used here (methyl salicylate is another such compound). Our results indicate that such treatments may result in improved susceptibility of apple to certain insects (such as Lacanobia), while the goal of such treatments is to improve plant resistance to insects. Again, this is somewhat speculative and field experiments with such SAR agents should be conducted with these findings in mind.

4. Effect of apple variety on Lacanobia development.

Weights of pupae were smallest with Granny Smith foliage and greatest with Red Delicious foliage. Development time was shortest with Golden and Red Delicious and longest with Granny Smith foliage. Survival to adult was highest with Red Delicious and lowest with Granny Smith. Other varieties were intermediate. There have been stated observations or conclusions over the past 6 years of preference or attractiveness of particular varieties of apple to Lacanobia fruitworm, based possibly on the severity of infestations. Previous studies of the incidence of Lacanobia in commercial apple orchards did not reveal a pattern, but did not disprove any preference for a given variety. These results do indicate the potential for faster growth, greater consumption of foliage, and higher survival of Lacanobia on certain varieties of apple compared to others, particularly Red Delicious.

5. Effect of insect damage to apple leaves on Lacanobia development.

Mean weight gained by larvae fed undamaged apple leaves was 6.1 mg per larva, compared to 8.4 mg per larva fed caterpillar-damaged apple leaves. These data were not significantly different by a t-test ($p = 0.33$, $df = 8$). The trend however, of higher weight gain on damaged foliage, is in line with the results of feeding trials with apple foliage treated with methyl jasmonate, with greater larval weight gain on treated foliage.

6. Potato as food for Lacanobia.

Potato is the best host plant tested in series of comparisons over 4 years of crops and weeds as food for Lacanobia larvae. In laboratory assays, larvae developed fastest, with the largest pupal weights, and highest survival rates to adult, compared to other plants tested, including apple. In the 7 day weight gain assay, Lacanobia larvae gained more weight in the 7 day period when fed potato (> 80 mg/larva) compared to larvae fed apple foliage (20 mg/larva). Also reported here is the identification of Lacanobia larvae in collections of insects made from commercial potato fields defoliated by caterpillars. Those collections were a mixture of Lacanobia, bertha armyworm, and variegated cutworm, but are of interest because of the superior performance of Lacanobia in the laboratory on potato foliage.

This information is of significance in relation to the regional population dynamics of Lacanobia fruitworm. This insect is extremely abundant in pheromone, blacklight, and feeding attractant traps throughout the Yakima Valley and much of the Columbia Basin, in areas with Lacanobia problems in apple orchards and in areas without problems, in years where they are a problem, and in years when they do not appear to be a problem. Lacanobia can feed on many types of plants, and may be reproducing on other irrigated crops in eastern Washington in addition to apple. This is the first record of it's pest status on potato, and suggests a possible role of potato in contributing to regionally high populations of Lacanobia. The conditions under which apple orchards are attacked are yet unknown.

B. **Behavior of codling moth**

1. Larval choices in arena assay**.**

In evaluating Golden Delicious fruit and foliage in winter, Pandemis leafroller larvae more often chose foliage to feed on (65%) instead of fruit (15%) or both foliage and fruit (20%). Similarly, Lacanobia larvae fed primarily on foliage (90%), compared to fruit (10%) or both foliage and fruit (0%). In evaluating Fuji fruit and foliage in early summer, Pandemis leafroller larvae again chose foliage most of the time (80%) , compared to fruit (10%), or both foliage and fruit (5%). Lacanobia larvae also chose Fuji foliage (75%), over Fuji fruit (5%) or both foliage and fruit (20%) most of the time.

These results support the field observations that most food consumed by both species is foliage, not fruit, and indicate that even in close quarters with both fruit and foliage at hand, most larvae of both species feed on foliage. This experiment also demonstrates the usefulness of this arena assay design as a way to detect or document changes in choices made by larvae of both species with changes in the quality of apple leaves and changes in the maturity of apple fruit with the growing season.

2. Adult oviposition in response to pear in flight tunnel.

Codling moths in a container in the flight tunnel layed numerous eggs during the four hour assay period regardless of treatment, with similar numbers of eggs laid downwind of pear, infested pear, or no fruit. Previous experiments demonstrated a consistently higher attraction response of codling moth to infested apple, compared to uninfested apple, but did not evaluate numbers of eggs laid under those circumstances. It is generally considered that plant or host chemicals involved in host finding (attractants) might not be the same as plant chemicals that stimulate oviposition.

C. **Chemical Defenses of Apple**.

Apple foliage posseses terpenoid compounds and protease inhibitors, in addition to other chemicals that probably help defend against attack by insects. We have repeatedly found strong increases in terpenoid compounds in fruit infested by codling moth, and to a lesser extent in fruit fed upon by leafroller larvae.

We were unable to correlate protease inhibitor activity in methyl jasmonate treated apple leaves with reduced performance (lowered weight gain, slower development) of Lacanobia larvae on those leaves. However, methyl jasmonate treated leaves did have increased levels of protein content, which could explain the faster weight gain of larvae fed foliage that had been treated with methyl jasmonate.

It is not known if apple foliage responds the same to different reported inducers of SAR or chemical defenses, and we do not fully understand the chemical changes that take place or their effects on insect pests of apple. Clearly though, the effects of these treatments are not very predictable and appropriate studies should be conducted with all pests of interest, when SAR treatments are to be considered as a means of protecting crops from diseases and insect attack.

Budget:

Project title:	Insect Responses to Induced Apple Defensive Chemistry
PI:	Peter J. Landolt
Project duration:	3 years.
Current year:	2004
Project total (3 years):	$64,400

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Total	$23,000	$24,400	$17,000

Current Year breakdown

Item	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Salaries	$18,000	$18,600	$15,000
Benefits			
Equipment			
Supplies	4,000	2,000	2,000
Travel	1,000		
Total	$23,000	$24,400	$17,000

FINAL REPORT

Project Title: Killing stations for Leafrollers on apple and pear.

PI: Peter Landolt

Organization: USDA, ARS, Yakima Agricultural Research Laboratory, Wapato

Cooperator(s): Jay Brunner, WSU, Wenatchee

Objectives:
1. Quantify mortality rates of leafroller moths attracted to pheromone lures.
2. Determine repellency or deterrence of PLR to permethrin/teflon grease formulation.
3. In small plots, determine effects of killing stations on captures of PLR in monitoring traps.
4. Determine response of OBLR to feeding attractant. This objective was added when high populations of OBLR were found locally.

Significant Findings:
1. Rate of contact and mortality of leafrollers was very high in laboratory tests that exposed moths to killing stations baited with pheromone lures.
2. Numbers of moths in pheromone monitoring traps was reduced significantly in plots with 50 killing stations per acre, indicating a knockdown of the population of moths in the orchard.
3. A commercially prepared killing station was very effective in killing contacting leafroller moths and holds promise for use in future experiments.
4. Oblique banded leafroller moths are attracted to controlled release dispensers that emit acetic acid, with an optimum release rate that is higher than that for either codling moth or Pandemis leafroller.

Methods

Objectives 1 & 2. Mortality of leafrollers contacting killing stations/repellency of stations.

Two assay designs were used to evaluate Pandemis leafroller response to killing stations. Moths were tested in a flight tunnel to determine rates of attraction and mortality of tested moths. A touch test was also used to evaluate the toxicity of killing stations, including field exposed or aged killing stations. This test involved handling moths with forceps and touching an antenna, leg, or wing tip to the killing station surface and then holding the moth in a vial for observations. Moth mortality was assessed at intervals up to 24 hours.

Badminton shuttlecock killing stations were tested after exposure in apple orchards, and the Suterra killing station also was tested after 2 weeks exposure in the field.

Objective 3. Knockdown of PLR in treated plots

Six apple orchard plots that were each one acre in area were each monitored with a blacklight trap, a pheromone trap and a feeding attractant trap. Three of these plots were treated with 50 killing stations with pheromone, and three received no killing stations (negative control). Killing stations were badminton shuttlecocks coated with a formulation of permethrin, and with pheromone lures (septa) pinned inside the rubber bulb of the shuttlecock. Effectiveness of killing stations was evaluated by comparing numbers of leafroller moths captured in monitoring traps after killing stations were deployed, in treated versus control plots. This experiment was repeated during the second flight of Pandemis leafroller.

Objective 4. Leafroller response to feeding attractant.

To document a response by OBLR to acetic acid and to determine the optimum release rate of acetic acid for trapping OBLR, a test of varied amounts of acetic acid was conducted. Two tests were

conducted; one comparing vial holes of 0.5 to 6 mm in diameter, and the other comparing vial holes of 3 to 25 mm in diameter. Traps were placed in apple trees in commercial orchards, using a randomized complete block experimental design. Three different apple orchards were used for these experiments.

A comparison was also made of acetic acid and molasses to determine if all attractiveness of molasses to PLR could be explained by PLR attraction to acetic acid. Acetic acid was dispensed from polypropylene vials with 3 mm holes. 200 ml of 10% molasses in water was used as the molasses bait. Agrisense dome traps were used because they can hold the molasses bait in the bottom reservoir of the trap. The test was conducted in an apple orchard during the second moth flight.

Results and Discussion:

Objectives 1 & 2. Mortality of leafrollers contacting killing stations/repellency of stations.

All PLR moths responding to pheromone lures in a flight tunnel contacted the killing station (shuttlecock) and either died or became paralyzed. All PLR moths placed in contact with killing stations subsequently died (24 hour test), compared to none of the controls. , and retained its effectiveness in killing PLR. Because assays of attraction, contact, and mortality were so successful, it was not necessary to set up additional experiments to test for repellency.

Both the badminton birdie killing stations and the Suterra killing stations remained lethal to contacting leafrollers after 7 days and 14 days in the field respectively. For both designs, mortality of PLR placed in contact with killing stations was close to 100% following field exposure.

Objective 3. Knockdown of PLR in treated plots.

Numbers of PLR in pheromone traps used to monitor moth activity in plots were greatly reduced in treated plots compared to control plots following the placement of fifty killing stations per one acre plot (Figure 1). These results were quite similar in all plots in both flights (June and August). A reduction in PLR in treated plots was not seen in blacklight trap catches however. These results are preliminary, but support the hypothesis that PLR numbers can be greatly reduced by killing stations. In similar tests conducted with killing stations against Lacanobia and against alfalfa looper, one acre plots were insufficient to overcome problems of immigration from outside plots, while 5 acre plots appeared to suffice. These encouraging results with the reduction in leafroller moths in pheromone traps with treated plots are however preliminary in that we do not know if this is a result of the death of a high percentage of males, or mating disruption because of 50 pheromone lures placed per acre in the plots. This is quite dramatically fewer that the 1000+ units per acre used in field trials of codling moth lure and kill formulations. This question of the mechanism (mortality or disruption) should be sorted out in subsequent testing in larger plots, as it was in the 5 acre Lacanobia killing station plots.

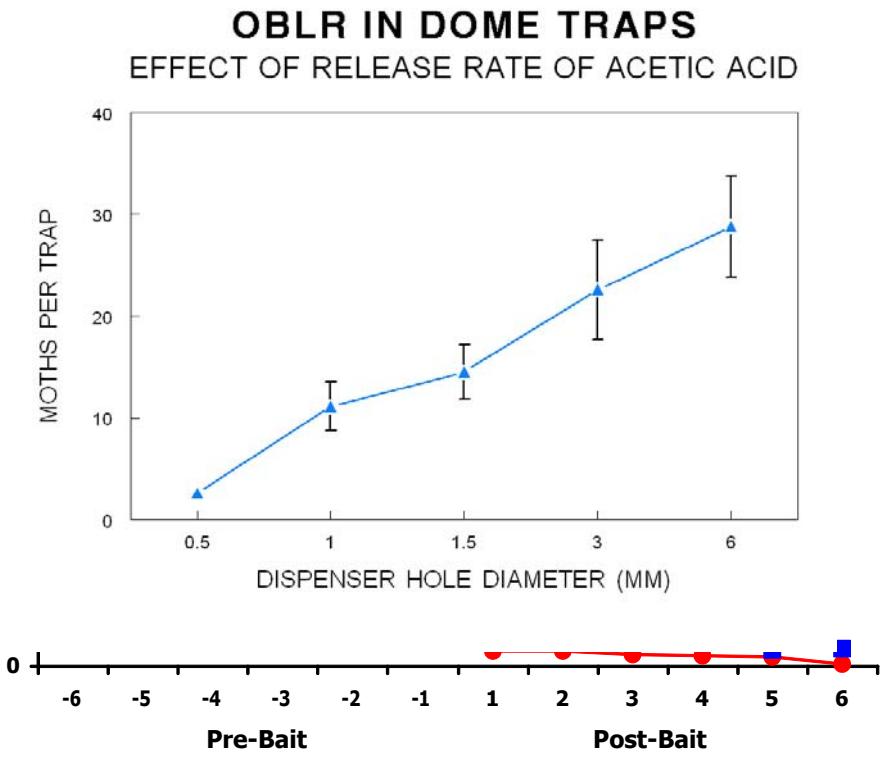


Figure 1. Mean numbers of Pandemis leafroller moths in pheromone traps used to monitor apple orchard plots in which killing stations were deployed.

Objective 4. Leafroller response to feeding attractant.

Greatest numbers of male and female OBLR were captured in AgriSense dome traps baited with polypropylene vials containing acetic acid released through 6 mm diameter holes (Figure 2). These vials release 2 milligrams of acetic acid per hour under laboratory conditions. This compares to an optimum of 1 milligram per hour for Pandemis leafrollers captured in traps, and 0.5 milligrams per hour for codling moth captured in traps. Both males and females of OBLR were captured in traps baited with acetic acid at a ratio of about one to one.

More Pandemis leafroller moths were captured in the Agrisense dome traps baited with vials releasing acetic acid than with 10% fermented aqueous solutions of molasses. These results indicate that all of the attractiveness of molasses to PLR could be due solely to the release of acetic acid from microbial fermenting of sugars in the molasses solution, and that further efforts to find co-attractants for PLR in fermented molasses are unlikely to be fruitful.

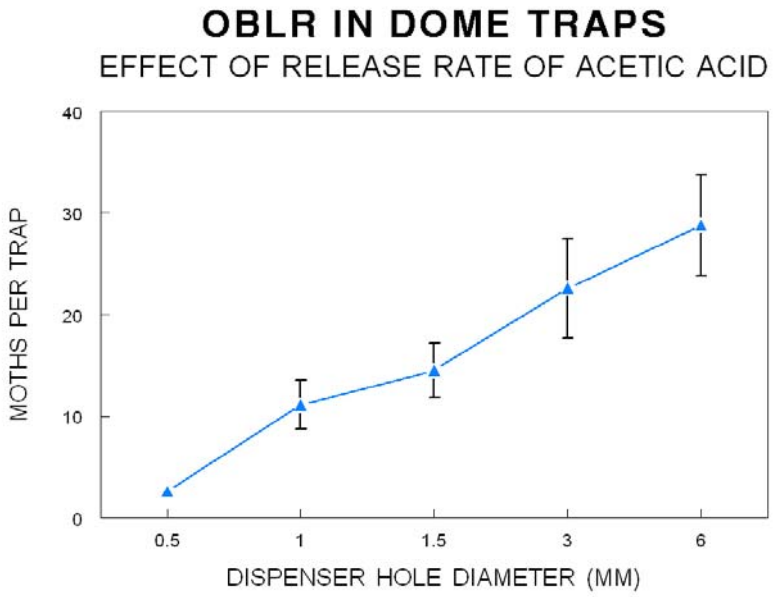


Figure 2. Mean numbers of OBLR moths captured in Agrisense dome traps baited with vials releasing acetic acid through holes in the vial lid, of varied diameters.

Budget:

Project Title:	Killing Stations for Leafrollers on Apple and Pear.
PI:	Peter J. Landolt
Project Duration:	2003.
Current Year:	2003.
Project Total:	$10,000

Year	Year 1
Salaries	$8,000
Supplies	$2,000
Travel	
Total	$10,000

FINAL REPORT
WTFRC Project #AE-02-223 **WSU Project #13C-3643-4366**

Project title: Examining the mechanisms of mating disruption in codling moth and leafrollers

PI: Vincent P. Jones, Associate Entomologist

Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA
(509) 663-8181 ext. 273, vpjones@wsu.edu
Co-PIs and affiliations: Jay F. Brunner, Entomologist and Director, WSU TFREC, Wenatchee, WA
Vincent Hebert, Assistant Entomologist, WSU FEQL, Richland, WA

Cooperator: Rick Hilton, Oregon State University

Objectives:
1. Determine the actual release rates of commercial dispensers used for CM and LR MD.
2. Measure the delays in mating seen in the field in CM and LR MD plots and develop a dose-response to establish a link between delay in mating and CM and LR population biology.
3. Determine the effect of delayed mating on life history of CM and LR.
4. Examine delays in mating of CM from orchards were MD problems cannot be traced to poor timing, low rates, or other obvious factors and compare it to data taken in Objective 2.

Significant findings:
- A volatile trapping system has been developed, validated and used in conjunction with residual analysis to help examine performance of five different commercial dispensers in Wenatchee and Medford, Oregon.
- Laboratory and field cage studies showed that OBLR males are still able to find females under MD conditions. However, the average time to find the females is significantly greater under MD.
- Laboratory and field cage studies showed that under MD conditions CM males never found the female within 1 night. However, using the virgin female traps in MD orchards, we were able to capture low levels of males.
- Studies on delayed mating with OBLR will be completed shortly. Initial findings show that delayed mating effects are composed of two factors, 1) an increase in sterile pairings as age increases and 2) a reduction in total egg production.
- We also found in preliminary studies that, in OBLR, male age at time of mating was an important factor in population growth. If a 4-day old male was paired with a 4-day old female, reproduction was half that of a 1- to 2-day old male paired with a 4-day old female.
- Codling moth studies will require more replicates, but egg production was significantly reduced for females experiencing a delay of 4 or more days.
- Mortality factors acting in the field have a much greater effect than lab studies suggest because the mortality rate in the field is much higher than under lab conditions. However, this can be predicted using the life table approach.

Objective 1.
Methods:
Pheromone dispensers were sent directly from the manufacturers to Dr. Hebert's lab. The dispensers were stored as per manufacturers' instructions until all five types had arrived. Twenty dispensers of each type were removed from their packaging, 10 to be used for residual analysis and 10 for volatile

trapping analysis at the FEQL. Two hundred dispensers of each type were packaged in blue ice and sent via overnight mail to Wenatchee, where 100 dispensers of each type were re-packaged and sent to Medford, Oregon, on the same day. Twenty dispensers of each type were immediately packaged and returned to FEQL from Wenatchee and Medford for day '0' analysis (to check what was actually put in the field). Dispensers were placed in orchards on May 5 (Wenatchee) and May 3 (Medford). In Wenatchee, 200 dispensers of each type were placed by hand in tree canopies at TFREC. Every 14 days after the initial placement, 10 dispensers of each type were randomly collected for volatile trapping analysis. In addition, 20 dispensers of each type were randomly collected at both sites every 28 days, half of which were analyzed for residual pheromone content and half frozen for later use if necessary. All dispensers were stored in sealed Mylar bags and frozen until shipment to the FEQL. All shipments were packaged with blue ice and sent to FEQL via next day FedEx for residual analysis and volatile trapping analysis.

Fig. 1. Volatile trapping system for aged pheromone dispensers.

The residual analysis procedures were the same as those followed in 2002. The volatile trapping method was newly developed by Elizabeth Tomaszewska in Dr. Vince Hebert's laboratory. The new system was devised, validated and reviewed by experts in the pheromone research area. The volatile trapping system (VTS) (Fig. 1) involves passing clean air over dispensers of different ages and trapping the pheromone released under constant conditions onto a substrate over a period of 2 hours. Standard procedures were then used to extract the pheromone from the substrate and identify the amount trapped using gas chromatography-mass spectrometry analysis. At present, data for both residual and VTS samples are available only for the 0-day, 14-day, and each subsequent 28-day interval. The remaining VTS samples and 14-day intervals will be reported later. Residual analysis and volatile trapping methods data are contained in a report by the FEQL laboratory and will be made available upon request.

Results – Residual Analyses:

The average loading rate of all dispensers was within the limits indicated by each product's label (see Manufacturer M-0 – Figs. 2A and 2B). There was no significant drop in codlemone levels from the M-0 samples and the day '0' samples when dispensers were placed in the field at either location.

NoMate CM: There was a slow decline in the residual codlemone during the first 28 days at Wenatchee and a slightly more rapid decline at Medford. From day 28 to day 140 at Wenatchee and day 28 to 112 at Medford there was a fairly linear decline in residual pheromone (Figs. 2A and 2B). The average calculated release rate in Wenatchee and Medford over a 140-day period was 0.75 and 0.81 mg per day, respectively. The NoMate-CM dispenser was efficient in release of codlemone, with an average of 79% (Wenatchee) and 86% (Medford) expended over the duration of the test. There was a high degree of consistency of residual codlemone in the NoMate-CM dispensers on all sample dates.

Isomate-C Plus: There was a gradual decline in the residual codlemone over the 140-day period in

both locations (Figs. 2A and 2B). This dispenser released an average of 71% (Wenatchee) and 77% (Medford) of codlemone over the duration of the test. The average estimated release rate in Wenatchee and Medford over a 140-day period was 0.67 and 0.70 mg per day, respectively. There was a high degree of consistency of residual codlemone in the Isomate-C Plus dispensers on all sample dates.

Isomate CTT: There was a gradual decline in the residual codlemone over the 140-day period in both Wenatchee and Medford (Figs. 2A and 2B). The average estimated release rate over the 140-day period in Wenatchee and Medford was 1.05 and 1.15 mg per day, respectively. This calculated release rate is lower than in 2002 even though the 2003 season was hotter. Isomate-CTT released an average of 57% (Wenatchee) and 62% (Medford) of codlemone over the duration of the test. There was a high degree of consistency of residual codlemone in the Isomate-CTT dispensers on all sample dates.

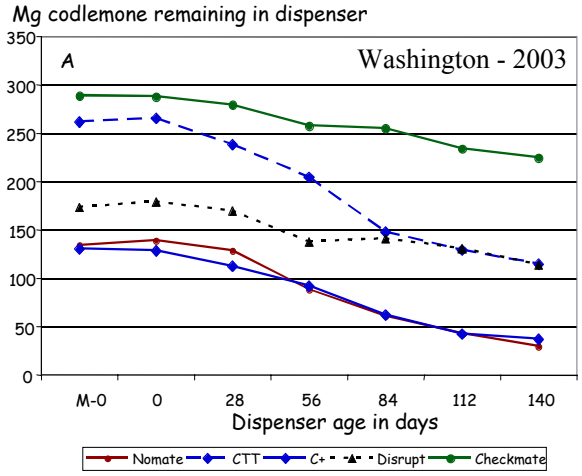
CheckMate CM XL1000: There was a very slow decline in the residual codlemone over the 140-day period in both Wenatchee and Medford (Figs. 2A and 2B). The average estimated release rate over the 140-day period in Wenatchee and Medford was 0.46 and 0.58 mg per day, respectively. This dispenser released an average of 22% (Wenatchee) and 28% (Medford) of codlemone over the duration of the test. The low level of pheromone release from this dispenser does not make it very efficient in terms of the use of the active ingredient. There was a high degree of variation of residual codlemone content in these dispensers as they aged. A similar behavior of pheromone release was noted from this dispenser in 2002.

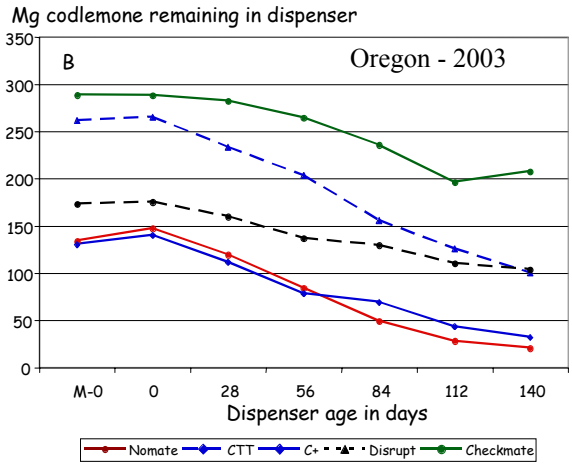
Disrupt CM: There was a very slow and erratic change in the residual codlemone over the 140-day period in Wenatchee and Medford (Figs. 2A and 2B). The average estimated release rate over the 140-day period in Wenatchee and Medford was 0.42 and 0.50 mg per day, respectively. This dispenser released an average of only 36% (Wenatchee) and 41% (Medford) of codlemone over the duration of the test. There was a high degree of variation from dispenser to dispenser in the residual codlemone as they aged. This dispenser is not very efficient in releasing pheromone over the season.

Results – Volatile Trapping:

It is possible, as shown above, to estimate a release rate of codlemone per day from residual data. That estimate of release rate is calculated by taking the difference in residual pheromone at the beginning and end of the study and dividing by the number of days. That provides an average release rate estimate over the entire period where conditions

Fig. 2. Residual data are from analyses of hand-applied pheromone dispensers in 2003 from Washington (A) and Oregon (B). Data show the average mg of codlemone per dispenser, based on a 5-dispenser sample, collected at various ages. 'M-0' is the analysis of dispensers upon arrival from the manufacturers, '0' is the sample of dispensers at the time they were initially placed in the field and the other samples are from dispensers aged the number of days shown.





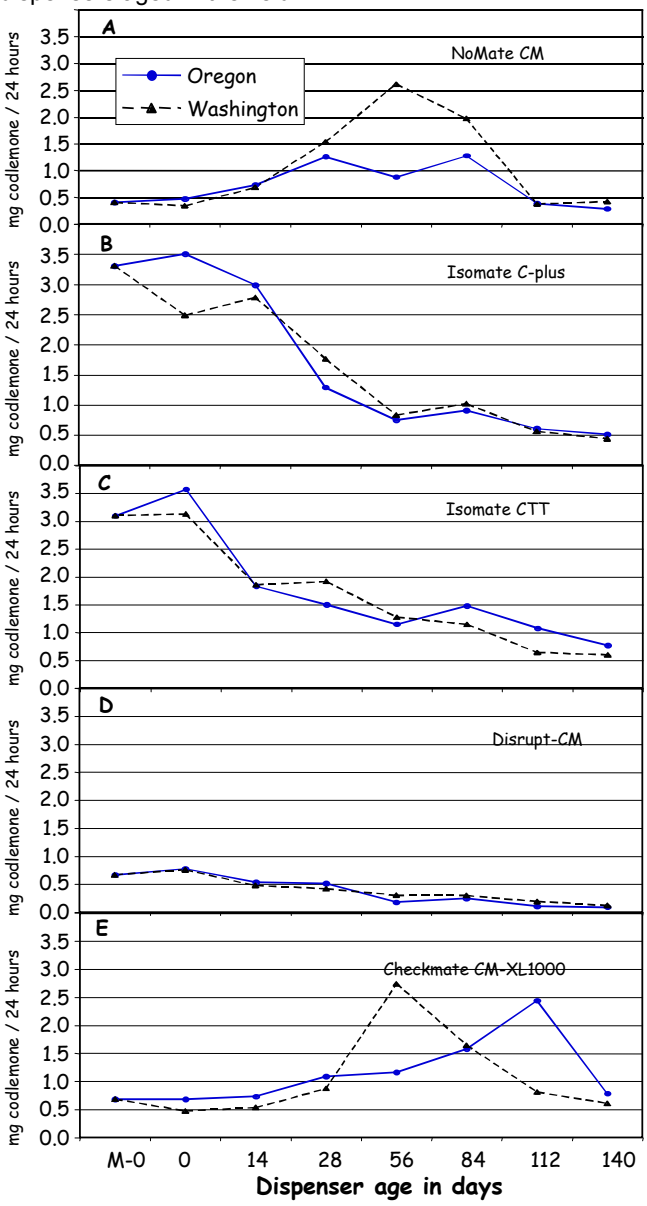
would vary a great deal. The VTS method provides an estimate of the codlemone release behavior of a particular dispenser of known age under constant conditions (room temperature, uniform air flow rate and humidity). Data collected by the VTS method are presented for each dispenser type in Figs. 3A-E.

NoMate CM: The release rate of codlemone was about 0.5 mg/day in the new dispensers and the rate increased with age of the dispenser in both locations, but especially in Wenatchee, where the release rates were between 1.0 and 2.5 mg/day for much of the season. The release rates on the last two sample dates declined back to the levels of the new dispensers. It is possible that the migration of pheromone within the dispenser to the surface was accelerated by the hot temperatures of mid- and late summer and as the dispenser load was diminished the rate of release slowed, but it was still at levels that should have been adequate to provide mating disruption of codling moth. The release rates of the NoMate dispensers from both locations were similar with the exception of the very high release rate in Wenatchee from the day 56 dispensers.

Isomate-C Plus: The release rate of codlemone was high from the M-0 and 0-day dispensers, varying from 3.0-3.5 mg/day. There was a slight decline in the release rate from the 14-day dispensers, but the rate was still high at almost 3.0 mg codlemone/day. After this initial high release rate, the release rates declined from about 1.5 mg codlemone/day for the 28-day dispensers to between 1.0 and 0.5 mg codlemone/day for the remainder of the test. It would appear that the Isomate-C Plus dispenser has a large amount of pheromone ready to volatilize when it is initially placed in the field. However, after the initial burst of pheromone, the rate of codlemone release declines to a lower but steady rate.

Isomate CTT: The release rate of codlemone was very similar to that of the Isomate-C Plus dispenser, that is, high from the M-0 and 0-day dispensers, varying from 3.0-3.5 mg codlemone/day. There was a slight decline in the release rate from the 14-day dispensers to about 2.0 mg codlemone/day. After this initial high release rate, the release rates declined from about 2.0 mg codlemone/day for the 28-day dispensers to between 1.5 and 0.5 mg codlemone/day for the remainder of the test. The release rates from older dispensers were higher for the Isomate CTT than Isomate-C Plus, which would be expected given their design, that is, to use fewer per acre because of a higher release rate. Like the Isomate-C Plus dispenser, the Isomate CTT dispenser evidently

Fig. 3. Average mg of codlemone released per dispenser in 24-hours (estimated) using the VTS method on different dispensers aged in the field.



has a large amount of pheromone ready to volatilize when placed in the field, and after a time the rate of codlemone release declines to a lower but steady rate.

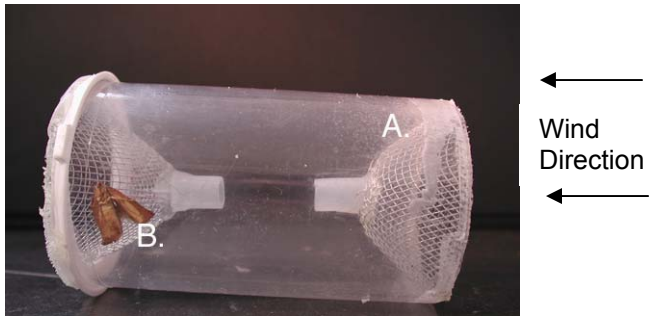
Disrupt CM: The release rate of codlemone from the Disrupt CM dispenser was low from the beginning of the study and declined slightly as the age of dispensers increased. New dispensers released between 0.5 and 1.0 mg of codlemone/day. The release rate of day-14 and day-28 dispensers was about 0.5 mg codlemone/day, but thereafter the release rate declined until it was between 0.1 and 0.2 mg codlemone/day. It is doubtful that under normal pressure from codling moth a dispenser releasing this amount of pheromone, especially one recommending the use of 200 dispensers per acre, would provide adequate control. The pheromone release behavior of Disrupt CM revealed by the VTS method helps explain the results from the residual study. The dispenser, especially the older dispensers, is just not releasing pheromone.

Checkmate CM-XL1000: The release rate of codlemone from the Checkmate CM dispenser was 0.5 and 1.0 mg of codlemone/day from new and day-14 dispensers, respectively. The rate of codlemone release increased by day-28 dispensers and then varied dramatically, though in different patterns by location, from day-56 through day-112 dispensers. Dates with the highest average release rates also had very high variability between dispensers, indicating that some dispensers were releasing at a very high rate while others were not.

The VTS method has the advantage of being a non-destructive method of assessing pheromone release rate from a device. It also could have the advantage of being able to measure the release rate of pheromone from different dispensers under different conditions, especially air flow and temperatures. With newer analytical equipment, it might be faster to evaluate the behavior of hand-applied dispensers using VTS, as the turn-around time of sampling could be short and possibly the cost of analysis greatly reduced.

Objective 2. The virgin female traps used to determine the delay in mating were inefficient at male capture and during the first generation we had numerous females escape, especially codling moth. During the second generation our modifications to the traps virtually eliminated the escapes, but we were unable to capture large numbers of males in either control or treated areas. During the latter part of the second generation, observations suggested that the problem was related to the position within the mating tube where the females were calling. If the females called from the upwind side of the trap (Fig. 4 – position "A"), males would enter and mate with the females. If they called from the downwind side and moved towards the edge of the funnel (Fig. 4 – position "B"), males would tend to try to mate through the screen of the funnel and not enter the cage. We are still working on this, but late in the season we simply placed the virgin female trap within a normal delta trap complete with sticky liner. This allowed us to check if the males would find the females, although actual mating was not being measured unless the males entered the trap. However, it did tell us that the males can locate the females, and placing large numbers of these traps in the orchard and tracking when exactly the first male moth was caught in the trap, compared to the age of the virgin females, allowed us to determine the delay of mating in the field. We will pursue this using funding from the RAMP/IFAFS grant this year.

Fig. 4. Effect of female calling position on trap catch using the virgin female mating tube.



We have not finished the dose-response information for codling moth or LR, in part because of the problems associated with the virgin female trap. However, we have performed lab and field studies to determine the effect of MD on mating and the density of moths in different size cages on mating success under both MD and non-MD conditions.

In the lab wind tunnels, trials were completed for OBLR and CM at male densities of 3 males, 2 males and 1 male (Table 1). With OBLR, there was a reduction in the percentage mating when 3 males were present compared to having either 1 or 2 males. The time for at least one of the males to find the female was greatest when only one male was present, followed by having 3 males present, and 2 males present had the shortest time. When the pheromone source (nine 10x lures spaced evenly at the top of the cage) was added to the cages to simulate MD, no matings were observed on the video in any of the trials (the tape only lasts 90 minutes). However, spermatophores were found in all the different male densities (Table 1). The pheromone source did not inhibit mating, as higher percentages of females mated with 3 males with the pheromone source present than without, but it did increase the time required by males to locate calling females.

In the lab experiments with CM, mating success decreased with increasing male density. One of the females in this trial had been mated by more than one male (double spermatophore). The time required for a female to be located was shortest for 2 males, intermediate when 3 males were present and greatest when 1 male was present (Table 1). These results suggest that although it takes longer for single males to locate females (52 minutes on average), mating success is higher at lower male densities. When we added the pheromone source to the wind tunnels no CM females were observed mating, and none acquired spermatophores overnight.

In the field cages, we also evaluated mating success of CM and OBLR at the same male densities of 3, 2 and 1 male per female. Of the OBLR females we evaluated, 89% exhibited calling behavior. With 3 males per female, 8% of calling females were mated within 4 nights. With 2 males, 12% were mated within 4 nights, and with 1 male 13% were mated within 4 nights. These results suggest that mating success increased as the male-to-female ratio was decreased. Most matings occurred on either the first or second night when 3 males were present, but some matings occurred on night 3 when 1 or 2 males were present, suggesting that it took slightly longer for females to be mated with fewer males. With the Isomate dispensers in place, the proportion of mating OBLR females increased over the control value with 3 males in the cage. This result parallels the findings of the lab wind tunnels.

Table 1. Percentage mating and time required in laboratory wind tunnels.

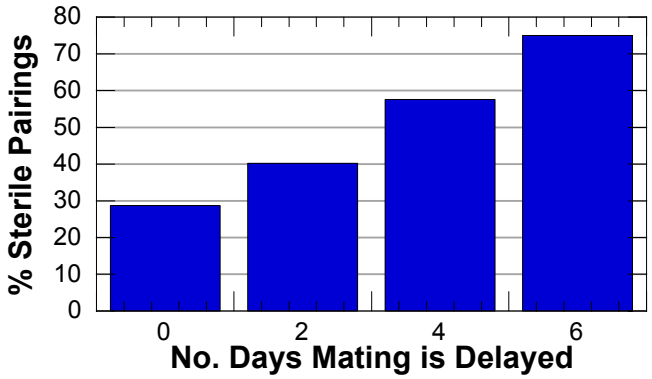
No. Males in Tunnel	% Calling Females that Mated	Average Time (min) for Males to Locate Female	Treatment	Moth Species
3	8	35	Non-MD	OBLR
2	18	28	Non-MD	OBLR
1	13	>90	Non-MD	OBLR
3	15	>90	MD	OBLR
2	6	> 90	MD	OBLR
1	16	>90	MD	OBLR
3	30	17	Non-MD	CM
2	28	13	Non-MD	CM
1	46	52	Non-MD	CM
3	0	–	MD	CM
2	0	–	MD	CM
1	0	–	MD	CM

More data will be needed to evaluate mating success in the presence of MD with the other male densities.

An average of 81% of the CM females in the cages called. With 3 males present, 10% of calling female CM mated, with 2 males 8% mated, and with 1 male 13% of female CM mated overnight. Mating tended to occur within the first 3 nights with each male density. When the Isomate dispensers were applied to emulate a MD treatment no mating was observed.

Is summary, the results of the lab and field components of these experiments suggest that lower male densities increase mating success for CM and OBLR when no competing pheromone sources are present. CM MD technology proved more efficient for mating suppression than OBLR MD technology. In both the lab and field experiments the application of pheromone resulted in complete mating suppression for CM with the different male densities. Although more data are needed, our results to date suggest that OBLR mating success may increase under MD at higher male densities.

Fig. 5. Effect of increasing delay in mating of female OBLR on sterility of the mating pairs



Objective 3.
OBLR and CM analysis are nearly complete. We have not been able to perform any of the work with PLR because our colony has production problems associated with disease. OBLR data will be complete by the time of the research review, while the codling moth data will be complete by mid-February.

For both CM and OBLR, we segregated the pupae by sex and placed them in separate cages. Individual female pupae were placed in separate cages with honey and water, and all individuals emerging on a given date were randomly assigned to one of four treatment groups (0, 2, 4 or 6 days delay before pairing). When females reached the desired age they were paired with 1- to 2-day old virgin males. Female egg production and mortality were measured daily, and life tables were used for analysis to see the effect of delayed mating on population growth.

Results OBLR:
A rough analysis of the data immediately showed that the effect of delayed mating on OBLR was composed of two factors, 1) an increase in the percentage of pairs that are sterile with increasing age of the female (Fig. 5) and 2) a reduction in total egg production, unrelated to the first factor. Using all the data in the analysis, we found that the net reproductive rate dropped dramatically as the delay in mating increased, and the effect was still considerable even when the sterile pairings were removed and the life table recomputed (Table 2).

We also have some preliminary data on OBLR mating where the males are aged 4 days (instead of being 1-2 days old like the other studies). While the data require more replication that should be completed by the research review, the current information suggests that pairing a 4-day old virgin female with a 4-day old virgin male causes a drop in the net fertility rate of nearly 50% compared to using a 4-day old virgin female and a 1- to 2-day old virgin male.

Table 2. Effect of delayed mating on reproduction of OBLR

All Pairs	Number of Days Mating was Delayed				Percent of Control Value		
Population Parameter	**0**	**2**	**4**	**6**	**2**	**4**	**6**
Net fertility rate	169.4	116.3	62.5	17.7	69	37	10
fertile eggs/female/day	17.5	12.2	6.8	1.7	70	39	10
Mean age net fertility	42.8	44.2	46.2	48.2	103.4	108.0	112.7
Mean Age Hatch	45.5	44.0	52.0	58.8	96.6	114.2	129.1
Net Reproductive Rate	84.7	58.2	31.2	8.8	68.7	36.9	10.4
Population Double Time	6.7	7.6	9.3	15.4	112.9	139.3	229.6
Generation Time	42.8	44.3	46.3	48.3	103.4	108.0	112.7
No Sterile Pairs							
Net fertility rate	236.3	94.5	63.8	59.5	40.0	27.0	25.2
fertile eggs/female/day	23.8	9.6	6.0	5.4	40.5	25.1	22.6
Mean age net fertility	42.8	44.3	46.3	47.6	103.6	108.2	111.3
Mean Age Hatch	52.5	44.0	52.0	51.0	83.7	99.0	97.1
Net Reproductive Rate	118.2	47.3	31.9	29.7	40.0	27.0	25.2
Population Double Time	6.2	8.0	9.3	9.7	128.3	149.2	156.6
Generation Time	42.8	44.4	46.4	47.7	103.7	108.3	111.3

Results CM:

Codling moth had a different response to delayed mating than OBLR. With OBLR, the increase in delay of mating reduced all the population growth parameters rather dramatically. With codling moth, we found that the percentage of sterile pairs did not rise with the increase in mating delay (Fig. 6). This data set is rather small compared to the OBLR data set, so we are running more replicates to see if this trend holds up. Secondly, the data show that there is little difference between the 0 and 2-day delay in terms of population growth but a large difference between 2 and 4 days and little difference between delays of 4 and 6 days (Fig. 7). When considering all pairs (even sterile ones), the 0 day delay had a slightly higher net fertility rate, but when the sterile pairs were dropped the trend was reversed. However, with increasing delay in mating the mean age of net fertility, mean hatch rate and generation times all increased.

Overall:

The laboratory survival of adult females is far in excess of the likely longevity of females in the

Fig. 6. Effect of increasing delay in mating of female CM on sterility of the mating pairs

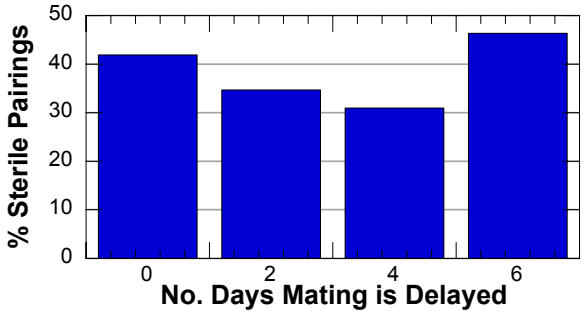
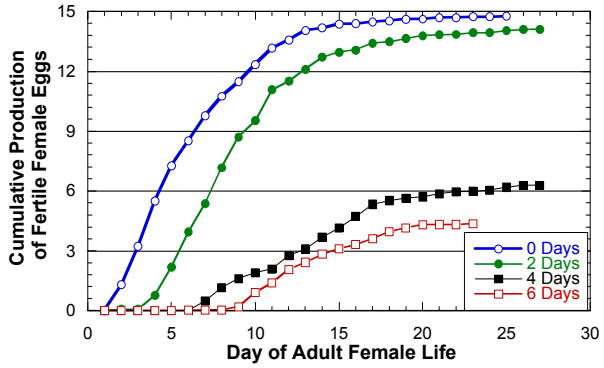


Fig. 7. Effect of delayed mating on codling moth production of fertile female eggs



wild. For example, our data from the mating tubes show that ≈ 78% of CM females die by 8 days. Thus, graphs like Fig. 7 can be used to help determine the difference in reproductive rate. For example, if codling moth females rarely live past 8 days of age, it becomes obvious that when females experience a delay longer than 4 days it is immaterial to population growth and that females experiencing no delay have about a 50% increase in reproduction compared to those experiencing a 2-day delay.

The other major difference between OBLR and CM is the extreme difference in reproductive rate. For example, the net fertility rate of OBLR for females mating on the date of emergence is nearly 4x that of codling moth females experiencing no delay. While the declines in reproduction are larger and more impressive for OBLR, the huge difference in initial reproductive rate makes it more difficult to control than CM if all other mortality factors are equal.

Objective 4. Although we located and set up the mating tubes in orchards with high densities of both codling moth and OBLR, the mating tubes were inefficient and worked primarily in the control areas. Although both CM and OBLR were caught in both areas, the numbers caught in the MD areas were too low to use to determine the delay in mating. We will continue to refine the mating tube and hope to use it to determine delays but will pursue this under our RAMP/IFAFS funding. For more information on the problems see Objective 2, first paragraph.

Budget:

Project title: Examining the mechanisms of mating disruption in codling moth and leafrollers

PI: Vincent P. Jones

Project duration: 2 years

Project total: $98,130

Category	**Year 1 (2002)**	**Year 2 (2003)**
Salaries (Associate in Research)[1]	$17,500	$18,200
Benefits (30%)	5,250	5,460
Wages	11,000	11,000
Benefits (16%)	1,760	1,760
Equipment	0	0
Materials and supplies[2]	1,900	1,900
Within-state travel[3]	1,200	1,200
Chemical analysis[4]	10,000	10,000
Total	$48,610	$49,520

[1] Associated with this project alone, half-time support from this project.
[2] Supplies included rearing supplies, telecom charges, traps, miscellaneous lab and field supplies.
[3] Included rental of a vehicle for this project for 3 months, gas, and upkeep.
[4] This funding enabled us to expand the RAMP/IFAFS proposal to include the role of dispenser technology. This funding was needed for the chemical analysis and not to fund any new equipment. Cost per sample was roughly $100.

Note: This funding was matched 1:1 by the AWII RAMP/IFAFS funding.

FINAL REPORT
WTFRC PROJECT # AE-03-333A

TITLE:	Assessing the Chemical Release Behavior of Mating Disruption Products in Orchard Air
PRINCIPAL INVESTIGATOR:	Vincent R. Hebert, Residue Laboratory Director, Food and Environmental Quality Laboratory, WSU TriCities
COLLABORATORS:	Vincent P. Jones, Associate Entomologist, Jay F. Brunner, Entomologist, Mike Doeer, Research Scientist WSU-TFREC

Mating disruption has become an important integrated pest management tool for controlling codling moth injury in apple and pear orchards in the Pacific Northwest. The mating can be interrupted, or at least delayed, when there is a sufficient amount of pheromone present in the orchard air interfering with the male's capability to locate females. Unfortunately, in certain areas suppression in mating through chemical disruption appears to have diminished and may be attributable to the declining seasonal use of organophosphorus insecticides that complement mating disruption (Jones, 2002). Additionally, commercially available hand-held dispensers and sprayable formulations may not provide for efficacious pheromone release (i.e., either too much pheromone released too soon or too little release over extended time intervals) leading to insufficient season-long suppression of moth mating (Brunner, 2002a). Although the number of sprayable applications and/or number of hand-applied dispensers per acre for season-long control is being studied (Brunner, 2002b), the chemical concentration in orchard air that results in mating disruption has not been thoroughly investigated. Indirect techniques such as 1) gravimetric measurement 2) volatile trapping of vapors under laboratory chamber conditions, or 3) residual analysis of field-aged dispensers provide some notion of pheromone release but can not be interpreted as useful information on air concentration. Direct measurements of ambient pheromone concentrations in orchard air will be critically important for comparing different formulations and systems (sprayable or hand-applied) of pheromone application. The ability to measure pheromone concentration in the ambient air can provide a mechanism to more directly relate pheromone delivery system release to pest activity and crop injury.

2003 PROPOSAL OBJECTIVES:

1. Develop sensitive analytical approaches for directly measuring pheromone release from commercially available codling moth mating disruption systems
2. Measure codlemone [(*trans,trans*)8,10 Dodecadien-1-ol] concentrations in orchard air at Isomate C+ dispenser application rates of 100, 200, 400, and 800 dispensers/acre
3. Provide air concentration data that can be used in combination with dispenser release data for assessing the performance and efficacy of mating disruption products.

SIGNIFICANT FINDINGS:

- Capability to detect codlemone to the mid-picogram (trillionth of a gram) per cubic meter of air after high volume air sampling with gas chromatographic (GC) mass spectroscopy
- Codlemone found to oxidize readily under atmospheric conditions but was stable when trapped on the air-sampling medium
- Demonstrated in the orchard environment that airborne codlemone concentrations could be measured at application rates as low as 100 dispensers/Acre
- We observed sequentially higher codlemone concentrations in the orchard air environment after 100, 200, and 400 dispenser/A applications

- The highest rate of application (800 dispenser/A) did not result in increased codlemone concentrations in the orchard air
- High air temperatures in late July 2003 appeared to have a significant influence on chemical release leading to higher ambient orchard concentrations, more so than increasing application rates
- Chemical breakthrough lowered PUF trapping efficiency especially on hot days; therefore the generated air data should be viewed as lower bound estimates.

MATERIALS AND METHODS

Objective 1:

Instrument sensitivity: To quantify codlemone residues in air, original air sampling, extraction and instrumentational methods had to be developed for detecting this pheromone to the mid-to-high pigogram per cubic meter range. We first evaluated the capability of our recently acquired GC/MS in detecting and quantifying codlemone at very low concentrations. This instrument was purchased with specialized accessories that could increase the sensitivity for substances like codlemone.

Trapping efficiency: A series of experiments first were then performed to identify an efficient trapping adsorbent that could remove codlemone from air at very high (> 150 L/min) air-sampling rates. We tested two adsorbents XAD (a polystyrene resin) and PUF (a polyurethane foam).

Codlemone reactivity: Before attempting orchard evaluations, we conducted a series of specialized laboratory experiments at the University of Nevada to measure the rate of loss of codlemone under sunlight conditions and in air. These experiments were essential for understanding the atmospheric environmental fate of this highly reactive substance.

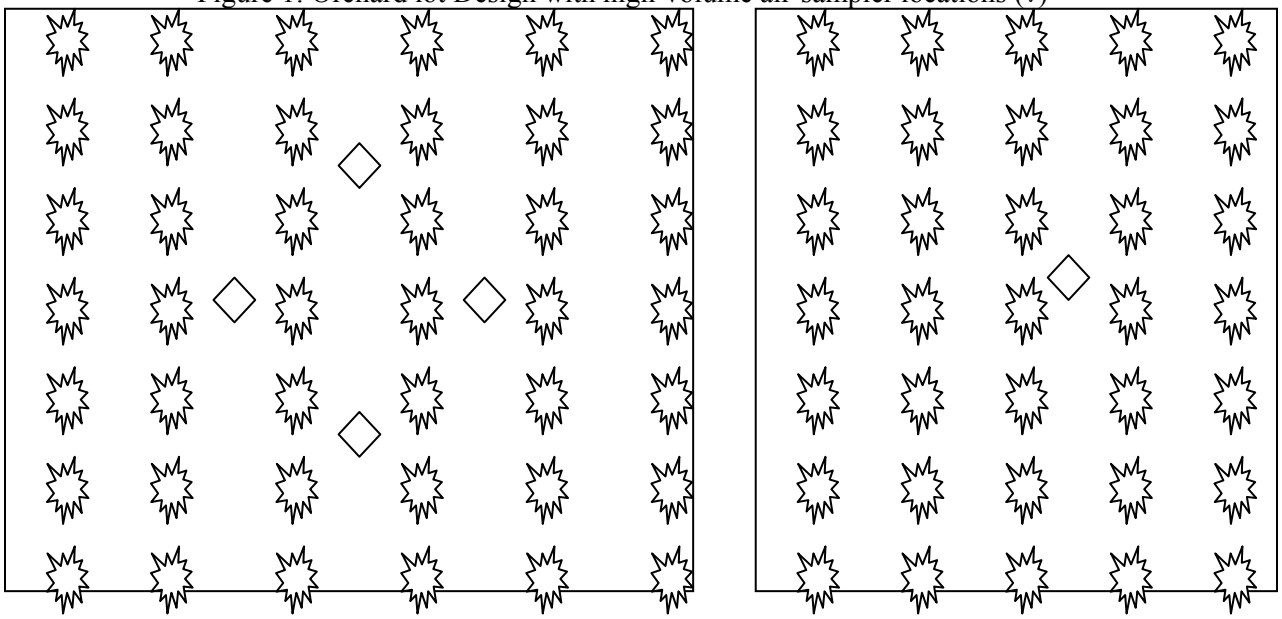
Analytical Method: The following analytical method was developed and validated before orchard air sampling:

1. Place the exposed PUF sample into a hot solvent extractor for a period of 3-hours using hexane as the extraction solvent
2. Reduce the volume of the solvent to near dryness then bring up to final volume in hexane for GC/MS determination.
3. Add the internal standard (50 μg/ml myristic acid methyl ester solution in hexane) to the final volume of the sample.
4. Analyze the extract by GC/MS using SIM (single ion monitoring mode) tuned to ions m/z 68, 81, 182 for codlemone and ions m/z 87, 143, 242 for myristic acid methyl ester.
5. For each set of samples ran on the GC/MS, at least one spiked sample was prepared by injecting a known volume of codlemone solution into the PUF. The spiked samples were extracted and analyzed in the same manner as the unknowns and control samples. Reagent blank, PUF blank samples were also routinely analyzed during the course of the study.

Objective 2: The following orchard air-sampling procedures were performed at the WSU TFREC orchards to measure airborne codlemone from Isomate C+ dispensers at various application rates:

Test Plot Design: A treatment (Block 18) and control orchard plot were established at the WSU TFREC. Enough physical distance separated the treatment from control plot to minimize possible cross-boundary source contamination (Figure 1). Four high volume air samplers were situated within close proximity to each other (ca. 50-75 feet) near the center of the treatment plot. One air sampler was placed near the center of the control plot.

Figure 1: Orchard lot Design with high-volume air-sampler locations (◊)



Test Plot Control Plot

Chemical Treatment Program: Four application rates were sequentially performed at ca. 1-week intervals. The first 100 dispensers per acre treatment were applied to Block 18 on 7/11/03. On 7/18/03, the second series of 100 dispensers were applied (total 200 dispensers per acre). On 7/25/03, an additional 200 dispensers were applied (400 dispensers per acre) and finally on 8/1/03 an additional 400 dispensers were added to Block 18 (total 800 dispensers per acre). All dispensers used in this orchard program were aged outdoors for 1-week prior to application to minimize the pulse release effect that is usually observed for Isomate dispensers.

Air Sampling: High volume Anderson air samplers were employed to extract codlemone from orchard air. Four replicate samplers were positioned at ground level in the orchard test plot and 1 sampler in the control orchard plot (Figure 1). After each weekly dispenser application, air samples were collected three times, usually at daily intervals, before the next dispenser application was performed.

For each air-sampling event, a polyurethane foam (PUF) cartridge was inserted into the air sampler and calibrated by TFREC personnel according to standard methods. The high-volume air samplers ran for a period of ca. 5-6 hours at an average air extraction rate of ca. 140-L min^{-1} (i.e., average air mass sampled ≈ ca. 46 m^3). The exposed PUF cartridges will be immediately placed in cold storage and subsequently delivered to WSU's Food and Environmental Quality Laboratory for codlemone determination.

Field Data Book: A field data book was constructed that provides the information needed to document and construct all phases of field sampling, cold storage, shipment, and chain of custody.

Meteorological Information: Meteorological data was collected over the experimental time frame at the WSU, TFREC weather station.

Chemical Analyses: All analyses were performed according to the methods listed in Objective 1.

RESULTS AND DISCUSSION:

Objective 1:

Instrument sensitivity: Our preliminary GC/MS findings were highly encouraging. We could reliably quantify pigrogram (part per trillion) range concentrations of codlemone. In other words, the instrument could be set up in a sensitive and selective mode to pull the needle out of a haystack.

Trapping efficiency: The next question to resolve was; could a trapping medium effectively pull codlemone out of the air when using our air-sampling equipment? In our evaluations, the XAD polystyrene adsorbent provided lower recoveries (ca 65%) than PUF (ca 95%). As a result, we then chose to evaluate the breakthrough capability of PUF to retain codlemone in the adsorbent over a 3-hour period at a high volume air-sampling rate of over 200-L/min. The recovery of codlemone spiked onto the PUF was quantitative (ca 105%) over this air-sampling interval thus demonstrating the capability for this adsorbent to both efficiently remove codlemone from the air and retain this substance on the trap over extended air-sampling intervals.

Codlemone reactivity: How fast does codlemone photoreact or oxidize in air after it is released from the dispenser? We found in our investigations at the University of Nevada that airborne codlemone was surprisingly stable under atmospheric sunlight conditions but oxidized rapidly (half-life ≈ 0.5 hours) in purified filtered air. From a chemical ecology perspective, fast reactivity is highly desirable, but the fast air oxidation triggered the need to examine its behavior when trapped on PUF. Experiments were then conducted at the FEQL to compare recoveries of codlemone when using a non-oxidizing inert gas (nitrogen) and when using air. This series of experiments showed that there was no appreciable difference in recovery. In other words, codlemone should not further oxidize when trapped on the PUF over the orchard air-sampling time frame.

Analytical Method: Preliminary field air-sampling studies conducted at the FEQL demonstrated that detectable residues could be observed at concentrations equivalent to ca 200 picograms per cubic meter of air. Conservatively, we set our limit for reliably quantitating codlemone in air at 1 nanogram (1 part per billion mass) per cubic meter or air. With this level of sensitivity, we anticipated that we should be able to detect/quantify airborne release of codlemone from hand-applied dispensers in the orchard environment at rates as low as 100 dispensers per acre.

Objective 2: Table 1 and Figure 2 presents the results from the Block 18 dispenser evaluations.

Orchard Data Summary:

100 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 5, and 7 days following the first 100-dispenser/A application showed fairly consistent codlemone air concentration behavior ranging from 1 to 1.5 nanograms per cubic meter of air. Of the 12 sampling observations, there was one replicate sample failure. The averaged mid-day air temperature for these three sampling events was 29.4 $\pm$ 2 C.

200 dispenser codlemone air data: Results from the averaged three air sampling dates taken 4, 5, and 6 days following the second 100-dispenser/A application showed less consistent codlemone air concentration behavior that ranged from 1.1 to 2.3 nanograms per cubic meter of air. Of the 12 sampling observations, there was also one replicate sample failure. The averaged mid-day air temperature for these three sampling events was 36.1 $\pm$ 2 C.

400 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 4, and 5 days following the third application by inserting 200 dispensers/A showed reasonably consistent codlemone air concentration behavior ranging from 6.7 to 8.7 nanograms per cubic meter of air. Of the 12 sampling observations, there no replicate sample failures. The averaged mid-day air temperature for these three sampling events was 38.3 $\pm$ 0.6 C.

800 dispenser codlemone air data: Results from the averaged three air sampling dates taken 3, 4, and 11 days following the third application by inserting an additional 400 dispensers/A showed somewhat variable codlemone air concentration behavior ranging from 3.8 to 4.9 nanograms per cubic meter of air. Of the 12 sampling observations, there no replicate sample failures. The averaged mid-day air

temperature for these three sampling events was 29.6 ± 1.4 C, much cooler than the 400 dispenser air sampling period.

There was not a direct linear relationship between increased numbers of dispensers and measured airborne codlemone, although sequentially higher air concentrations in the orchard air environment were observed after 100, 200, and 400 dispenser/A applications. The highest rate of application (800 dispenser/A) did not result in increased codlemone concentrations in the orchard air. This was not a surprising observation since the very high late July air temperatures likely had a significant influence on chemical release on the 400 dispenser/acre application rate leading to very high ambient orchard concentrations of codlemone. Although the analytical method proved sensitive and reliable (laboratory spikes average recoveries 91 ± 8% ; n = 12), we did experience chemical breakthrough in the air samples, especially on the hotter days in July. Field spike recoveries ran near the end of the field study were ca. 40% of expected. As a result, the collected air data cannot be entirely viewed as absolutely quantitative due to less than desirable trapping efficiency on hot days. The generated air codlemone data, however, should be viewed as a lower bound residue estimate of actual orchard air concentrations at the various application rates.

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- Jones, W. 2002. *Pest Management Practices Survey 2000 Report*. WSU Tree Fruit Research and Extension Center. Wenatchee, WA. http://opus.tfrec.wsu.edu/~wjones/Survey2000/.

BUDGET:

Project title:	**Chemical Release Behavior of Mating Disruption Products**
PI:	**Vince Hebert**
Project duration:	**2003**

Salaries		
Ag Res Tech I		
(This will be a new half time position)		
April – September 2003		**$6,795**
Wages		
Time slip @ $10/hour, 800 hours		**$8,000**
Benefits		
Ag Res Tech I @ 57%	3873	
Time Slip @ 16%	1,280	
	========	
		$5,153
Goods & Services		
High volume air samplers, 3 @ $2300		**$14,900**
Solvents, gases, glassware, reagents		
Travel		
12 trips to Wenatchee @ 240 miles R/T		**$994**
TOTAL		**$35,842**

Table 1: Codlemone measured concentrations in orchard air

Air Sample ID	Date of Sampling	Averaged Codlemone Concentration (ng/m^3 ± SD)*	Mid-day air temperature (C)****
100 dispensers/acre			
07-14-03 T1 to T4	07-14-03	1.1 ± 0.20	28
07-16-03 T1 to T4**	07-16-03	1.5 ± 0.20	29
07-17-03 T1 to T4	07-17-03	1.1 ± 0.09	31
200 dispensers/acre			
07-22-03 T2 to T4	07-22-03	2.3 ± 0.75	38
07-23-03 T2 to T4	07-23-03	2.0 ± 0.45	36
07-24-03 T1 to T4***	07-24-03	1.1 ± 0.22	34
400 dispensers/acre			
07-28-03 T1 to T4	07-28-03	7.7 ± 2.0	38
07-29-03 T1 to T4	07-29-03	8.8 ± 1.4	38
07-30-03 T1 to T4	07-30-03	6.8 ± 2.6	39
800 dispensers/acre			
08-04-03 T1 to T4	08-04-03	3.8 ± 2.0	29
08-05-03 T1 to T4	08-05-03	4.9 ± 1.4	31
08-12-03 T1 to T4	08-12-03	4.7 ± 0.6	28

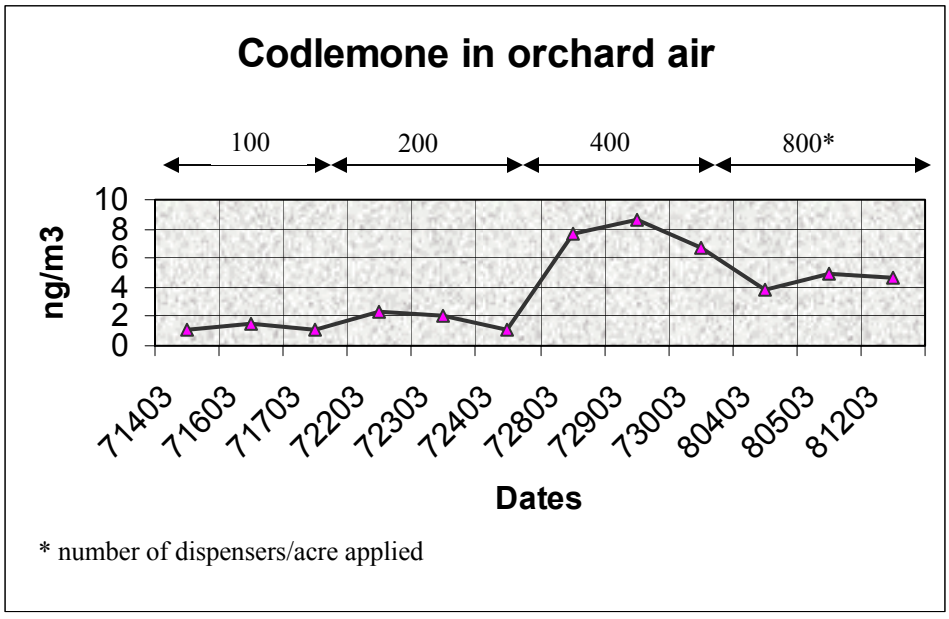
* The average concentration and standard deviation taken from 4 replicate air samples on that date

** Sampler 07-16-03 T1 failed and was not included in the averaged air concentration

*** Sampler 07-24-03-T3 failed and was not included in the averaged air concentration

**** Temperature reading at 2 pm on each of the air sampling dates

Figure 2: Codlemone measured air concentrations



FINAL REPORT
WTFRC Project # AE-03-332 **WSU Project #13C-3643-6385**

Project title:	Survey of codling moth organophosphate resistance and associated tolerances to other pesticides
PI:	John E. Dunley, Associate Entomologist
Organization:	WSU Tree Fruit Research and Extension Center
Co-PIs and affiliations:	Alan L. Knight, Research Entomologist, USDA-ARS, Wapato, WA; Bruce Greenfield, Agricultural Research Technologist III, WSU-TFREC, Wenatchee, WA

Objectives:
1. Survey codling moth populations throughout the state for levels of azinphosmethyl resistance.
2. Determine levels of cross-resistance to select insecticides.
3. Develop an adult bioassay for the chloronicotinyl insecticides and survey levels of tolerance around the state.

Significant findings:

1. Levels of azinphosmethyl (Guthion) resistance were low to moderate in codling moth populations throughout the state.
2. Populations high enough to screen against multiple chemistries had only low levels of resistance, preventing meaningful cross-resistance data for pyrethroids or chlorpyrifos (Lorsban).
3. A population with high azinphosmethyl resistance was identified near Manson during second-generation flight.
4. There is potential cross-resistance with acetamiprid (Assail), although the data are too variable to be conclusive.

Methods:

Levels of azinphosmethyl resistance in codling moth populations were sampled using standard bioassay techniques (Riedl et al. 1986). Adult males were collected using pheromone trap liners evenly coated with 1 ml of Tanglefoot®, loaded with a pheromone source (rubber septum permeated with 1 mg or 10 mg codlemone, depending on the presence of pheromone mating confusion), and placed in the upper third of the orchard canopy to maximize trap catch. Only male moths were collected, as the sex pheromone used in the traps only attracts male moths. During first and second codling moth flights, near the peak flight, between 200 and 400 traps were placed in an orchard prior to dusk and collected the following day at dawn. Traps containing moths were transported to the lab and immediately bioassayed using the topical application bioassay method (1 μg insecticide concentration per moth). Moths that oriented ventral side down in the traps were either repositioned dorsally or removed. Moths that did not respond with vigorous leg movements following the application of the pesticide were removed from the experiment.

Codling moth populations were sampled from 16 orchards from the major growing areas in the state. As time and resources permitted, orchards that experienced problems in past years and those experiencing unusually high trap catch (>20 moths per trap per week) were identified and sampled during the first flight. Some of those orchards were revisited during second flight, and newly identified orchards were also sampled.

Population responses were compared by probit analysis. Probit regression lines were estimated using the probit option of POLO (POLO 1987). Likelihood ratio tests were used to test hypotheses of equality and parallelism in response lines. Lethal concentration ratios were calculated according to Robertson and Priesler (1992); ratios with confidence limits (CL) not encompassing 1.0 will be considered significantly different ($\alpha = 0.05$).

Results and discussion:

Unpredictable and unfavorable weather made sampling difficult in 2003, particularly because of extremely cold weather during the first flight. This limited the number of successful bioassays, and many trapping efforts (approximately 40%) did not yield enough moths for bioassay.

Despite some extremely high trap catches (both from trap counts provided by referring fieldmen, and from bioassay trap yield), all orchards sampled during the first flight were Guthion susceptible (Table 1). Thus, Lorsban (Table 3) and esfenvalerate (Asana) (Table 4) were tested only in orchards with susceptible populations, and data regarding cross-resistance were not found. Assail bioassays were conducted against Guthion-susceptible populations during the first flight. However, during the second flight, a Guthion-resistant population was identified in Manson. This allowed testing of Assail to determine potential levels of cross-resistance. Fenpropathrin (Danitol) and thiacloprid (Calypso) were not tested against any populations (trap nights were limited in the Guthion-resistant orchard, as the grower treated immediately after samples were collected).

Table 1. Bioassay results for Guthion tested against first flight (overwintering flight) of codling moth populations in Washington State, 2003. No statistically significant differences were found among populations.

Location	LC50 (μg ai/moth)	Resistance ratio
Prosser	0.09[1]	1.1[1]
Grandview	0.13	1.6
S. Wapato	0.26[1]	3.3[1]
Wapato	0.08	--
N. Wapato	0.20	2.5
Yakima	0.17	2.1
Quincy	0.25[1]	3.1
WSU-TFREC	0.12	1.5
East Wenatchee	0.23[1]	2.9[1]
Peshastin	0.15[1]	1.6[1]

[1] Estimates only; data do not fit probit model adequately for statistical analyses.

Table 2. Bioassay results for Guthion tested against second flight (summer flight) of codling moth populations in Washington State, 2003.

Location	LC50 (µg ai/moth)	Resistance ratio
Prosser	0.13	1.2
Grandview	0.16	1.5
S. Wapato	0.22[1]	na[1]
Wapato-1	0.15	1.4
Wapato-2	0.2[1]	na[1]
N. Wapato	0.25[1]	na[1]
Quincy	0.24	3.0
WSU-TFREC	0.11	--
East Wenatchee	0.21[1]	1.9[1]
Peshastin	0.13	1.6
Manson-1	0.54*	4.9*
Manson-2	0.31	2.8

[1] Estimates only; data do not fit probit model adequately for statistical analyses.
*Significantly different from the susceptible population (WSU-TFREC).

Table 3. Bioassay results for Lorsban tested against select codling moth populations in Washington State, 2003. No statistically significant differences were found among populations.

Location	LC50 (µg ai/moth)	Resistance ratio
Prosser	0.028	-1.8
Grandview	0.03	-1.7
WSU-TFREC	0.05	1.0

Table 4. Bioassay results for Asana tested against select codling moth populations in Washington State, 2003. No statistically significant differences were found among populations.

Location	LC50 (µg ai/moth)	Resistance ratio
Prosser	0.06	1.2
Grandview	0.03	0.6
WSU-TFREC	0.05	--

Table 5. Bioassay results for Assail tested against select codling moth populations in Washington State, 2003. No statistically significant differences were found among populations.

Location	LC50 (µg ai/moth)	Resistance ratio
Prosser	0.32[1]	na[1]
Grandview	0.11	1.0
WSU-TFREC	0.11	--
Manson-1	0.76[1]	6.9[1]
Manson-2	0.27[1]	2.5[1]

[1] Estimates only; data do not fit probit model adequately for statistical analyses.

Guthion resistance was found to be low to moderate in orchards sampled throughout the state. The lack of significant resistance in many orchards with high trap catch and unacceptably high infestation points toward operational factors as a likely cause. These factors include possibilities such as inadequate pest control measures leading to higher populations, failure to respond to higher populations with increased rates and decreased spray intervals, and low levels of resistance reducing residual activity of Guthion. Nevertheless, it appears that a rapid shift to Guthion resistance is not the cause of increased codling moth damage in many orchards.

However, identification of a significantly high Guthion-resistant population, along with observed damage following three Guthion applications in that orchard, indicates that Guthion resistance can and does occur at problematic levels in the region. The level of resistance in the Manson population is equal to some of the highest levels of resistance found in the Sacramento Delta in the 1990s. In those cases, codling moth control was marginally maintained at <2% infestation at harvest by using four Guthion applications at 3 lbs. each, with additional applications of other materials between flight peaks and prior to harvest.

The adult bioassay for Assail appears to be adequate; however, adjustments to the method should be examined in the future (such as increasing the exposure time, altering the moth holding temperature, etc.). Bioassay results for Assail were quite variable in this study. Unfortunately, the results for the Manson Guthion-resistant population were not precise enough to determine statistical confidence of cross-resistance with Assail. Thus, it cannot be concluded from this study that cross-resistance exists between the compounds. However, with the inference of cross-resistance between Guthion and Assail, additional studies should be conducted of field efficacy of Assail in Guthion-resistant orchards.

Budget:

Project title: Survey of codling moth organophosphate resistance and associated tolerances to other pesticides
PI: John E. Dunley
Project duration: 1 year
Current year: 2003
Project total (1 year): $14,550

Item	**Year 1 (2003)**
Salaries[1]	**$ 8,150**
Benefits (27%)	**2,200**
Wages[2]	**2,000**
Benefits (16%)	**320**
Supplies[3]	**380**
Travel[4]	**1,500**
Total	**$14,550**

[1] A portion of the salary for Bruce Greenfield's Ag Tech position.
[2] Time-slip wages.
[3] Supplies: pheromone traps and liners.
[4] Travel: loca
No additional l travel to research plots only.
funding supported this project in 2003.

FINAL REPORT

Project Title: Understanding Codling Moth Behavior – A Prerequisite to Effective MD

PI: Alan Knight, Research Entomologist
Organization: USDA, ARS, Wapato, WA

Objectives:

1. Examine the effect on fecundity and fertility of multiple mating by both female and male codling moth and determine the occurrence of multiple mating under field conditions.
2. Examine the mating status of CM populations in replicated orchards treated with 200-400 Isomate C+ dispensers per acre, 0.25 – 1.0 Suterra Puffers per acre, 1.6 – 4.0 Pheromone Mops per acre, 1200 drops of Last Call™ per acre, and 10.0 g A.I. sprayable codlemone per acre.
3. Examine the efficacy of dispensers emitting codlemone + isomers versus codlemone in replicated plots.

Significant findings:

- Under laboratory conditions multiple mated codling moth females laid more eggs than females that mated only once.
- The size of spermatophores passed by males decreased with subsequent matings, but no associated reduction in the number of eggs laid by females was observed.
- Under field conditions: < 25% of females were found to be virgin in orchards treated with sex pheromone dispensers compared with 5% in conventional orchards; the occurrence of multiple mate females was low in all orchards during the first generation and much lower in sex pheromone-treated orchards than conventional orchards in the second generation.
- Experienced males were responsible for nearly half of all mating that occur in one conventional orchard during both generations and in the first generation in an orchard treated with sex pheromone dispensers. Experienced males accounted for >90% of mating in the second generation in this later orchard. This suggests that a small proportion of the male population ("Super Males") may be able to locate and mate with females in orchards despite their treatment with sex pheromones.
- Using four trapping methods (DA-baited traps, interception traps, virgin female-baited traps, and virgin female-baited funnel traps) no difference in the mating success of codling moth was found in replicated plots treated with either standard sex pheromone dispensers or dispensers loaded with an equilibrium blend of codlemone isomers.
- The lowest percentage of virgin female codling moth was found in orchards treated with attract and kill but these were in high pest pressure orchards. The use of microencapsulated sprayables appeared to be ineffective in preventing mating. Increasing the dispenser density of hand-applied dispensers did not increase the low percentage of virgin moths captured.
- The most effective sex pheromone-based approaches in preventing mating were strategies using widely spaced high emission pheromone sources such as puffers and Pheromone Mops. Orchards treated with the higher rate of Pheromone Mops had the highest percentage of virgin females.

Methods:

Laboratory studies were conducted to examine the effect on fecundity and egg fertility of multiple mating by male and female codling moth. Cups were set up with one virgin female and three males. After seven days the female was dissected and after an additional seven days the number of eggs laid and hatched were counted in each cup. In the sequential male mating test males were placed in a cup with a new virgin female moth every 24 hours for ten days. After seven days each female was

dissected to determine her mating status, and after seven additional days, the number of eggs laid and hatched in the cup were counted. These data were analyzed with ANOVA.

Four orchards treated with sex pheromone dispensers and four untreated orchards were monitored with delta traps baited with DA lures. All females were dissected to determine their mating status and the number of spermatophores deposited. The sizes of all spermatophores were measured in two orchards.

Delta traps baited with the DA lure were placed in a large number of orchards under various types of sex pheromone-based programs. Each orchard was monitored with 2-10 traps at a density of 1 trap per hectare. The following pheromone treatments were evaluated: 200 and 400 Isomate C+ dispensers per acre, 100 and 200 Isomate C-tt dispensers per acre, 1.0 and 0.25 Suterra Puffers per acre, 1.6 and 4.0 Pheromone Mops per acre, 1,200 drops of Last Call™ per acre, and sprays of 10.0 g A.I. codlemone of Suterra's microencapsulated sprayable formulation. All female moths were dissected.

The sex pheromone of codling moth, codlemone, was chemically isomerized and loaded (250.0 mg) into Checkmate membrane dispensers by Suterra LLC (Bend, OR). Replicated 5-acre apple plots were treated with either 400 Checkmate 1000XL dispensers or these experimental dispensers per acre. Plots were monitored weekly with virgin female-baited and lure-baited traps. In addition, the use of interception traps and virgin female-baited funnel traps were used to assess mating under these two pheromone treatments. Fruit injury was not assessed in this study due to the loss of nearly all the fruit in these orchards following thinning. The sex pheromone content of new dispensers and for dispensers aged 75 d in the field were analyzed by Suterra personnel. Both dispensers released on average 1.2 mg per day of codlemone + isomers. The standard dispensers initially contained < 5.0% isomers but this increased to 10% at the end of the trial. The isomerized dispensers contained about 39% isomers throughout the trial.

Results and discussion:
Growers who apply dispensers loaded with the sex pheromone of codling moth attempt to disrupt mating of this pest. However, we have shown consistently that the percentage of virgin female moths in sex pheromone-treated orchards is low. In addition, to mating several factors could be important such as the occurrence of multiple mating by either sex. For example, our laboratory data suggests that reducing the incidence of multiple mating by female moths can further reduce the growth of codling moth's populations between generations (Table 1). Unfortunately, we did not find that sperm depletion occurs with males that mate more than once (Table 2). In fact, we almost saw a significant increase in fecundity with mating by experienced versus inexperienced males. Field data showed that multiple mating is reduced in sex pheromone-treated orchards and there is an increase in the proportion of females mated by experienced males, especially during the second generation (Table 3).

Differences in the proportion of mated females in orchards under various sex pheromone-based control strategies were interesting (Table 4). Reduction in codling moth mating in orchards treated with sprayables was negligible. The utility of increasing the density of hand-applied dispensers appears to be marginal. However, a delay in egg hatch (=delay in mating) was detected this year in several orchards treated with a high rate of dispensers. It will be interesting to determine if this delay also occurs in orchards treated with fewer dispensers.

Interestingly, the highest virginity rates for codling moth occurred in orchards treated with widely spaced high emission pheromone sources, i.e. puffers and Pheromone Mops (Table 4). The standard array of puffers places them around the border of the orchard. We have shown that this is not an optimal arrangement as much of the pheromone is wasted. The I.-H.E.L.P. approach uses an internal grid of puffers spaced 50 meters from the edge and 100 meters apart while the perimeter of the

orchard is treated with hand-applied dispensers. Pheromone Mops are clusters of 50 Isomate C-tt dispensers that are placed at a density of 1.6 – 4.0 per acre beginning 25 meters from the edge. These are also placed in an internal grid (30 –35 m apart) in the orchard and the orchard's perimeter is treated with hand-applied dispensers. Growers have generally used the Pheromone Mops at the 1.6 per acre rate but our new data suggest that the higher rate may be more effective.

Data gathered on trap shutdown and mating measured by four trapping approaches failed to detect any difference in the efficacy of standard dispensers and dispensers emitting an isomerized blend of sex pheromone (Tables 5 and 6). These results differ from our previous studies with the isomer blend contained in rope dispensers provided by Pacific Biocontrol. The change in the isomeric ratio of the standard dispensers during this test may have confuted these results. Ideally the isomers should have comprised < 5.0% of the blend during the entire experiment.

Budget:

Understanding Codling Moth Behavior – a Prerequisite to Effective MD
Alan Knight

Project Duration: 2003
Current Year: 2003

	Year 1 (2003)
Salary[1]	$20,000
Benefits	$4,000
Supplies	$6,000
Travel[2]	$1,000
Total	$31,000

This work was supported by complementary funding of $38,000 from IFAES and RAMP.

[1] Seasonal employment of Ted and Caleb Goehry.

[2] Local travel to research plots only.

Table 1. The effects of the number of times female codling moths were mated on the number of eggs laid and hatching.

# times females mated	# reps	Mean (SE) # eggs laid	Mean (SE) # eggs hatched	Mean(SE) % egg hatch
1	89	74.4 (5.9)b	36.9 (3.9)	43.1 (2.7)
2	61	90.1 (8.1)ab	48.8 (6.2)	46.4 (3.2)
3	31	108.7 (14.2)a	57.0 (10.3)	44.7 (4.6)
4	22	74.6 (11.7)b	36.7 (8.6)	39.3 (5.0)
Statistical analysis:		$P < 0.05$	$P = 0.11$	$P = 0.70$

Table 2. The effects of the order of mating by male with virgin female codling moth on the size of the spermatophores and number of eggs laid and hatching.

Order of male mating	# reps	Mean (SE) width of sperm-atophore	Mean (SE) # eggs laid	Mean (SE) #egg hatched	Mean (SE) % egg hatch
1st	62	1.78 (0.02)a	67.0 (5.8)	37.4 (4.5)	48.8 (3.9)
2nd	34	1.31 (0.04)b	73.9 (6.0)	38.8 (4.2)	49.9 (4.3)
3rd	21	1.12 (0.030c	95.1 (8.1)	47.7 (7.3)	45.4 (5.0)
4th	14	1.06 (0.04)c	68.7 (10.6)	29.4 (7.4)	32.6 (6.2)
Statistical analysis:		$P < 0.001$	$P = 0.06$	$P = 0.40$	$P = 0.20$

Table 3. Data on mating by codling moth in orchards treated conventionally or with sex pheromone dispensers during both generations, 2003.

	Mean (SE) percent					
	1st Generation			2nd Generation		
Orchard treatment	Virgin females	Multiple-mated females	Small spermatophores[a]	Virgin females	Multiple-mated females	Small spermatophores[a]
Conv.	5.4 (2.4)	2.8 (1.1)	42.2	5.8 (2.3)	31.5 (1.2)	55.4
MD	21.5 (9.9)	0.6 (0.6)	45.9	24.2 (9.6)	5.2 (3.3)	91.7

[a] Spermatophores < 1.6 mm were categorized as small. Data for spermatophores size was collected from one orchard of each treatment type. Other data were gathered from four orchards of each type.

Table 4. Summary of codling moth catches in 5-acre plots treated either Checkmate 1000XL, dispensers loaded with an experimental isomerized or in untreated blocks. The test was conducted from 2 May through June 13, 2003.

	Mean (SE) catch per trap				
	Pheromone Lure	DA Lure			
Treatment	Male	Female	Male	Total	Mean (SE) proportion mated female
Control	346.0(21.2)	22.7(3.3)	40.3(11.5)	63.0(13.1)	0.86(0.01)
Checkmate 1000XL	175.2(28.7)	45.2(5.7)	60.4(9.7)	105.5(13.3)	0.86(0.04)
Checkmate Isomer	328.5(24.3)	62.1(6.9)	70.0(10.3)	132.1(16.6)	0.86(0.01)

Table 5. Comparison of female moths in three different types of traps within replicated 5-acre plots (n = 3) treated with Checkmate XL100 or Checkmate Isomer at 400 dispensers per acre versus untreated control plots.

Treatment	% mated females caught on interception traps	% female-baited sticky traps catching moths	% female-baited live traps catching moths
Checkmate XL1000	72.9	48.0	9.5
Checkmate Isomer	78.2	37.0	21.7
Untreated	83.4	63.0	20.5

Table 6. Mating success of female codling moth under different pheromone programs in Washington State during 2002-2003.

Pheromone program	# replicates	Mean # females per trap per season[a]	% virginity[a]
Last Call A&K paste	16	33.5	9.1
Conventional	31	30.2	16.2
Isomate C+ 200/acre	145	7.5	18.2
Suterra Sprayable	12	5.3	19.0
Isomate C+ 400/acre	101	13.0	21.5
Isomate C-tt 200/acre	10	2.5	24.0
Isomate C-tt 100/acre	12	1.3	25.0
Pheromone Mops 1.6/acre	9	4.0	27.8
Perimeter Puffers	44	1.3	32.7
I-HELP Puffers	16	1.0	43.0
Pheromone Mops 4.0/acre	9	4.7	47.6

[a] Female moths were captured in delta traps baited with the Pherocon DA lure.

FINAL REPORT

Project Title: Monitoring Codling Moth with the DA Lure

PI: Alan Knight, Research Entomologist
Organization: USDA, ARS, Wapato, WA

Co-PI: Doug Light, Research Entomologist, USDA, Albany, CA

Objectives:

1. Evaluate the use of DA-baited traps to establish a female codling moth Biofix plus the accumulation of 155 DD for the start of egg hatch.
2. Evaluate the use of measuring daily high temperatures across three-day periods to improve the selection of Biofix for either males or females.
3. Construct a new degree-day model for predicting codling moth injury that incorporates the influence of daily accumulation of degree-days on female mating and egg lay.
4. Evaluate the efficacy of this new model compared with the current WSU model across several apple orchards situated in central Washington.

Significant findings:

- The use of the DA lure to establish a female moth Biofix followed by 155 degree-days was found for a third year to be roughly equivalent to the current use of male catch in pheromone traps plus 250 degree-days to predict the beginning of codling moth egg hatch.

- A color-coded Biofix system based on three-day periods of maximum daily temperatures was successful in assigning the Biofix dates for males and females in all eight sites plus in a similar study conducted in Medford. This approach, on average, did not improve the standard Biofix selection protocol that is based on weekly counts of moth catches in traps.

- Moth flight and egg hatch were found to continue throughout the summer in unmanaged orchards. A decline in activity (no cessation) occurred between 900 – 1,000 degree-days after Biofix. Unhatched codling moth eggs were found in these orchards every week during the season.

- A new predictive phenology model was developed for codling moth that includes the nonlinear effects of daily degree-day accumulations to control the rate of female mating and the number of eggs laid. These curves were generated from a series of laboratory tests conducted under fluctuating temperatures in environmental chambers.

- The new model improved the prediction of cumulative fruit injury during the first generation in four of the five sites fully evaluated. The model delays the accumulation of fruit injury compared with the WSU model due to the restraining influence of cool spring temperatures. The accumulation of 20% of the total fruit injury was approximately 110-150 DD later than that predicted by the WSU model. In addition the distribution of injury over the entire generation was shifted later with 80% of the injury occurring 190-230 DD later than predicted by the WSU model.

- The current WSU model was more accurate than the new model for one site that can be characterized by three important factors: establishment of a rather late Biofix (13 May), the orchard was neither sprayed with insecticides or treated with sex pheromones, and the moth

population was highly susceptible to Guthion (population originally derived from field-releases of the USDA laboratory colony).

- A significant delay in the occurrence of fruit injury was found in three orchards treated with a full rate of Isomate-C+ dispensers (400/acre). The first detection of fruit injury in these sites was 4-8 d later than predicted by the male Biofix.

Methods:
Studies were conducted to establish the utility of using maximum daily temperatures to select the correct Biofix for both male and female emergence. Data from the literature as well as our previous studies found that using an activity threshold of 55°F for males and 62°F for females at dusk could be used. Maximum temperatures typically are 12°F higher than dusk temperatures thus a day was scored as 'orange' if the maximum temperature was >67°F and 'Red' if > 74°F. A male Biofix event was triggered if the average of a three-day period was orange and a female event was triggered by a red event. Calendars were generated for each site and updated until fruit injury was first detected. These were mailed to interested pest managers at each site.

Laboratory studies were conducted to examine the non-linear relationship of daily accumulated DD and female CM mating success and egg laying using environmental chambers programmed to fit typical fluctuating spring diurnal temperatures. Nonlinear equations were fit to these data. The emergence curve for male codling moth summarized in a table was fit to a Weibull equation and a similar curve was fit for females using a 50 degree-day delay due to protandry. The model was constructed using an Excel spreadsheet. Accumulated daily degree-days advanced the emergence of each sex. The efficiency of mating based on degree-days was used to transform virgin to mated females. The number of eggs laid by each mated female was adjusted based on daily degree-days. Constant mortality factors were included in the model for adults, eggs and neonates. The model runs from the Biofix to mid July. The model was validated with data collected from 8 apple orchards in Washington. Temperatures were recorded at each site and blocks were monitored with two sex pheromone-baited (Biolure 10X) and DA-baited delta traps checked weekly. At each site 2-4 trees with moderate crop load were chosen and all injured fruit were removed each week from 23 May until 21 July. The output of the WSU and new model were compared with these field data (cumulative emergence and cumulative egg hatch).

Results and discussion:
The goal of this project was to improve grower's ability to predict the emergence and activity of female codling moth and the timing of the resulting fruit injury. This improvement will allow pest managers to more effectively target insecticide sprays and monitoring efforts for codling moth. Growers are accustomed to timing insecticide sprays based on the establishment of a Biofix that is defined as the beginning of sustained catch of male moths in a sex pheromone-baited trap. Typically traps are checked weekly and this approach generally works well. We have shown during the past three years that the DA lure (pear ester) can be used to effectively monitor female codling moth and have tested the use of an alternative Biofix approach. This approach predicts the beginning of egg hatch at 155 degree-days after the beginning of sustained catch of female moths. In addition, the identification of a true Biofix can be improved by considering the pattern of daily maximum (dusk temperatures) that is suitable for flight and mating activities. We proposed last year to use a color-coded scheme to establish a Biofix for codling moth and this year we evaluated this approach for improving both the male and female Biofix date.

Data collected from eight orchards this year found that on average including the effects of maximum temperature did not improve the selection of a true Biofix date (Table 1). However, we noted that in the three orchards treated with sex pheromone dispensers both approaches to establish a male Biofix

performed worse than non-pheromone-treated orchards. This may be due to a delay in mating that occurs in orchards treated with sex pheromone where egg lay is delayed and the date of first egg hatch is delayed relative to the timing of male activity in traps. A similar shift in the accuracy of the female-based Biofix dates was not observed (Table 1).

Codling moth adult activity in high-pressure sites occurs all season with only a short decrease (no cessation) in activity between generations at 900 – 1,000 degree-days after Biofix (Fig. 1). The current WSU model predicts two distinct flight periods with 90% emergence occurring within 500 degree-days after Biofix. This significant difference between the current model and field data has a strong impact on grower's ability to manage codling moth with insecticides. The model was developed nearly 30 years ago when the population density of codling moth was generally low in orchards, prior to the use of sex pheromones for mating disruption, and prior to the detection of widespread Guthion resistance. It appears that the model no longer provides an accurate portrayal of codling moth emergence in some orchards. A second limitation of the current model is that it uses a fixed curve for the occurrence of egg hatch that mirrors the curve for moth emergence (the numbers and timing of injured fruit directly follows the emergence and density of adults). Thus, if different emergence and egg hatch curves exist among orchards, the model will fit the data for some orchards and not for others.

As a reasonable starting point in developing the new model we fit the emergence of moths to the curve used in the current model. However, the new model includes the emergence curves for both sexes with the start of female emergence timed 50-70 degree-days after the start of male emergence. Future modifications of this model will allow us to shift emergence earlier or later to better fit the cumulative curve of egg hatch collected from field data. In addition, we can adjust the emergence curve of adults during the second generation where there does not seem to be protandry occurring. The new model allows the timing and magnitude of female mating and egg lay to vary among years and orchards due to differences in daily temperatures. We conducted a large number of tests to establish the form of these models linking daily temperatures with mating success (Fig. 2) and egg lay (Fig. 3). These equations were inserted into a simple Excel spreadsheet model. We found that in orchards with an early Biofix it was more likely to have cool temperatures following Biofix and this resulted in a larger delay in the egg hatch curve relative to the WSU model. In an orchard with a late Biofix the daily temperatures are typically warmer and females would be more likely to mate and lay a fuller complement of eggs.

The model was tested in eight apple sites in Washington where we monitored temperatures, trapped moths, and sampled for fruit injury each week. These data were summarized to generate data sets to compare the new model with the current WSU model. Data from six sites are presented in Fig. 5. Table 2 shows that the WSU model only approximated the data from 1 site (E. Moxee: late Biofix [13 May], unsprayed, no mating disruption, and no resistance to Guthion). The accumulation of 20% injury in the other sites was approximately 100 degree-days later in the field than predicted by the WSU model; and the three orchards treated with sex pheromones were delayed even later (150 degree-days). It is difficult to evaluate the data from Brewster because of the impact of sprays on the population that likely limited the occurrence of injury later in the flight period. The data from Orondo suggest that the occurrence of resistance to Guthion in this population may have delayed the timing of emergence and fruit injury observed in this orchard. A similar delay in the emergence of adults reared from overwintering bands was measured under laboratory conditions.

Results gathered from this study during 2003 are very interesting. It is clear that codling moth adults are active all summer and that the current model significantly underestimates the extent of adult activity later in the flight curve. We have developed a new model that is more flexible in predicting female activity in orchards between sites and between years. Further work is needed to adjust the emergence curve in the model and to add the second-generation emergence curve. In

addition, further investigations into factors influencing the phenology of codling moth are needed. We have suggested that a combination of several factors may influence the observed curves of cumulative emergence and egg hatch. These can include, the resistance level of the population to Guthion, the occurrence of an early or late Biofix, whether the orchard is treated with sex pheromones, and the quantitative temporal influences of insecticides. Considerably more field data needs to be collected to fully understand the importance of these factors within orchards in Washington. For example, specific models can be developed for orchards based on codling moth's status of resistance and the use of sex pheromones. The influence of sprays on moth mortality can also be included in the model.

Budget:
Project title: Monitoring Codling Moth with the DA Lure
PI: Alan Knight
Project duration: 2003
Current year: 2003

	Year 1 (2003)
Salary[1]	**$10,000**
Benefits	**$3,000**
Supplies	**$1,000**
Travel[2]	**$1,000**
Total	**$15,000**

This work was complemented by $12,000 received from IFAES and RAMP.
[1] This funding provided 25% of Brad Christianson's position.
[2] Local travel to research plots only.

Table 1. Comparison of four methods used to predict the occurrence of fruit injury by codling moth: sustained male capture in a sex pheromone-baited trap, sustained female capture in a DA-baited trap, a three day period with the median high temperature > 67.4°F (orange alert) and a three day period with a median high temperature > 72.4°F (red alert).

		Date (days off prediction of 1st injury)			
Site	Date of 1st injury	Orange alert	Sustained male catch	Red alert	Sustained female catch
Moxee (MD)	6/2	5/2 (4)	4/30 (6)	5/12 (6)	5/15 (4)
E. Wenatchee (MD)	6/2	5/2 (4)	4/28 (5)	5/12 (1)	5/28 (4)
E. Parker (MD)	6/2	4/22 (8)	4/20 (9)	5/22 (2)	5/20 (4)
E. Moxee	6/9	5/13 (4)	5/22 (2)	5/23 (6)	6/4 (3)
Orondo	5/28	4/21 (4)	4/23 (8)	5/10 (3)	5/12 (2)
W. Parker	5/23	4/21 (4)	4/20 (0)	5/21 (1)	4/29 (2)
Zillah	5/28	5/2 (0)	5/5 (0)	5/13 (1)	5/13 (1)
Brewster	5/28	4/20 (4)	4/28 (0)	5/10 (1)	5/27 (8)
Mean difference all sites		3.6	3.0	2.6	3.5
Mean difference MD sites		5.3	6.7	3.0	4.0

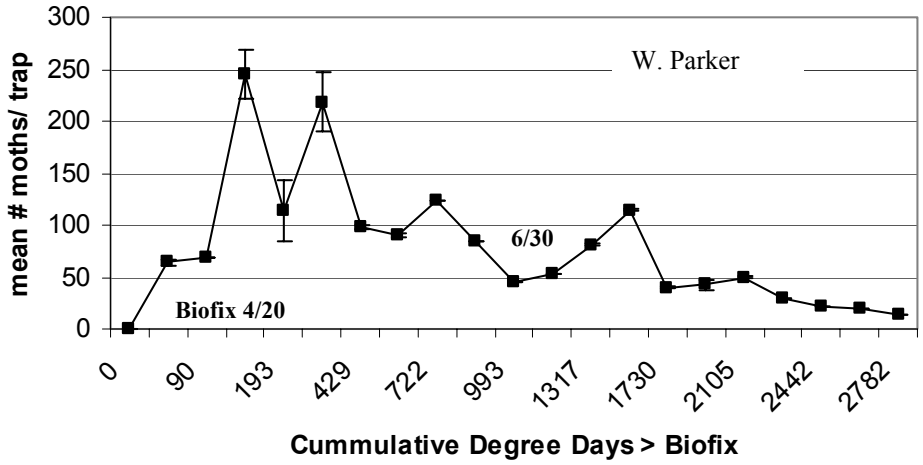
Table 2. Summary of proportion of codling moth fruit injury during the first generation compared with the WSU model

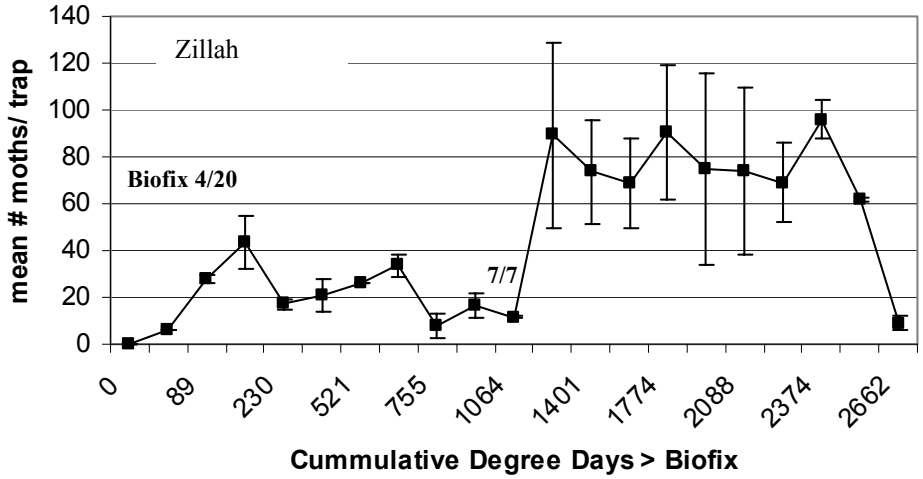
			DD total accumulated after Biofix	
Location	Under MD	OP resistance	20% injury	80% injury
WSU Model	-	-	360	640
E Moxee	no	no	370	580
Brewster[a]	no	?	460	610
Orondo	no	yes	460	820
E. Wenatchee	yes	no	470	870
Moxee	yes	no	510	830
E. Parker	yes	no	510	870
W. Parker[b]	no	yes	-	-
Zillah[b]	no	yes	-	-

[a] Orchard sprayed several times with Guthion.

[b] Due to low crop load in these orchards, all fruit were injured before the completion of first generation.

Fig. 1. Seasonal codling moth catch in sex-pheromone baited traps in 2003.





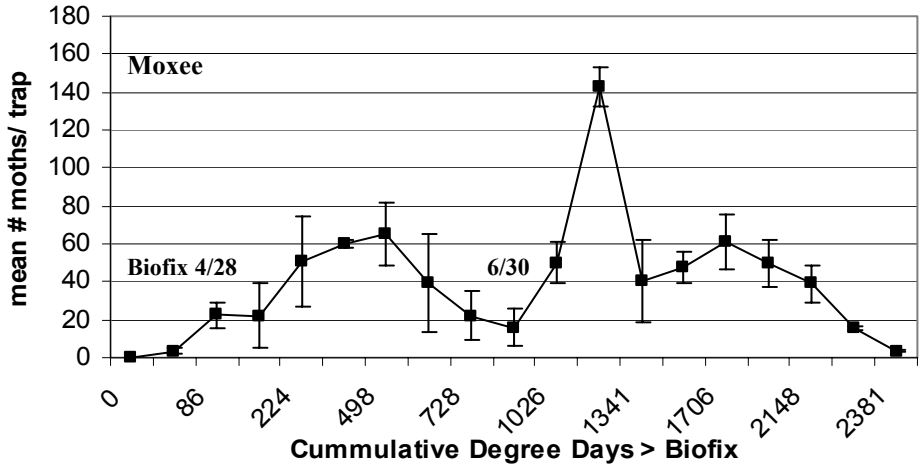


Figure 2. The proportion of females mating as a function of the daily accumulation of degree days.

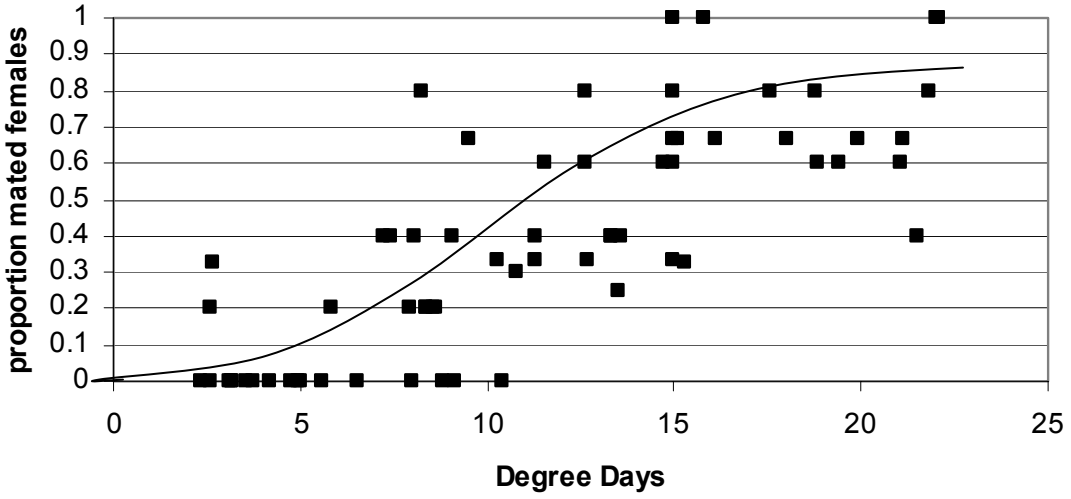


Figure 3. Number of eggs laid per mated female as a function of daily accumulated degree days.

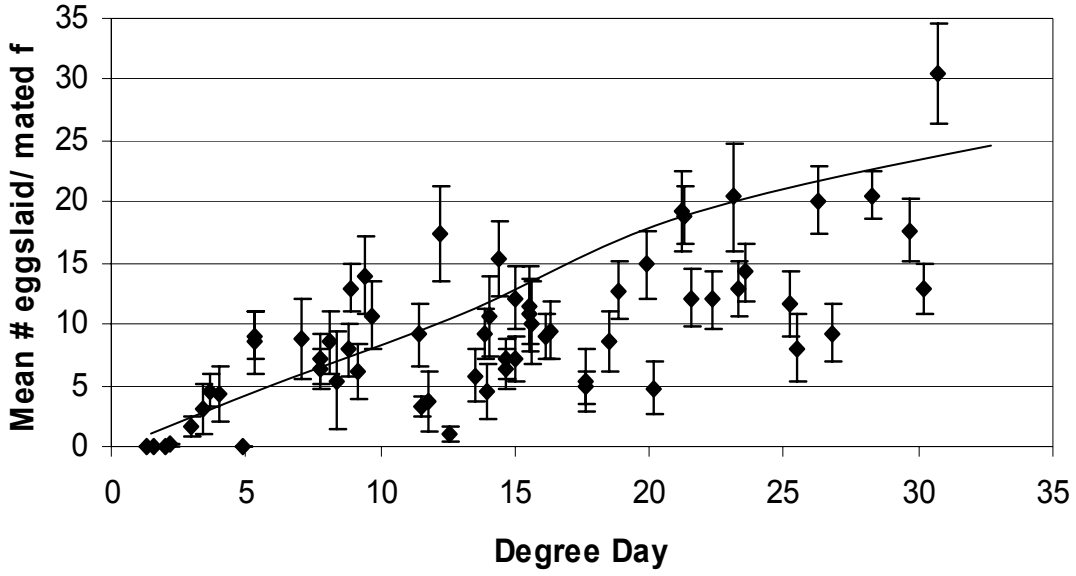


Figure 4. Comparison of the predicted cumulative emergence and egg hatch (fruit injury) of the WSU model and the new model.

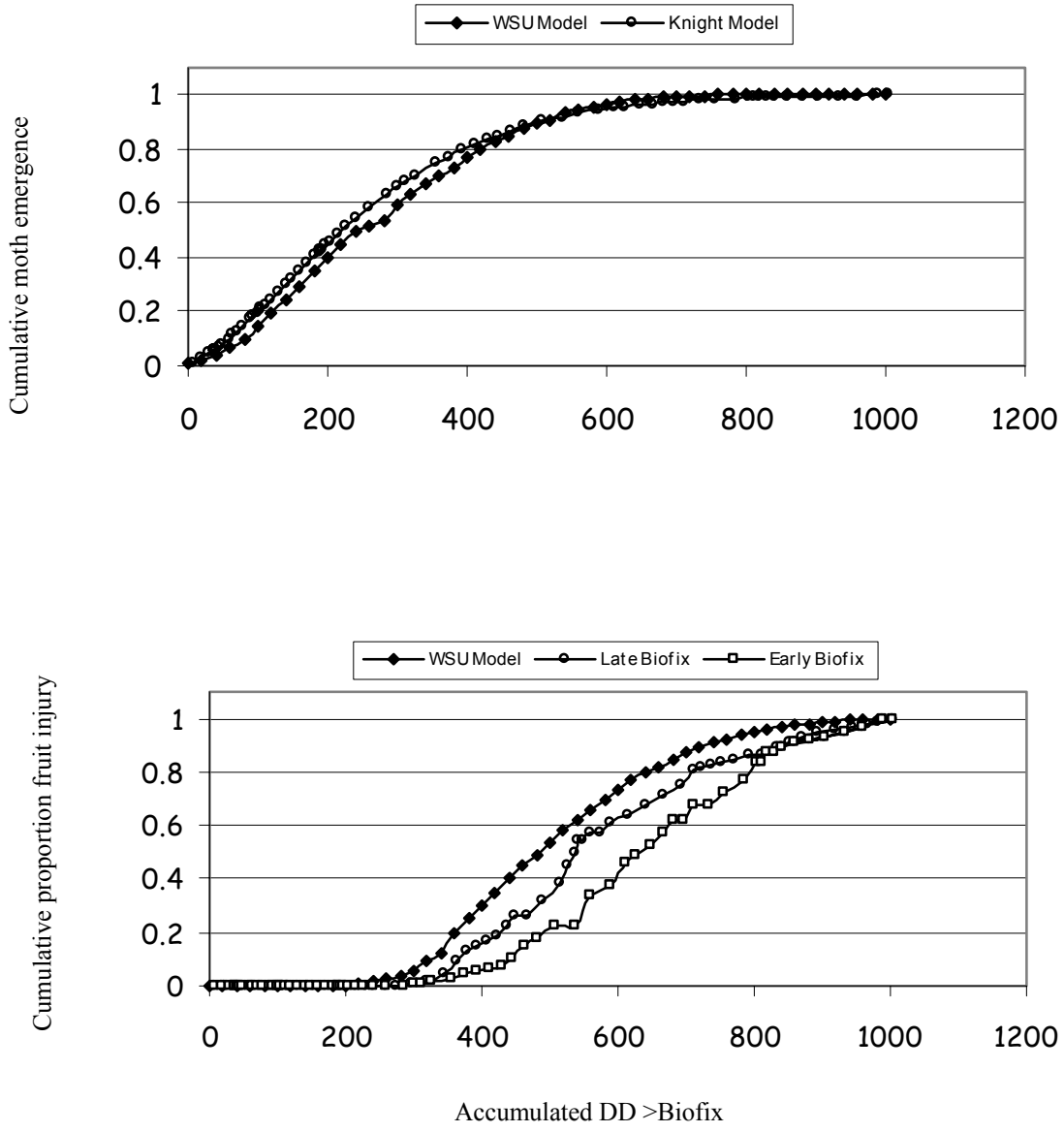


Figure 5. Comparison of both the current WSU model and a new model predicting the timing of first generation codling moth injury versus field data from six apple orchard sites in Washington.

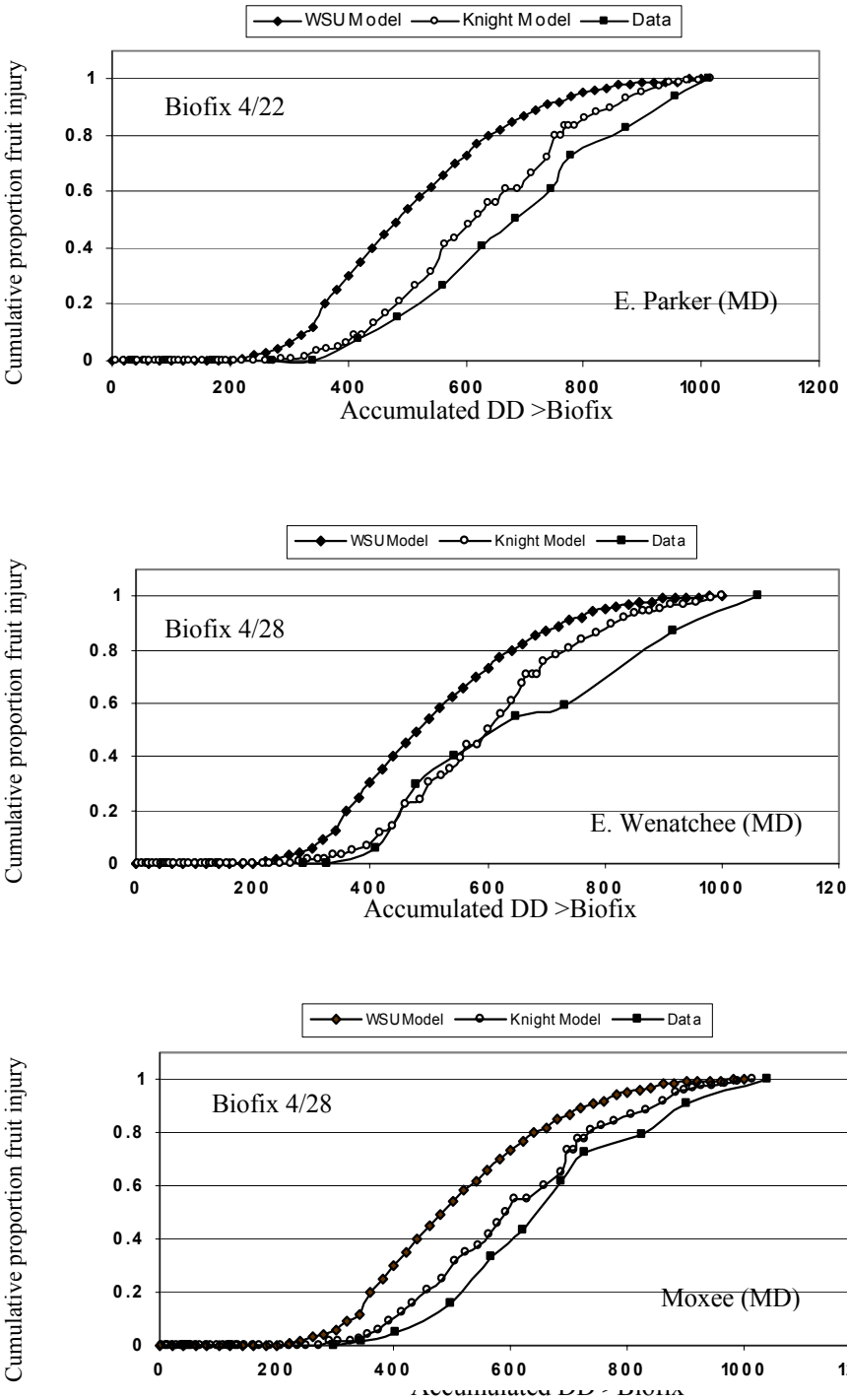
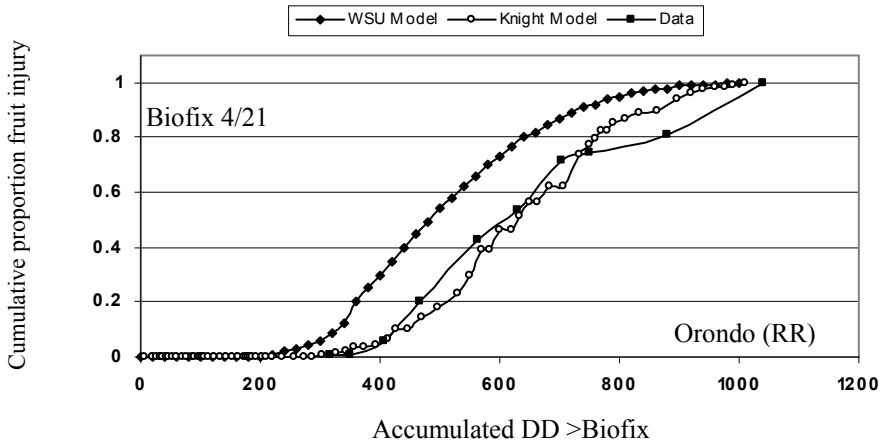
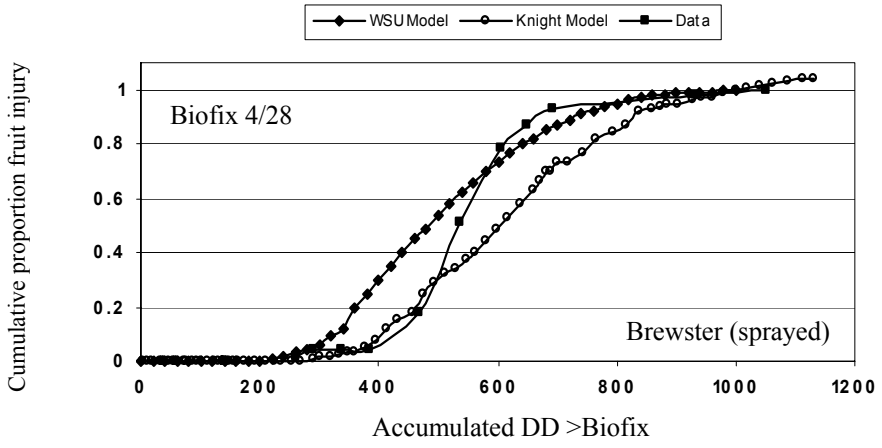
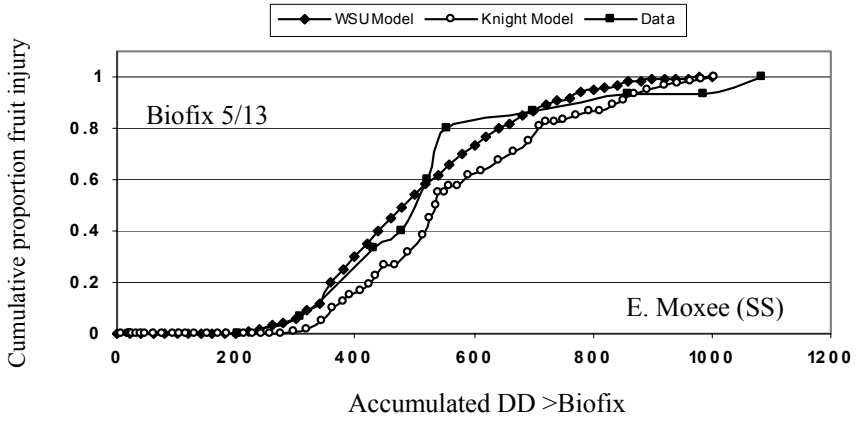


Figure 5. Continued







FINAL REPORT

Project Title: Removing Female Codling Moths From Orchards

PI: Alan Knight, Research Entomologist
Organization: USDA, ARS, Wapato, WA

Co-PI: Doug Light, Research Entomologist, Albany, CA

Objectives:

1. Refine the use of the DA-based killing stations to manage CM.
2. Evaluate the use of light traps to manage codling moth.
3. Evaluate the relative attractiveness of female CM to select apple and pear volatiles alone and in combination with DA.

Significant findings:

- Studies to identify a suitable long-lasting adhesive for a killing station found that adding insecticide to Teflon axel grease generated 100% mortality of codling moths in a 1-s touch test during 8 weeks of field aging.

- A replicated test using killing stations (a grid of 24 within a 1-acre plot) was established in an apple orchard treated with sex pheromone dispensers. Horizontal killing stations (5x5" hinged, flat cards) were baited with a dual sex pheromone/DA lure. Mean moth catch in DA-baited traps of each sex were reduced 50-75% compared with similar traps in the untreated plots. However, differences in fruit injury between treatments could not be accurately assessed due to highly variable and low crop load among plots and high levels of fruit injury.

- A subsequent field test found that the 5x5" horizontal killing station was a poor trap design when compared with the large delta trap used in our previous studies.

- Light traps filled with a hydrophobic formulation of kaolin (certified for use in organic orchards) caught an equivalent number of codling moth as standard light traps baited with an insecticide strip and caught significantly more large moths, such as noctuids.

- One half of a certified organic apple orchard (7 acres) treated with sex pheromone dispensers was also trapped with a grid of 10 light traps. Fifty female codling moths were caught in light traps from 9 June to 28 July. Fruit injury was low in both sides of the orchard.

- DA-baited delta traps caught more moths than unbaited interception traps early in the season. However, from late June until early September this trend was reversed as an unbaited interception trap caught 3 times more moths than a DA-baited delta trap.

- Baiting interception traps and light traps with the DA lure substantially increased the capture of both sexes of codling moth.

- The addition of adjacent apple fruit and foliage synergized the attractiveness of the DA lure for female codling moths flown in a flight tunnel. The specific active components of this blend have not yet been identified.

Methods:
We evaluated the toxicity of esfenvalerate mixed at 6.0% (w/w) in Teflon axel grease (Reese Teflon Hitch Ball Lube, Oakville, Ontario) for adult codling moths. Dr. Landolt had previously reported the effectiveness of this grease in his studies with the Lacanobia fruitworm. Chilled moths were touched for 1 s to a card coated with a thin film of grease and mortality was assessed after 24 h. The card was aged in the field and tested each week for 10 weeks.

A hinged, flat 5x5" card was selected as a suitable killing station due to its low cost, ease of application, and from previous research that showed the capture of female codling moth was reduced on similar cards sized less than 5x5". The effectiveness of these cards as traps compared with a diamond and a delta trap were evaluated in field trials from 30 June – 11 July. All traps (n = 15) were coated with Tanglefoot and baited with a dual DA / sex pheromone lure. The number of moths caught for each trap was compared with ANOVA.

The effectiveness of the lure and kill approach for codling moth was assessed in four 1-acre plots established on 5 - 7 May within a 35-acre orchard treated with sex pheromone dispensers (400 Checkmate 1000XL, Suterra, LLC, Bend, OR). Three delta traps (two DA and one sex pheromone-baited) were placed in each plot including the paired untreated (sex pheromone alone) plots. The four paired untreated plots were situated ca. 75 m from the treated plots. Fruit injury was assessed on 10 June by sampling 600 fruit per plot.

Standard light traps (Ron Britt & Associates, Yakima, WA) were either filled with a hydrophobic kaolin formulation (M96-018) or an insecticide-impregnated strip. Weekly moth catches were compared over a 6-week interval with repeated-measures ANOVA.

A seven-acre organic apple orchard treated with a full rate of Isomate C+ (400 dispensers per acre) was split into two halves and one half was treated with an array of 10 light traps connected to a deep-cycle 12V battery. Five of the light traps were baited with a DA lure. The two plots were also monitored with sex pheromone and DA-baited delta traps. Fruit injury was assessed in mid July by inspecting 2,000 fruit from each half of the orchard. The number of male and female moths caught in each type of trap was summarized and fruit injury was assessed at mid-season in both blocks.

Four high-pressure organic apple orchards were monitored with sex pheromone and DA-baited delta traps, interception traps, and light traps. The data were subdivided into two time periods: early season and the remainder of the season. During the early season period we compared only the sex pheromone and DA-baited delta traps with interception traps. Then beginning on 17 June we included a comparison of light traps and we examined the effect of baiting both light traps and interception traps with DA lures.

A successful protocol was developed to assess the attractiveness of the pear ester for adult codling moth using a flight tunnel. The addition of foliage and/or fruit on the attractiveness of the DA lure was evaluated in a series of tests. On each date five male and female moths were released in the flight tunnel at the beginning of the scotophase (lights-off) and the number of moths captured in a diamond trap baited with a 1.0 mg DA lure was counted the following day (20 h later). Studies were conducted with the lure only, adjacent fruit and foliage but no lure, and lure plus adjacent fruit and foliage.

Results and discussion:
The Teflon grease + 6.0% Asana was highly effective in killing codling moth adults for 8 weeks (Figure 1). Fifteen percent of moths survived a 1-s touch with the grease after 9 and 10 weeks. This suggests that a killing station can be easily constructed to provide good killing potential for an entire

generation. Additional work is needed to develop a killing station that can be effective for the entire season (16+ weeks).

Reductions in moth catches in the plots treated with the killing stations was significant for both male and female moths in the DA-baited trap but no difference in capture was found for males in the pheromone trap (Table 1). Crop load in these eight plots was highly variable due to poor thinning practices. Less than 10% of trees had any fruit and these averaged < 30 fruit per tree. Fruit injury on 10 June varied from 27 – 60% among plots with most fruits having multiple injuries. The study was terminated on this date due to the unacceptable level of fruit injury, the low and variable crop load among plots, and because additional data showed that the 5x5" card was an ineffective killing station due to its small size.

Mean moth catch ($\pm$ SE) on the 5x5" cards (1.0 $\pm$ 0.3) was significantly lower than in a standard diamond trap (7.3 $\pm$ 1.1) and delta trap (3.3 $\pm$ 0.9), $F = 14.62$; df = 2, 42; $P < 0.0001$. The selection of this card as a suitable killing station due to its low expense was a poor choice. Further work is needed to optimize the shape and size of the killing station for codling moth.

Using a 1-2" layer of kaolin powder in a light trap was an effective method to retain codling moth: mean (SE) catch in traps with powder = 19.1 (4.2) versus 17.6 (4.5) in traps with an insecticide strip, $P = 0.96$. A significantly greater number of larger moths (Noctuidae and other moth families) were caught in the light traps with kaolin (73.6 [8.4] versus the killing strip (44.9 [4.2], $F = 8.85$; df = 1, 6; $P < 0.05$. This approach will allow light traps to be used in certified organic orchards as a supplementary tool for managing and monitoring codling moth and perhaps Lacanobia fruitworm. Unfortunately, in the study to assess the value of light traps to supplement mating disruption, the orchard was initially treated with a series of light traps that were ineffective in catching anything. These traps were replaced on 9 June. Following this we caught a total of 50 female moths in the 10 light traps and 80% of these were in the traps baited with a DA lure (Table 2). Catches of moths in the pheromone and DA-baited traps were similar between the two portions of this orchard and fruit injury in mid July was low throughout this orchard. The objective of this study was to become familiar with the use of light traps for management of codling moth. The biggest problems we encountered with using light traps, besides our false start with defective traps, was the required regular maintenance of the batteries. The use of light traps is a reasonable approach to supplement other control strategies for codling moth, especially if traps can be hard wired to a dependable power source.

Early in the season the DA-baited trap was more effective than an interception trap in capturing both sexes of codling moth but caught significantly fewer males than a pheromone-baited (Biolure 10X) trap (Table 3). The pheromone-baited trap caught a similar number of males as a light trap from late June until early September (Table 4). In comparison, the DA-baited trap performed poorly. During this time period the interception trap caught nearly 3-times as many moths as the DA-baited delta trap (Table 4). In this study the light trap caught only 30% as many females as male moths. Baiting the light and interception traps with a DA lure increased the captures of both sexes by ca. 80-100 moths per trap. Captures of female moths was increased nearly 9-times on a baited interception trap versus the baited delta trap. These data with the DA lure are encouraging and suggest that management of codling moth by female lure and kill has potential. Further improvement in the parameters of a killing station for codling moth may be the key to effective control of this pest.

Placing the diamond trap in the middle of the flight tunnel improved the recapture of codling moth in the flight tunnel. In addition, placing apple fruit and foliage on either side of the trap increased the capture of female codling moth (Table 5). These studies are ongoing.

Budget:
Project title: Removing Female Codling Moths From Orchards
PI: Alan Knight
Project Duration: 2003
Current Year: 2003

	Year 1 (2003)
Salary[1]	**$12,000**
Benefits	**$1,000**
Supplies	**$2,000**
Total	**$15,000**

This work was complemented by a $5,000 grant from IFAES and RAMP.
[1] Two summer employees (GS-3) for 4 months.

Table 1. Comparison of moth catches in 1-acre plots treated with sex pheromone dispensers plus 24 killing stations versus plots treated with only sex pheromone dispensers, 9 May - 13 June 2003, n = 4.

	Mean (SE) catch per trap		
	Pheromone Lure	DA-lure	
Treatment	Male	Male	Female
Pheromone + Killing Stations	162.0 (24.5)	26.0 (3.5)	28.6 (4.6)
Pheromone	189.6 (14.5)	65.3 (6.7)	56.7 (4.8)
Paired t-test, df = 3	$P = 0.46$	$P < 0.05$	$P < 0.05$

Table 2. Comparison of moth catches and fruit injury in an organic apple orchard under a full rate of mating disruption that was split into half and supplemented with or without the addition of ten light traps from 9 June – 28 July. Five of the light traps were baited with a DA lure.

Sampling index	Management supplemented with 10 light traps	No light traps
Pheromone trap	10.0 males per trap	9.0 males per trap
DA trap	4.7 males + 2.0 females per trap	4.0 males + 3.0 females per trap
Light trap	12.6 males + 2.0 females per trap	-
Light trap + DA	12.8 males + 8.0 females per trap	-
% fruit injury	0.20	0.30

Table 3. Early season catches of codling moth in several types of traps placed in four orchards, 7 May – 13 June 2003.

	Mean (SE) moth catch per trap		
Trap	Male	Female	Total
Pheromone-baited delta trap	300.6 (27.5)a	-	300.6 (27.5)a
DA-baited delta trap	52.7 (12.1)ab	40.7 (13.4)	93.4 (24.1)ab
Interception trap	31.8 (11.4)b	24.3 (4.5)	56.1 (15.1)b
Kruskal-Wallis One-way ANOVA	$X^2 = 8.0$ $P < 0.05$	$X^2 = 0.33$ $P = 0.56$	$X^2 = 8.00$ $P < 0.05$

Table 4. Comparison of captures of male and female codling moths from 20 June to 5 September in a number of traps placed in four orchards.

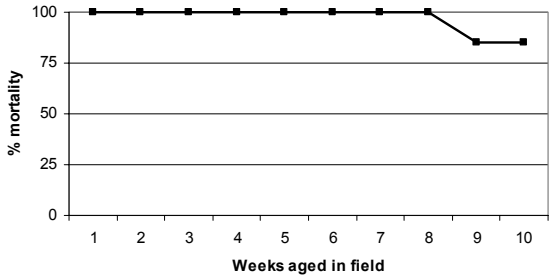
	Mean (SE) moth catch per trap		
Trap	Male	Female	Total
Pheromone-baited delta trap	517.5 (80.3)a	-	517.5 (80.3)ab
DA-baited delta trap	22.9 (6.8)b	17.9 (6.7)b	40.8 (13.1)b
Light trap	588.8 (216.4)a	169.8 (57.8)ab	758.5 (268.0)a
Light trap + DA lure	661.0 (223.9)a	251.3 (70.9)a	912.3 (292.2)a
Interception trap	71.8 (21.0)ab	50.7 (10.7)ab	122.5 (27.8)ab
Interception trap + DA lure	140.7 (39.6)ab	156.0 (52.7)ab	296.7 (80.1)ab
Kruskal-Wallis One-way ANOVA	$X^2 = 18.5$ $P < 0.01$	$X^2 = 14.1$ $P < 0.01$	$X^2 = 17.2$ $P < 0.01$

Table 5. The proportion of virgin male and female moths recaptured in a sticky diamond trap following release in a flight tunnel, 5 males and 5 females per trial.

Treatment	# trials	Proportion of males recaptured in trap	Proportion of females recaptured in trap[a]
Lure in trap only	5	0.28	0.04
Lure in trap + adjacent fruit and foliage	11	0.20	0.26
No lure in trap with adjacent fruit and foliage	5	0.08	0.12

[a] 87% recaptured females were mated.

Figure 1. Mortality of adult codling moth following a 1-s touch to a Teflon paste mixed with 6.0% esfenvalerate. Paste was aged in the field from 6 May to 15 July.



FINAL REPORT
WTFRC Project # AE-01-32

TITLE: Development of operational strategies for practical control of codling moth neonate larvae and prepupae using codling moth granulovirus (CpGV) and *Steinernema feltiae* (Nematoda).

PI: Lawrence A. Lacey

ORGANIZATION: USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA

Objectives:

Granulovirus

1. Quantify virus counts and larvicidal activity for codling moth (CM).
2. Assess the effects of storage of CpGV formulations at 2, 25, and 35˚C.
3. Develop a laboratory exposure and bioassay system for assessing the protective effects CpGV formulation on sensitivity of the virus to solar radiation.
4. Assess the persistence and efficacy of three CpGV products recently certified for use in organic production in an experimental orchard; Cyd-X (Certis), Virosoft (Biotepp), Carpovirusine (Sumitomo)
5. Monitor the season-long performance of Cyd-X used by several commercial organic growers.

Nematodes

1. Determine temperature effects on the effectiveness of spring and fall applications of *Steinernema carpocapsae* and *S. feltiae* to control overwintering CM larvae.
2. Compare hand gun application of nematodes with that of airblast sprayer application.
3. Assess the effects of irrigation type on efficacy of *S. carpocapsae* and *S. feltiae* in apple and pear orchards.
4. Assess the effects of mulches on the activity and persistence of *S. carpocapsae* and *S. feltiae* in apple orchards.
5. Demonstrate the utility of *S. feltiae* for control of cocooned CM larvae in fruit bins.
6. Determine if adjuvants can improve the activity of nematodes in fruit bins.
7. Evaluate drencher-applied treatment of bins with nematodes.
8. Conduct treatments of infested fruit bins in a packing line drop tank.

Significant findings:

1. The Virosoft and Cyd-X formulations of granulovirus stored well at 25ºC (77ºF) and 35ºC (95ºF) maintaining larvicidal activity for over 20 weeks.
2. Although CpGV is susceptible to solar deactivation, significant larvicidal activity was observed up to 14 days after application of the 3 commercial products.
3. Weekly applications of CpGV timed during peak CM egg hatch will likely provide effective population suppression for growers with moderate to low CM pressure.
4. Entomopathogenic nematodes can provide control of overwintering CM when temperatures are 15˚C (60˚F) and higher and moisture is maintained. In trellised apples and conventional pears this moisture was adequately maintained by pre and post wetting of trees using existing irrigation.
5. Mulches offer promise to extend the persistence and activity of nematode larvicidal activity.
6. Entomopathogenic nematodes provide a means of controlling cocooned CM in fruit bins when the bins are immersed in the drop tank or passed through a drencher apparatus. Bins will have to be stored at out of sunlight and strong air currents at 60ºC or higher for 24 hours in order for the nematodes to be most effective.

Codling moth granulovirus – quantitative laboratory studies on product quality

Methods, Results and Discussion. Counts of the granules in each formulation were performed using a light microscope and a Petroff Hauser counting chamber. The virus granule counts reported on the label of each product and counts performed in the laboratory are presented in Table 1. The laboratory counts for the 3 products were comparable, but differed slightly from that reported in label information. We will supplement these counts with counts made using a scanning electron microscope in the near future.

Table 1. Label and laboratory counts of virus granules in each of three commercial formulations of the CM granulovirus.

Product	label counts per liter	laboratory counts per liter
Carpovirusine	minimum of 10^{13}	2.95×10^{13}
Cyd-X	3×10^{13}	2.02×10^{13}
Virosoft	4×10^{13}	2.53×10^{13}

Quantitative bioassays were performed on each of the products by exposing 30 neonate larvae to each of 7 concentrations of virus on artificial media as described by Lacey et al. (2002). The bioassays were repeated on 5 separate dates. The larvicidal activity of the 3 products are similar based on the data derived from these bioassays. The approximate LC_{50} values for each product are reported in Table 2.

Table 2. Approximate concentration (LC_{50}) of each of three commercial formulations of CM granulovirus required to kill 50% of neonate larvae, based on quantitative bioassays and laboratory counts of virus granules.

Product	approximate LC_{50} (granules/mm^2)
Carpovirusine	25
Cyd-X	18
Virosoft	20

The shelf life of the 3 commercial formulations was studied by storing each of the three formulations at 2, 25, and 35ºC (35-95ºF) and regularly bioassaying 10^{-3} (one thousand fold) and 10^{-5} (one hundred thousand) dilutions of the products against neonate larvae after 2 to 20 weeks of storage using the procedures described by Lacey et al. (2002). Both the Virosoft and Cyd-X formulations produced nearly 100% mortality at both dilutions for all three storage regimes for 20 weeks. The Carpovirusine formulation stored well at 2ºC, but larvicidal steadily declined after relatively short term storage at 25 and 35ºC.

A bioassay system for determining the solar sensitivity of virus formulations was developed using sprayed apples that are exposed to simulated solar radiation and then challenged with neonate CM larvae. Prior to treatment with virus the apples are halved and the cut side sealed with molten wax after which aluminum foil is glued in place over the cut side using a hot glue gun. Apple halves are then sprayed in a DeVries spray cabinet using a thousand fold (10^{-3}) or hundred thousand fold dilution (10^{-5}) of virus plus surfactant (0.025% Silwet). The half apple allows an even distribution of virus over the surface of the fruit that would not be possible using whole apples. After drying, the apples are exposed to UV and other wavelengths of light equivalent to 10,800 KJ/m^2 in a solar simulator (Atlas) for 4 hours. Controls are sprayed with water and wetting agent and also exposed to 4 hours of simulated solar radiation. Treated controls are sprayed with virus, but not exposed to solar radiation. The individual apple halves are then infested with 5 neonate larvae and incubated for 10

days at 25ºC after which the survival of larvae and size of the larval entries are determined. We have obtained initial data with the Cyd-X and Carpovirusine formulations that validates the testing procedure and sets the stage for comprehensive comparative testing of all three formulations this winter. The mortality of larvae in virus treated (Carpovirusine, 10^{-3} dilution) apple halves that received 10,800 KJ/m2 was 50% compared to 97% in apples that were not exposed to solar radiation. These data indicate high sensitivity of the virus to the concentrated radiation even at high concentrations of the virus.

CpGV - field

Persistence of CpGV products

Methods. This study was conducted in a plot of 224 trees (spur-reds) at the USDA experimental orchard near Moxee, WA. Applications were made on 2 June and 14 July to seventy individual trees using a motorized backpack airblast sprayer (Stihl). Products were applied in a 3×2 factorial design according to Table 3; with 10 randomly selected trees per treatment. Trees were sprayed from multiple angles providing realistic coverage and a large tarp was used to confine treatments. Immediately after spraying and at 1, 3, 7, 10 and 14-day intervals 50 apples per treatment were removed and challenged with 5 neonate CM larvae using a standardized laboratory bioassay. After 10 days, apples were destructively sampled to quantify fruit damage and larval mortality.

Results. For the June application residual activity of all products (label rates; Table 3) remained highly effective (>80% larval mortality relative to controls) for 24 hours following application and moderately effective (>70%) after 72 hours (Figure 1). Significant activity in all treatments remained after 14 days, suggesting prolonged survival of the virus in UV-protected locations, such as the calyx of fruit. Fruit damage was also reduced; while overall >97% control larvae formed deep entries, <35% of CpGV-killed larvae's stings were >3mm. The second application showed similar results (data not shown).

Table 3. CpGV applications made to experimental plots at Moxee, 2003

Product	**Label rate (oz/ac)**	**Low rate (oz/ac)**	**High rate (oz/ac)**
Cyd-X (Certis)	1-6	3	6
Virosoft (Biotepp)	3.2	3.2	6
Carpovirusine (Sumitomo)	13.7	6	13.7

Grower assessments

Methods. The impact of Cyd-X as a primary control measure for CM within commercial organic orchards was monitored at five locations (Table 4). Individual growers applied Cyd-X treatments within label recommendations; i.e. 3-4 oz in 100-200 gal. + 6-12 oz wetting agent per acre. Applications were timed according to biofix information and sticky pheromone trap data, according to normal monitoring practices. Historically all sites had suffered repeated CM attacks with Cyd-X treatments generally confined to known 'hotspots' from 2002 where mating disruptors (200-400/ac) and some routine sanitation of infested fruit was also employed. Trees were sampled to estimate damage throughout the season. Damaged fruit was removed to determine the proportion of deep entries caused by larvae surviving treatment. Selected trees were banded to monitor overwintering generations.

Results and discussion. The grower assessments provide strong circumstantial evidence for the effectiveness of well-timed CpGV applications against CM outbreaks (Table 4). In all cases where 1st generation larvae were targeted and treated areas monitored, fruit damage was reduced or eliminated in the 2nd generation, with the majority of neonates killed throughout the season as indicated by the high proportion of failed entries or shallow stings. Adult catches in pheromone traps were also

significantly reduced in the second and third generation combined, while trap bands placed around trees indicated the overwintering generations remained low (data not shown). In one case (Moxee), a severe infestation was reduced (when compared to an oil check) but not adequately controlled by late season applications of the virus alone.

Figure 1. Live codling moth larvae recovered from apples previously treated with CpGV.

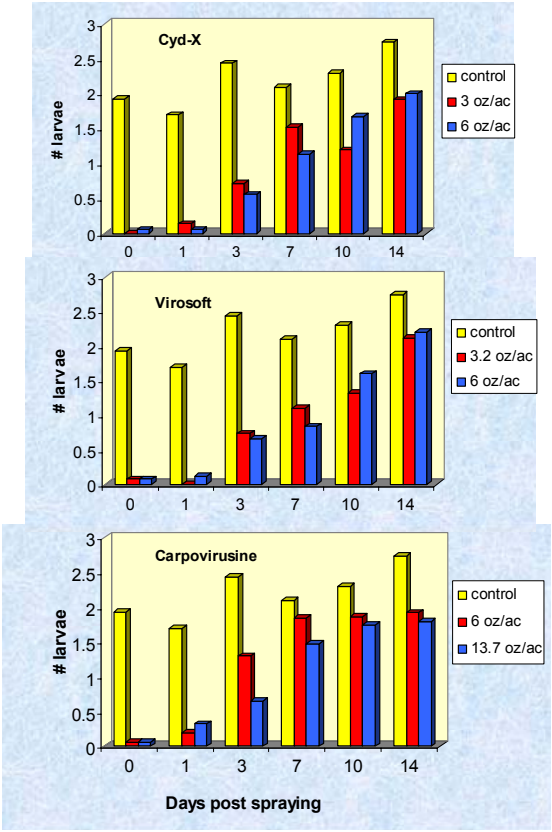


Table 4. Fruit injury and adult codling moth activity monitored in commercial orchards following various CpGV applications (2003 season)

Region (WA)	**Area monitored**	**# Applications[1]**		**# Damaged fruit/tree[2]**	**Proportion of deep entries**	**# Adults/trap**
Quincy	Braeburn (2.5 ac trellised)	1st gen.	1	0.04	0	5.4
		2nd/3rd gen.	1	-	Na	1
Royal City	Braeburn (3.9 ac)	1st gen.	5	0.76	0.03	30.3
		2nd/3rd gen.	4	0.21	0	0.5
Parker Hgt	Gala (3.5 ac trellised)	1st gen.	6	0.08	0.13	20.7
		2nd/3rd gen.	6	0.03	0.2	2.3
	Golden Del. (2.95 ac)	1st gen.	6	3.8	0.17	28.3
		2nd/3rd gen.	8	3.5	0.14	4.3
Mattawa	Red Del. (2.5 ac)	1st gen.	2	Na	Na	21.3
		2nd/3rd gen.	4	0.81	0.14	3.3
	Granny Smith (0.75 ac)	1st gen.	2	Na	Na	21
		2nd/3rd gen.	4	1.9	0.08	2

[1]Accumulated degree days; 1st generation = 220-1020, 2nd–3rd = 1100-2300
[2]A minimum of 30 trees assessed per generation

Field trials of nematodes for control of overwintering codling moth larvae.
Methods, Results and Discussion. Methods utilized in the evaluation of nematodes in our studies followed protocols outlined by Lacey et al. (2000). Applications of nematodes using a backpack sprayer at the rate of 1 million infective juvenile (IJs) nematodes per tree, in the fall resulted in near complete control of cocooned sentinel larvae in logs with both *S. carpocapsae and S. feltiae* when temperatures averaged 16˚C (Figure 2). However when temperatures averaged 8.2˚C, control remained high with only *S. feltiae*. For spring applications, the efficacy of both species was somewhat reduced due to lower sustained temperatures (Figure 3). Nevertheless, *S. feltiae* provided 70-80+% control of sentinel larvae in logs. In another study, hand gun applications were somewhat more effective than airblast sprayer applications due to more concentrated coverage of the trunk and scaffold branches, whereas the airblast sprayer covered a lot of area on the tree and orchard that was not infested with CM.

Figure 2. Fall orchard test

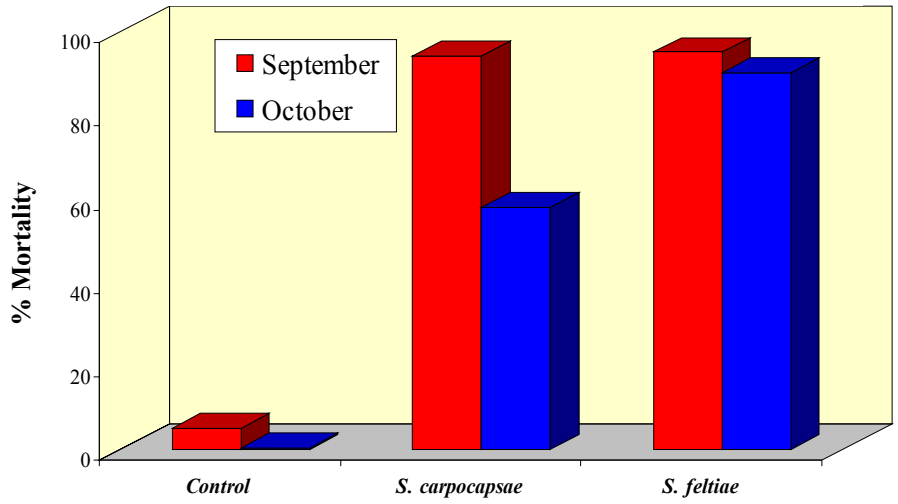
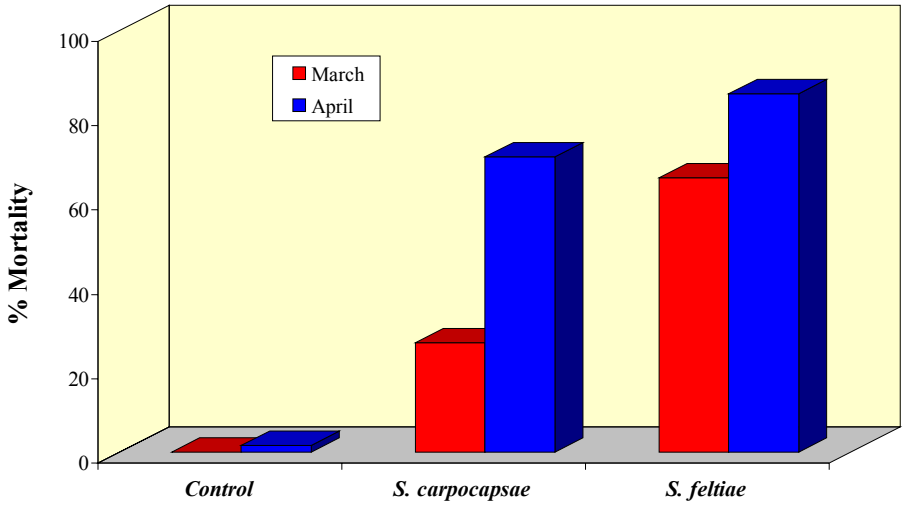


Figure 3. Spring orchard test



For the irrigation studies, fall applications in apple and pear of 1 billion infective juveniles (IJs)/ac provided good CM control using *S. carpocapsae* and moderate control for *S. feltiae* (Figures 4-5). In the pear orchard, cocooned CM sentinel larvae in pear wood were placed on tree trunks 4-5ft. above the ground simulating natural overwintering sites. Both existing and modified irrigation treatments provided sufficient moisture both before spraying and for up to 8 hrs post treatment (figures show pooled data). Control mortality 'within ground' reflect native infections.

Figure 4. Airblast sprayer applications of nematodes in a trellised apple orchard, with pre and post-spraying irrigation (Oct. 2003)

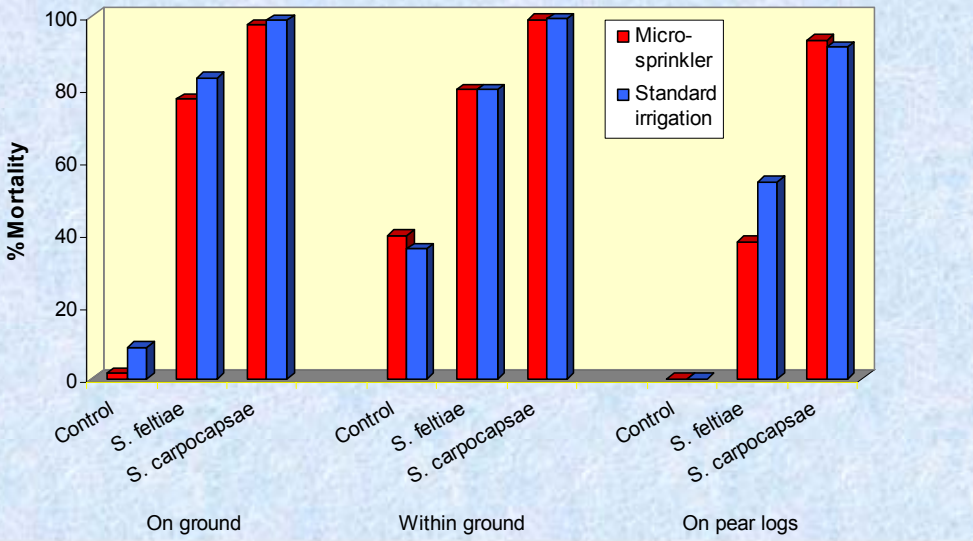
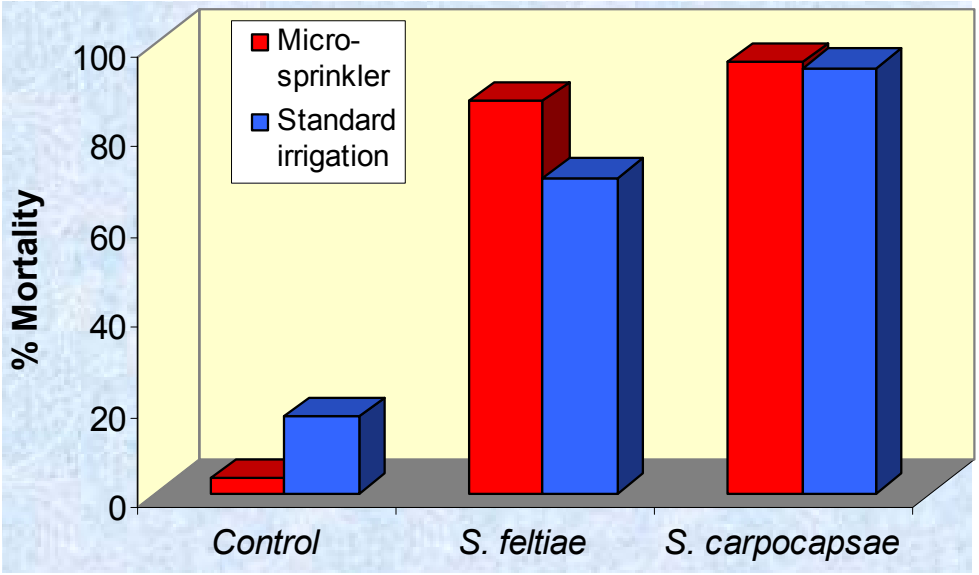
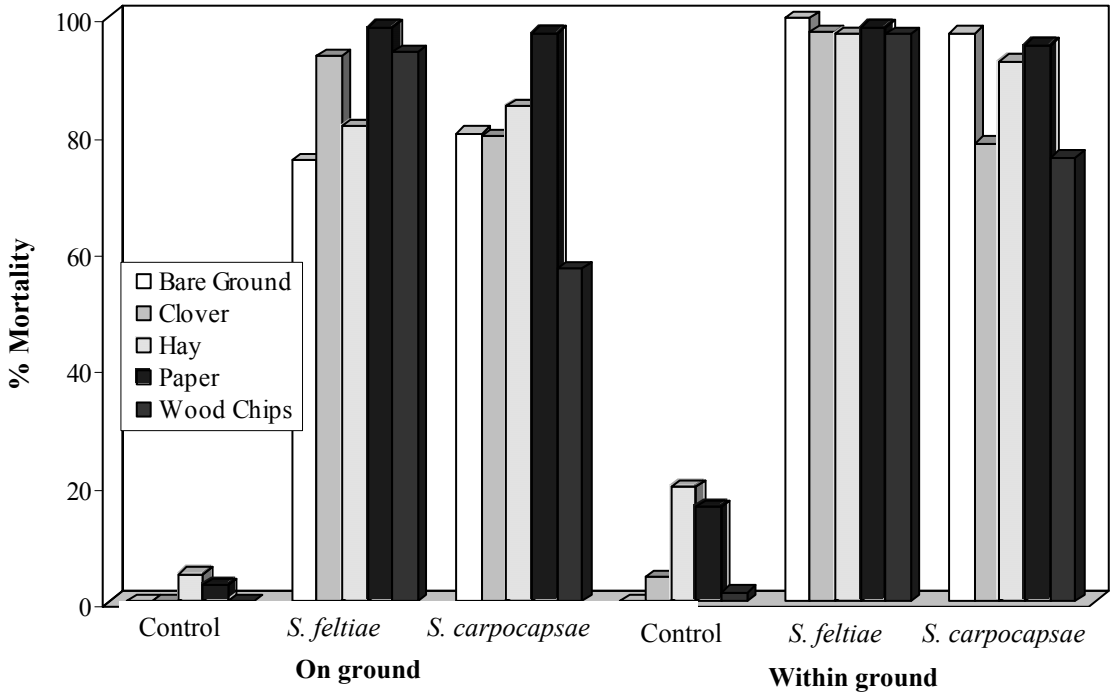


Figure 5. Airblast sprayer applications of nematodes in a pear orchard, with pre and post-spraying irrigation (Oct. 2003)



Steinernema carpocapsae and *S. feltiae* were applied to m^2 plots (100,000 IJs/m^2) within plots of trellised Red Delicious apples where the ground beneath the apples was covered with one of four different mulches (wood chips, shredded paper, hay, clover) or left bare. Two cardboard strips each containing approximately 20 cocooned larvae were placed beneath the mulch in each plot, one on the surface of the ground, the other in a groove within the soil. Both nematode species performed well (Figure 6) especially against larvae that were placed in grooves within the soil. The on ground larvae were well controlled by both nematodes under the paper mulch, but variable responses were observed for the other mulches. *S. carpocapsae* was less effective under wood chips. Nematode infection and mortality in control sentinels revealed the presence of native entomopathogenic nematodes.

Figure 6. Trials of *Steinernema feltiae* and *Steinernema carpocapsae* applied to 4 types of mulch



Nematodes for control of cocooned codling moth larvae in fruit bins.

Methods, Results and Discussion. Treatment of infested miniature fruit bins by submersion in suspensions of *S. feltiae* ranging from 10 to 100 IJs nematodes/ml produced 50 to 95 % mortality in cocooned CM larvae (Figure 7). This nematode species is active at cooler temperatures than *S. carpocapsae* and may provide treatment options when it is not possible to store treated bins at temperatures above 60ºF (15.5ºC). The addition of a wetting agent (Silwet) and/or a humectant (Sta-moist) to suspensions of 10 IJs/ml significantly improved the activity of the nematodes (Figure 8). The wetting agent, Silwet apparently enabled improved penetration of crevices in which the larvae spin their cocoons, while the Sta-moist slowed the rate of desiccation. Infested bins were treated in an experimental packing line drop tank (USDA laboratory in Wenatchee, WA) using 3 concentrations (10-50 IJs/ml) with each of 2 species of commercially available nematodes (*S. carpocapsae and S. feltiae*). There was a distinct dosage-mortality response with the *S. feltiae* preparation; the highest mortality (80+%) of cocooned CM larvae was observed using the 50 IJ/ml concentration. A clear dosage-mortality response was not observed for *S. carpocapsae* (Figure 9); high mortalities were observed at both the 10 and 50 IJ concentrations.

Drencher application of *S. feltiae* at 10 and 25 IJs/ml resulted in CM control that was comparable to that obtained in drop tank applications of the same concentrations (Table 5). This provides another treatment option if the bins can be stored at temperatures above 60ºF in a location where they will not dry rapidly.

Figure 7. **Mean % mortality of codling moth due to treatment with *Steinernema feltiae* at 10, 25, 50 & 100 IJ's/ml of drop tank water**

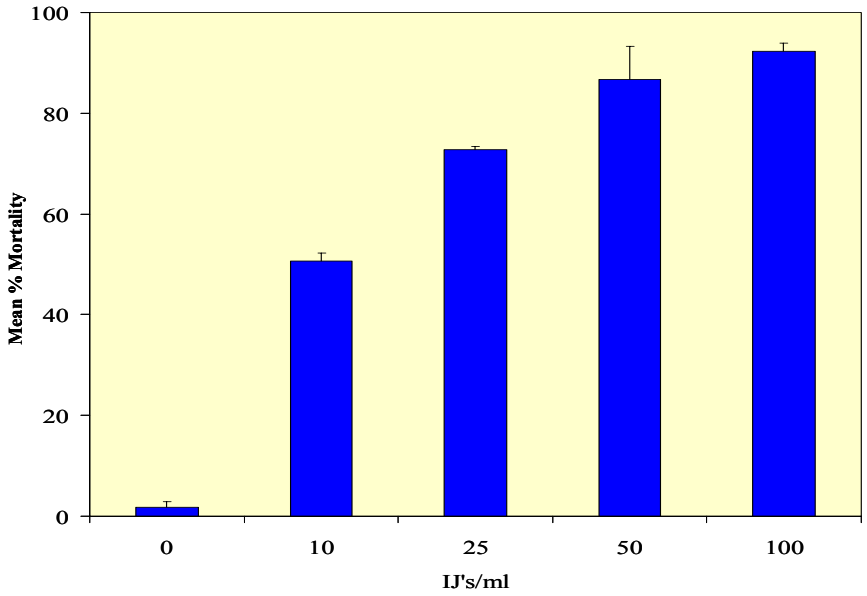


Figure 8. The effect of formulation components on the larvicidal activity of *Steinernema feltiae* at 10 infective juveniles per ml of water against cocooned larvae of *Cydia pomonella* in fruit bins in low and high humidity

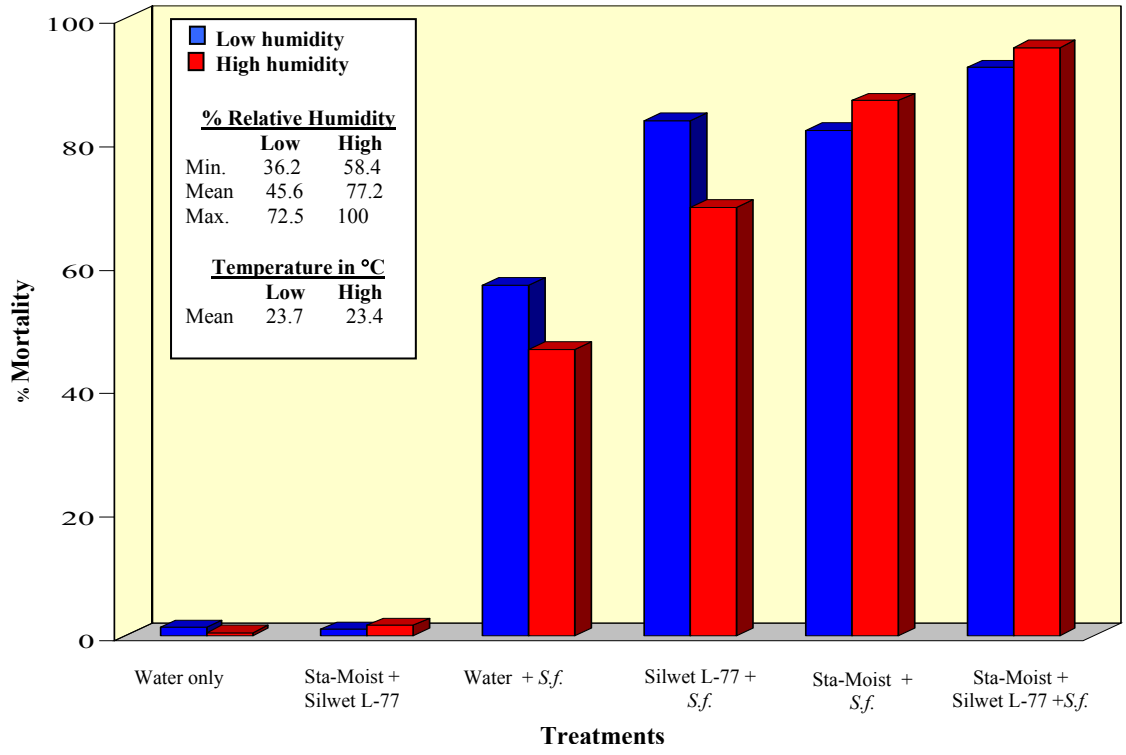


Figure 9. Mean % mortality of codling moth due to treatment with *Steinernema feltiae* and *Steinernema carpocapsae* at 10, 25 & 50 IJ's/ml of drop tank water

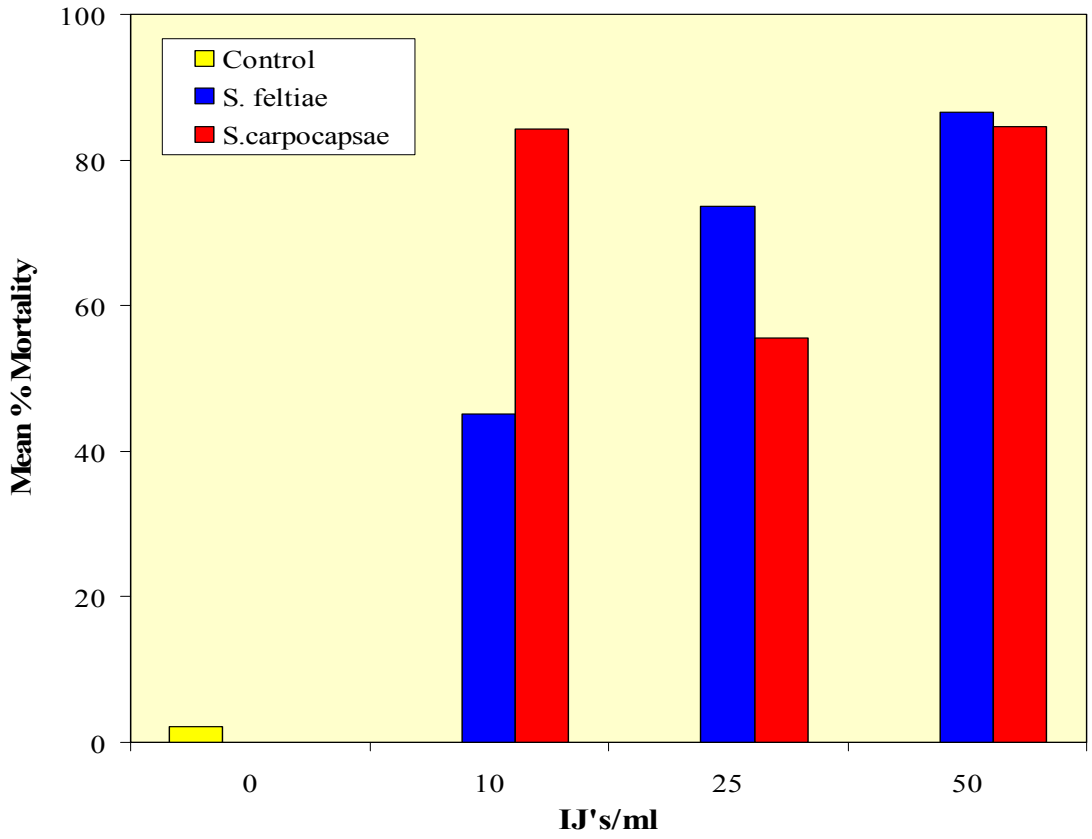


Table 5. Evaluation of drencher-applied *Steinernema feltiae* at two concentrations and two treatment times for control of codling moth larvae in fruit bins.

IJ/ml	Time (min)	Mean % mortality
10	2.5	46.4
	7	40.4
25	2.5	58.2
	7	62.9
0*		1.8

* Mean control mortality for two test dates.

Conclusions

Each of the three commercial formulations of CpGV show high levels of activity in laboratory bioassays. All three store well at cool temperatures, but the Cyd-X and Virosoft formulations can withstand moderate term storage at room temperature and higher. The solar testing procedure will enable comparison not only of the individual commercial formulations, but will also enable testing of adjuvants that could enhance uptake of more virus (phagostimulant) or improve solar protection.

Our results show that repeated applications of commercial CpGV formulations provide a valuable alternative for management of CM in Washington State and elsewhere. Weekly applications for many timed during peak egg hatch integrated with other strategies such as mating disruption or other soft pesticides will likely provide effective population suppression growers with moderate to low CM pressure. Future work at YARL will focus on optimizing the application rate and frequency of CpGV applications as well as improving the persistence and uptake through formulation.

Entomopathogenic nematodes can provide control of overwintering CM when temperatures are 15˚C (60˚F) and higher and moisture is maintained. In trellised apples and conventional pears this moisture was adequately maintained by pre and post wetting trees using existing irrigation. Mulches offer promise to extend the persistence and activity of nematode larvicidal activity. Optimizing the use of mulches in conjunction with entomopathogenic nematodes will be the subject of future research at the Yakima Laboratory.

Entomopathogenic nematodes offer a means of controlling cocooned CM in fruit bins without the use of chemical pesticides. Treatment of bins with suspensions of nematodes can be done while they are immersed in the drop tank or passed through a drencher apparatus. Bins will have to be stored at out of sunlight and strong air currents at 60ºC for 24 hours in order for the nematodes to be most effective.

References Cited

Lacey, L. A., A. Knight, and J. Huber. 2000. Microbial control of lepidopteran pests of apple orchards. In "Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests" (L.A. Lacey and H. K. Kaya, eds.) pp. 557-576. Kluwer Academic Publishers, Dordrecht.

Lacey, L.A., Vail P.V. and Hoffmann D.F. 2002. Comparative activity of baculoviruses against the codling moth *Cydia pomonella* and three other tortricid pests of tree fruit. *J. Invertebr. Pathol.* 80, 64-68.

Funding History

Title: Development of operational strategies for practical control of codling moth neonate larvae and prepupae using codling moth granulovirus (*Cp*GV) and *Steinernema spp.* (Nematoda).
PI: Lawrence A. Lacey, USDA-ARS, Yakima Agricultural Research Laboratory
Project duration: 3 years
Project total (3 yrs): $86,270

	2001	2002	2003
Salaries and wages (includes benefits)			
Technician, partial support for GS-5	20,700	$20,000	$25,000
Summer help, GS-3, 1 FTE (3 mos.)	5,185	5,185	3,500
Subtotal	$25,885	$25,185	$28,500
chemicals, plasticware, misc. materials	1,500	1,500	1,500
Subtotal	1,500	1,500	1,500
Total	$27,385	$28,885	$30,000

FINAL REPORT
WTFRC Project #AE-01-53 **WSU Project #13C-3643-6089**

Project title: New pest management programs for apple and pear

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee, WA; (509) 663-8181 ext. 238; jfb@wsu.edu

Co-PIs and affiliation: Elizabeth Beers, Entomologist; John Dunley, Associate Entomologist; and Vince Jones, Associate Entomologist, WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Cooperator: Mike Doerr, Senior Scientific Assistant, WSU-TFREC, Wenatchee, WA

Objectives:
1. Compare pest control at several sites using codling moth (CM) mating disruption as a base program supplemented with either "conventional" insecticides or newly registered selective insecticides.
2. Monitor CM and other pests using newest technology available to supplement monitoring by crop consultants.
3. Assess the impact of selected natural enemies on pests at each site under different pest control programs.
4. Evaluate the impact of different programs based on crop loss due to pests and costs of pest controls.
5. Use the demonstration sites as opportunities to educate growers and crop consultants on how different selective pest control programs work.

Significant findings - Apple:
1. In apple orchards that started with moderate to high CM pressure, populations declined in both OP and NO-OP blocks over the 3-year duration of the project.
2. In apple orchards that started with low to moderate CM pressure, populations did not increase over the duration of the project.
3. The average insect control cost of the NO-OP program was slightly higher than the OP program in the first year of the project but was lower or the same in the last two years.
4. Removing OP insecticides [essentially chlorpyrifos (Lorsban 4E)] from the pre-bloom control program did not result in increased problems with scale, aphids, mites or sucking bugs in the 15 apple orchards in our study.
5. There were NO unexpected increases in pest problems from "secondary" insects in either the OP or NO-OP blocks at any location. Secondary pest populations were low in all orchards, as was the level of injury to fruit at harvest.
6. Monitoring of codling moth, leafrollers and lacanobia fruitworm provided growers with information needed to respond with well-timed control measures where needed.
7. Codling moth captures in the 10X and DA lure-baited traps were similar in the first two years of the project in all orchards, but in 2003 two orchards had unusual or unexpected differences in DA captures compared to 10X lure-baited traps.
8. The percentage of female CM captured in DA-baited traps in both OP and NO-OP programs was similar in all years but tended to decline over time.
9. Management of lacanobia fruitworm became easy in both OP and NO-OP blocks with the introduction of newly registered insecticides.

10. Education programs have been specifically conducted, including intensive workshops and field days, to transmit the findings from this project.

Significant findings - Pear:

1. After two years of comparison between a "soft" and "conventional" pear pest management program there were no significant differences in most fruit quality measures.
2. The "soft" program was based on use of insect growth regulators in summer and Surround in pre-bloom.
3. The cost of the "soft" program is at this time more than the "conventional" program: (2002- SOFT $355/acre, CONV $335/acre; 2003- SOFT $401/acre, CONV $355/acre).
4. The biggest pest challenge for the "soft" program has been the control of pear rust mite.
5. After two years there has been no significant advantage in increased natural enemy densities to the "soft" control program over the "conventional" program.

APPLE

Methods:

The 15 apple sites established in Washington used the same monitoring methods established in year 1 (2001). Each site consisted of a grower and crop consultants willing to participate in the project. Each apple orchard was treated with CM mating disruption. Within each site, supplements to CM mating disruption were either conventional insecticides (OP supplements) or selective insecticides (NO-OP supplements). Controls for other pests followed a "traditional conventional" or a "selective alternatives" approach. With the exception of certain NO-OP products that might have been donated by the registrant, pest control costs were borne by the grower.

***Monitoring activities*:** Standardized monitoring activities developed under CAMP were be used in cooperation with crop consultants working at the sites to assess pest insects. Pheromone trapping was used for CM, leafroller and lacanobia fruitworm. Non-pheromone monitoring systems were used for CM.

***Assessment of biological control*:** Orchards at each site were monitored to compare effects of different programs on the kinds and abundance of natural enemies. Pest/natural enemy systems to be monitored were spider mites/predatory mites, aphids/parasitoids-predators and leafminer/parasitoids.

***Program evaluation*:** The value of different programs was evaluated by comparing the level of pest density based on monitoring activities, the level of natural enemy density, the amount of crop injury, and the amount of pesticides used and their costs in each program.

***Education*:** Educational events were held in each region where the demonstration study sites were located to provide a local value to the experiences of growers and crop consultants participating in the project.

Results and discussion – Apple:

Codling moth: Initially, a wide range of CM populations was present within the 15 AWII apple sites. In the first generation of 2001, CM traps (10X) averaged 4.0 (OP blocks) and 7.0 (NO-OP blocks) moths/trap and ranged from an average of 0 to 52 moths/trap for the season. Codling moth catches were lower in the second generation in most orchards in 2001, with an average of 1.7 (OP blocks) and 1.5 (NO-OP blocks) moths/trap. There were no differences in the season average capture in 10X lure-baited traps between programs, 5.6 ± 2.1 in the OP blocks and 8.5 ± 4.2 in the NO-OP blocks. There were no significant differences in codling moth captures in 10X traps between the OP and NO-OP treatment blocks in 2002 or 2003 (Fig. 1), and in most all blocks trap counts would be considered moderate to low by industry standards.

The DA lures attract both sexes of CM. Capture of moths in the DA lure-baited traps was low in 2001. In the first generation, captures in the OP and NO-OP blocks averaged 72% and 70% males, respectively, while second generation males accounted for 67% and 63%, respectively. The percentage of mated females was slightly lower in the OP compared to the NO-OP blocks for the first

generation in 2001, 32% versus 50%, respectively. The percentage of mated females increased in the second generation to 80% and 78%, respectively. This same trend was observed in 2002, although in 2003 the percentage of females mated was lower than in the previous two years. Average catch in DA lure-baited traps in the first generation was slightly lower than 10X pheromone lure-baited traps while, in the second generation, catch in DA lure-baited traps was slightly higher than in the pheromone lure-baited traps. This pattern was different in two of 15 orchards in 2003. In these unique cases CM capture in DA traps was about 10 times higher than in the pheromone-baited traps. However, the high level of moth capture in the DA lure-baited traps did not translate into high levels of fruit injury.

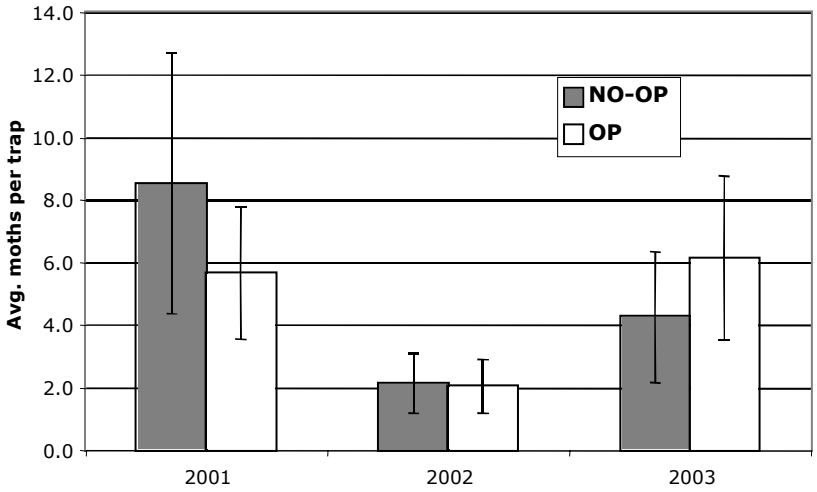


Figure 1. Average CM/trap for the season (10X lure-baited traps).

Leafroller: A wide range of leafrollers densities was detected in the AWII blocks in 2001. The range of moth capture per block was from 0 to 895 (OP mean 233±68 and NO-OP mean 149±62) for OBLR and 0 to 554 (OP mean 68±40 and NO-OP mean 65±31) for PLR. In 2002, the average OBLR moth captures were about the same as 2001 (OP mean 250±75 and NO-OP mean 167±61), while the PLR moth captures increased slightly (OP mean 139±49 and NO-OP mean 160±50). In 2003, average OBLR moth captures were lower than in 2001 or 2002 (OP mean 75±22 and NO-OP mean 121±37), and the PLR moth captures also decreased slightly compared to 2002 (OP mean 94±37 and NO-OP mean 124±66).

Leafroller pressure in each block was categorized using moth capture in a standard lure-baited trap as high: >200 moths, moderate: 100-200 moths, low: 50-99 moths, and very low: <50 moths per trap. The ratio of captures in the standard and low-load lure-baited trap in each block was used to determine if a leafroller population was internal (0 to 5), unclear (5 to 10) or external (>10). Using these criteria, OBLR populations were rated as being "high" in 6 OP blocks (three with an internal population source) and four NO-OP blocks (only one with an internal population source) in 2001, while PLR populations were rated as being "high" in only two blocks in both OP and NO-OP blocks. In 2002, OBLR populations were rated as being "high" in six OP blocks (all of which had an internal population source) and five NO-OP blocks (with four having an internal population source) while PLR populations were rated as being "high" in five OP and six NO-OP blocks (all of which were rated as having an internal population source). Most of the orchards with high OBLR populations were in the Columbia Basin, Quincy and Brewster areas. Orchards with high PLR populations were in the Wenatchee or Yakima valley areas. In 2003, OBLR populations were rated as being "high" in six OP blocks (four of which had an internal population source) and three NO-OP blocks (with two

having an internal population source) while PLR populations were rated as being "high" in four OP and two NO-OP blocks (with none being rated as having an internal population source). The perception that OBLR was becoming more common in all orchards over time was not as well supported by moth capture data as by the identification of larvae found at each location. The trend across the fruit industry is for an increase in the densities of OBLR in apple orchards while PLR densities have declined. This does not seem to be due to any specific pesticide program, conventional or soft, but could be due to OBLR being more fit (resistant) to all pesticides used in apple orchards and because they have a higher biological potential for increase and more alternate hosts than PLR.

Lacanobia fruitworm: This relatively new pest was monitored with a pheromone lure-baited trap, one per block. There was a wide range of total moths captured, but there were little differences between OP and NO-OP treatment blocks in any year. One problem with attempting to compare lacanobia populations with pheromone traps is the strong flying capability of these moths and their ability to move long distances. It is probably better to rely upon other measures, such as foliage feeding or fruit injury, to gauge the effect of different programs on this pest.

Field damage surveys

The AWII apple orchards were surveyed for damage by lepidopteran pests four times during the growing season: late May (leafroller feeding on shoots), early July (codling moth damage to fruit and lacanobia/cutworm feeding on shoots), early August (leafroller feeding on shoots), and late August/September (codling moth damage to fruit). The surveys showed a range of pest injury among the AWII orchards.

Codling moth (CM) surveys revealed very low levels of fruit damage in July in all years. The preharvest surveys typically showed slightly more CM damage than harvest bin samples. The preharvest on-tree fruit injury surveys were also of value because they provided a better idea of injury distribution in the orchard than bin samples. In most blocks where CM damage was found it was largely confined to orchard edges in both OP and NO-OP blocks. There were no significant differences in average CM damaged fruit between treatments in any year with either assessment method (Fig. 2).

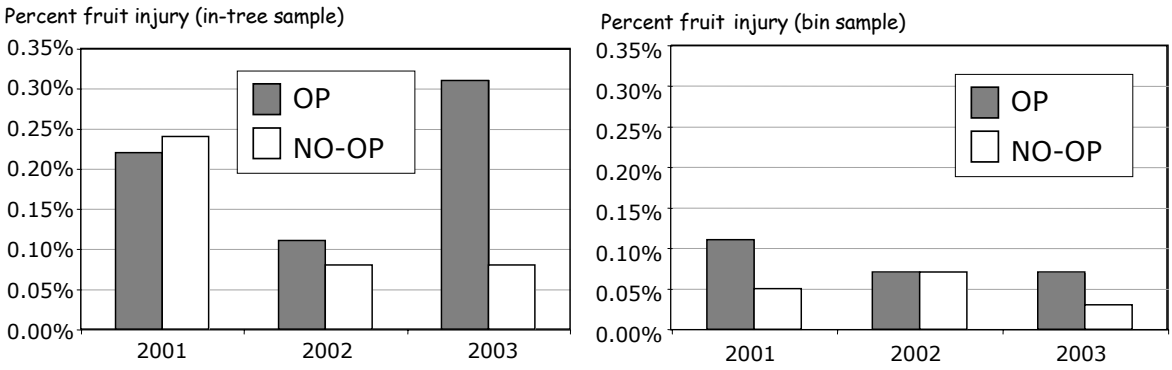


Figure 2. The average percentage of fruit injury from CM in OP or NO-OP blocks over three years as measured by in-tree assessments or bin samples.

Leafroller feeding on fruit was detected in 17, 13 and 16 of the 30 treatment-blocks in 2001, 2002 and 2003, respectively. Fruit injury by leafrollers was higher in the OP and NO-OP blocks in 2001, but there were no significant differences between treatments thereafter.

Fruit feeding by **cutworms** on fruit was detected in 15, 8 and 10 of the 30 treatment-blocks in 2001, 2002 and 2003, respectively. There was no difference in damage levels between treatments in any year.

Damage by **other pests** was sporadic and rare. **Stink bug** damage was a problem in some blocks in some years but was not a widespread problem throughout the AWII blocks. Likewise, **lygus** damage was reported from a few blocks each year, but the levels were always low. Damage by **campylomma** was reported from only two blocks (at 0.04% in each). No **San Jose scale** was found in any of the 30 treatment-blocks surveyed during the three years. **Thrips** damage was found in only one orchard of Granny Smith (0.2% fruit with marking in the OP block, 0.3% in the NO-OP). One orchard of Golden Delicious had **grape mealybug** infesting the fruit at harvest (1.4% infested fruit in the OP block, 0.8% in the NO-OP). Fruit damage levels in 2002 were quite low and similar to the levels found in 2001.

Pesticide use

All AWII apple blocks used CM mating disruption, most at rates close to 200 dispensers/acre, or about half the recommended full rate of the dispensers used. CM mating disruption is included as a single insecticide application based on the rate used (400 dispensers per acre = one full application and 200 dispenser per acre = 0.5 of an application), with cost based on the number of dispensers per acre. The data presented here include only insecticides and miticides, not fungicides, plant growth regulators or other non-insecticidal products. For example, carbaryl (Sevin) used for crop load management and Lime Sulfur or Rally used for disease suppression are not included in the data. The main organophosphate (OP) insecticides used in the OP treatment blocks were chlorpyrifos (Lorsban) [7 of 15 blocks] and azinphosmethyl (Guthion) [7 blocks]. For control of lepidopteran pests, the NO-OP blocks relied upon methoxyfenozide (Intrepid) [9 of 15 blocks] and pyriproxyfen (Esteem) [9 blocks]. The use of the more selective "soft" insecticides was not limited just to the NO-OP blocks; two OP blocks received methoxyfenozide and two OP blocks received pyriproxyfen, generally applied soon after bloom for leafroller control. Spinosad (Success) was used mostly in the OP blocks for leafroller control (eight OP blocks, one NO-OP block). Chloronicotinyl insecticides were used in both treatment blocks but to a greater extent in the OP blocks: imidacloprid (Provado) in two OP and one NO-OP blocks, thiamethoxam (Actara) in one OP and one NO-OP block, and acetamiprid (Assail) in six OP and three NO-OP blocks. Miticides were used in the OP blocks (three) but not in any of the NO-OP blocks.

The total number of insecticide applications was not significantly different between the OP and NO-OP programs in all project years (Table 1). The number of insecticides that were applied that controlled both CM and LR was higher in the NO-OP blocks in all years and was 0.5 more over the duration of the project (Table 1). By contrast, more of the LR-specific insecticides were applied to the OP program blocks than NO-OP blocks. There were no differences in the total number of "other" insecticides applied (mostly aphicides or controls for true bugs) in the OP and NO-OP blocks. Very few miticides were applied to any block, but there were slightly more in the OP blocks than NO-OP blocks.

Table 1. Average number of foliar insecticides applied per acre equivalent to AWII apple orchards, 2001-2003.

	CM+LR		Leafroller		Mites		Other		Total	
Year	OP	NO-OP	OP	NO-OP	OP	NO-OP	OP	NO-OP	OP	NO-OP
2001	1.9	2.4	1.3	0.6	0.1	0.1	1.8	2.4	5.0	5.5
2002	1.7	2.0	1.2	0.2	0.0	0.0	2.1	2.1	5.1	4.3
2003	2.2	2.7	1.1	0.1	0.2	0.0	2.1	1.6	5.6	4.3
3-year avg.	1.9	2.4	1.2	0.3	0.1	0.0	2.0	2.0	5.2	4.7

To compare the relative expense of the OP and NO-OP programs only insecticide data are used. These data do not include Sevin (carbaryl) because its main use is as to modify crop load. It no doubt

has some impact as an insecticide, but its impact is not thought to be an over-riding influence. In 2001, the cost of the OP program was $30 higher than the NO-OP program though there was no statistical difference between them (Table 2). In 2002, the average cost of the NO-OP program declined by $50 per acre and was lower than the OP program, but again the difference was not statistically significant. In 2003, the cost of both programs increased but was essentially the same for both programs. Oil was used about equally by both the OP and NO-OP programs. Guthion (azinphosmethyl) was the most commonly used insecticide, other than oil, in the OP blocks. In orchards that used Guthion, it t was used an average of almost two times per acre at a cost of nearly $25. Intrepid (methoxyfenozide) was the most commonly used insecticide in the NO-OP blocks. In orchards that used Intrepid, it was used an average of two times per acre at a cost of $50. The average cost of pheromone treatments in both OP and NO-OP blocks was $60 per acre.

Table 2. Average cost of foliar insecticides applied per acre to AWII apple orchards, 2001-2003.

Program	2001	2002	2003
OP	151±14.4	150±12.5	175±16.0
NO-OP	183±23.7	138±14.1	170±11.8

In 2001 and 2002, the average number of Sevin applications was between 1.5 and 1.7 per block in 9-12 of the 15 orchards, with essentially no differences between programs. In 2003, the average use of Sevin was lower, 1.2 times in 8 of 15 orchards. The average cost of Sevin treatments was between $10 and $15 per acre across the project with no differences between OP and NO-OP blocks.

Secondary pests and natural enemies

Personnel from the WSU-TFREC visited each orchard several times throughout the season to sample specifically for a number of secondary pests and natural enemies. These samples included campylomma, aphids, white apple leafhopper, leafminer and spider mites. Associated natural enemies were also sampled. There were no differences between treatments over the three years of the study.

PEAR

Methods:

The six pear sites established in Washington used the same monitoring methods established in year 2 (2002). Each site consisted of a grower and crop consultants willing to participate in the project. Within each site, the orchard was split into two pest management programs: conventional (CONV), using traditional practices, and soft (SOFT), arbitrarily limited to insecticides and miticides with IGR-like activity. Kaolin (Surround) was also included in the SOFT program and one conventional block.

***Monitoring activities*:** Standardized monitoring activities developed under CAMP were used to assess pest insects in cooperation with crop consultants working at the sites. Pheromone trapping was used for CM, leafroller and lacanobia fruitworm. Biweekly beating tray samples were used to assess adult pear psylla densities, and leaf-brushing was used to determine densities of pear psylla nymphs and mites.

***Assessment of biological control*:** Orchards at each site were monitored using beating trays to compare effects of different programs on the kinds and abundance of natural enemies. Pest/natural enemy systems monitored were spider mites/predatory mites, aphids/parasitoids-predators and leafminer/parasitoids.

***Program evaluation*:** The value of different programs was evaluated by comparing the level of pest density based on monitoring activities, the level of natural enemy density, the amount of crop injury, and the amount of pesticides used and their costs in each program.

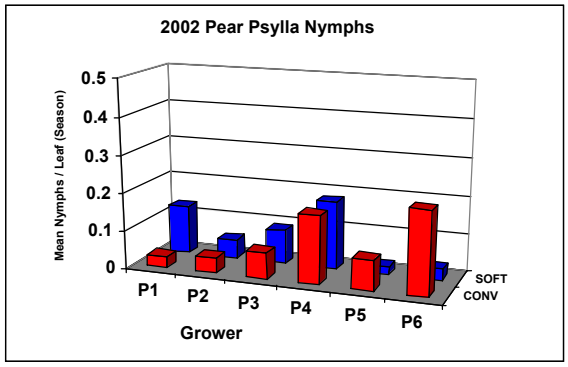
***Education*:** Educational events were held in each region where the demonstration study sites were located to provide a local value to the experiences of growers and crop consultants participating in the project.

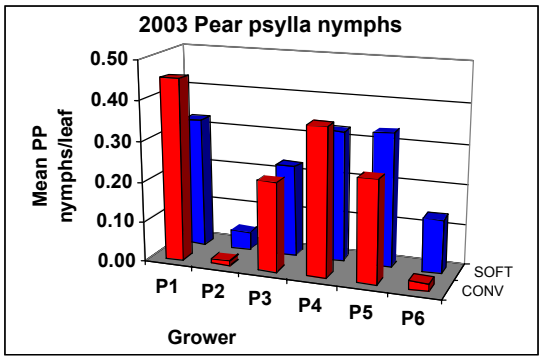
Results and discussion – Pear:

Pest densities

Pear psylla: Although pear psylla (PP) pressure as indicated by nymph densities was moderate to high across the AWII project, no significant difference was noted between treatment regimes. In 2003, the average PP fruit damage was moderate across all AWII orchards. However, two of the orchards (P2, P6) had very low damage levels, two had moderate damage (P1, P3) and two (P4, P5) had damage levels above what would be considered acceptable (>0.5%); no differences were found between management programs within each of the orchards. PP damage in the SOFT and CONV blocks was well correlated with PP densities.

Densities of pear psylla nymphs, 2002 and 2003.





Codling moth: CM adult captures in pheromone-baited traps during the first generation were generally low, with the notable exception of orchard P3. During the second generation, adult CM captures continued to increase in orchards P3 and P4. The elevated captures in these orchards were in both SOFT and CONV blocks and were not associated with treatment regimes.

Leafroller: There were no live leafrollers detected at any of the pear sites during the first or second larval sample in 2003. However, leafroller damage was noted in harvest bin samples in orchards P4, P5 and P6 (Wenatchee area blocks). In fact, the damage observed in the CONV block of orchard P6 was at 0.25%. Leafroller populations were measured indirectly with standard and low-load pheromone lures. On average, captures were slightly higher in the CONV blocks, but the differences in density and control between treatment regimes were not significant.

Mealybug (GMB) and pear rust mite (PRM): Sampling for these pests was difficult and did not provide a clear measure of the risk a block had for damage. GMB was generally not troublesome in the Yakima orchards. All Wenatchee area orchards had some level of GMB infestation at harvest. In orchard P4 a troublesome infestation was observed in the SOFT block, and in P6 an elevated infestation was noted in the CONV block. On average, no differences were noted between treatment regimes. PRM damage was generally very low across the AWII project, with a couple of exceptions. The SOFT blocks of orchards P1 and P3 each had elevated levels of damage, 0.76% and 0.68%, respectively. The ability to manage PRM infestations can be a limiting step in the full implementation of a soft spray program (IGR based or organically accepted) in Washington State pear orchards.

Natural enemies: Several beneficial arthropods were monitored with the limb-tap samples taken biweekly in the AWII pear project. Results indicated no obvious trend in the absolute number of beneficial insects between treatment regimes (CONV seasonal mean = 0.8 beneficials/beat tray, SOFT = 0.9). It may take more time and a better understanding of the disruptive effects of some new insecticides to manage pear orchards to take greater advantage of arthropod natural enemies.

Fruit damage

All treatment-blocks had 2,500 pears examined during harvest for pest damage (Table 3). Russet caused by **pear psylla** was detected in 10 of 12 treatment-blocks, but in only one block (P3 SOFT) did marked fruit exceed 0.4%. In the NCW orchards, psylla marking was consistently lower in the SOFT blocks, in line with the lower psylla adult and nymph counts found there. Fruit was considered marked if the cumulative area of psylla-caused russet exceeded the area of a nickel. **Grape mealybug** counts reflect fruits infested with nymphs, and these infestations were only found in NCW. Fruit infestation by GMB was of particular concern in orchard P4, especially in the SOFT treatment block. **Codling moth** damage was low; the damaged fruit was found mostly on block edges. **Leafroller** damage was quite low, if present at all. **Pear rust mite** damage was noted on the fruit in two orchards (P1 and P3) and was particularly prevalent in the SOFT block of P3; additional controls will be needed in 2003 to reduce this potentially serious pest. Other pest damage was found at low and variable amounts and appeared unrelated to the treatment program.

Table 3. Fruit evaluations at harvest, AWII pear orchards, 2002.

	Percent fruit injury									
	Pear psylla russet		Grape mealy bug nymphs		Codling moth		Leafroller		Pear rust mite	
Orchard	SOFT	CONV	SOFT	CONV	SOFT	CONV	SOFT	CONV	SOFT	CONV
2003	0.74	0.82	2.65	2.15	0.07	0.07	0.01	0.12	0.24	0.03
2002	0.36	0.23	6.02	3.63	0.07	0.17	0.05	0.07	1.54	0.07

Pesticide use and expense

The overall cost of the SOFT treatment regime was higher on average than the CONV in the second year (2003 – Table 4). The differences were driven by a combination of high CM and high PP pressure in a number of the orchards. However, in orchards P4 and P6 the cost of the SOFT treatment regime was substantially less than the CONV, and the cost range of the treatment regimes over the entire AWII project was similar. The distinction between the treatment regimes was driven by the use of Guthion, Lorsban and Assail for CM and Actara and AgriMek for PP in the CONV blocks versus Intrepid and Esteem for CM and Dimilin, Surround and azadirachtin for PP in the SOFT blocks. Generally the SOFT insecticides required more applications and thus cost slightly more.

Table 4. The average number of insecticides (2003) and cost of the different treatment regimes in 2002 and 2003.

	SOFT		CONV	
Year	# of apps	Total cost	# of apps	Total cost
2003 (Range)	15.2 (13-18)	$401.67 (226.74-525.39)	13.3 (11-18)	$355.35 (188.65-570.42)
2002		$355.00		$335.00

SUMMARY

Apples: For three years the use of a pheromone-based pest management program that eliminated the use of OP insecticides was successful in controlling key pests while experiencing no detrimental effects from increased injury to foliage or fruit from secondary pests. The number of insecticides applied to the OP and NO-OP program blocks was essentially the same over the three-year study and the cost of the insecticides was also similar, that is not statistically different. While there were no obvious negative results associated with the NO-OP program, there were no observable positive effects on natural enemy densities or, for that matter, reductions in use of insecticides to control secondary pests. While it is possible that the NO-OP program could eventually result in higher

densities of selected natural enemies, use of other pesticides, like Sevin and possibly some fungicides, could represent a significant barrier to achieving this goal.

Pears: Insect pest control in pear was maintained in soft management programs, arbitrarily limited to insect growth regulators, relative to conventional programs over a two-year period. Pear psylla and codling moth control was not significantly different either year. Grape mealybug and pear rust mite control remain problematic, as there are no soft materials available. Densities of natural enemies were expected to be higher in soft programs but were not found to be significantly different between programs. In both years of the project, numbers of applications as well as costs of the soft program were higher, though not significantly so. Thus, it appears that pest management is feasible when limiting pest management tactics to the more environmentally benign materials, although costs may increase. Further study is necessary to determine if long-term implementation can increase biological control and reduce overall costs.

Budget:

Project title: New pest management programs for apple and pear
PI: Jay F. Brunner,
Project duration: 3 years (2001-2003)
Three-year total: $278,059

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)	All years
Total from WTFRC	$89,760	$106,321	$94,978	$291,059
From IFAFS/RAMP	$55,000	$55,000	$55,000	$165,000