Washington Tree Fruit Research Commission

2003 Apple Entomology Research Review

January 27, 2003 Richland, WA

Time	Page	PI	Organization	Title	Duration
9:00		Brunner	WSU - Wenatchee	IFAFS/RAMP update	
9:05	1	Unruh	USDA - ARS, Wapato	Effects of new insecticides on natural enemies of apple pests	01-03
9:10	7	Unruh	USDA - ARS, Wapato	Genetic markers to identify pests	02-04
9:15	13	Beers	WSU - Wenatchee	Feeding, thresholds, & pheromone trapping of Campylomma	02-04
9:20	19	Brunner	WSU - Wenatchee	Stink bug behavior & control	02-04
9:25	25	Brunner	WSU - Wenatchee	Behavioral-based control tactics for apple pests	02-04
9:30	31	Brunner	WSU - Wenatchee	New pest management program	01-03
9:35	37	Jones	WSU - Wenatchee	Mechanisms of mating disruption in CM & leafroller	02-03
9:40	43	Jones	WSU - Wenatchee	Sampling plans for leafrollers & their natural enemies	01-03
9:45	49	Lacey	USDA - ARS, Wapato	Strategies to control CM using granulovirus & nematodes	01-03
9:50	55	Landolt	USDA - ARS, Wapato	Insect responses to induced defensive chemistry	01-03
9:55	58	Yee	USDA - ARS, Wapato	Optimizing ammonia with traps to manage apple maggot	01-03
10:00	64	Yee	USDA - ARS, Wapato	Re-evaluation of host use by apple maggot	02-03
10:05	68	Felsot	WSU - Richland	Pesticides exposure & drift - alternative sprayer technology	02-04
10:10-10:30		**BREAK**			
10:30	74	Beers	WSU - Prosser	Biology, ecology, & management of thrips	02-02
10:45	78	Alston	Utah State University	Plum curculio biology, range, & distribution	00
11:00	85	Walsh	WSU - Prosser	Lygus bug & western flower thrips ecology	01-02
11:15	93	Knight	USDA - ARS, Wapato	Bisexual monitoring of CM & establishing a female biofix	00-02
11:25	101	Knight	USDA - ARS, Wapato	Managing CM with bisexual attract & kill formulations	00-02
11:35	105	Knight	USDA - ARS, Wapato	Pheromones & attraction inhibitors for CM mating disruption	98-02
11:45	NA	Smirle	PARC	Leafroller resistance to Confirm, Intrepid, & Avaunt	00-02
12:00-1:30		**LUNCH**			
1:30	110	Landolt	USDA - ARS, Wapato	Development of feeding attractants for control of moth pests	00-02
1:40	115	Landolt	USDA - ARS, Wapato	Optimization of attractants for spotted cutworm	02-02
1:50	117	Unruh	USDA - ARS, Wapato	Leafroller biological control	99-02
2:05	123	Brunner	WSU - Wenatchee	Bait-based monitoring systems for lepidopteran pests	00-02
2:20	134	Pszczolkowski	WSU - Pullman	Enhancing for insecticides targeting lepidopteran larvae	00-02
2:35	144	Miliczky	USDA - ARS, Wapato	Biology & behavior of natural enemies of apple pests	01-02
2:50	153	Schrader	WSU - Wenatchee	RAYNOX for suppression of insects in apple & pear	00-02
3:05	164	Judd	PARC	Field wind tunnels to improve mating disruption systems	98-01
3:20	173	Hebert	WSU - Prosser	Bundled gas chromatograph with CI & MSD	02-02
3:30-5:30		**POSTER SESSION**			

CONTINUING PROJECT REPORT **YEAR 3/3**

TITLE: Effects of New Insecticides on Natural enemies: Acute toxicity and sub-lethal effects

CO-PIS
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Dave Horton, USDA-ARS Yakima
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COLLABORATORS
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OBJECTIVES (2003):

1. Test acute toxicity of the next 5 new insecticides to 9 arthropods using a combined topical, residue and per-os exposure method
2. Develop bioassay methods to measure sub-lethal effects on beneficial insects
3. Test sub-lethal effects in those cases where acute effects are trivial or short-lived.
4. Model acute and sub-lethal toxicity data to provide field testable predictions of pesticide effects (New objective since 2002)

Significant Findings:

- Bioassay procedures were developed for 5 of the 9 target arthropods: *Forficula auricularia, Chrysoperla carnea*, *Galenodromus occidentallis,and Colpoclypeus florus* and *Anthocorus nemoralis* and is under development for a 6th *(Deraeocoris brevis).*
- Acute toxicity from exposure to the first 5 new insecticides (Provado, Actara, Intrepid, Esteem, Success) was estimated for lacewing, predator mite and earwigs and for 3 and 2 insecticides for *Anthocoris* and *Colpoclypeus.*
- Sublethal bioassays have been done for all insecticides for egg, larval, and adult lacewings and adult predatory mite, and for one insecticide for Anthocoris bugs..
- *Galendromus occidentalis*: Provado and Actara both repelled and reduced fecundity. When these effects were combined, total egg production was reduced 0-66% by Actara and 0- 99% by Provado at field rates, depending on population tested. Surprisingly the lepidopteran insecticide, Success, caused a 44% reduction in total egg production. The IGRs Intepid and Esteem caused very moderate reductions total egg production. Fecundity loss was highly variable among populations and replicates as was the tendency of the mites to run off the treated surfaces.
- *Chrysoperla carnea*: Actara and Provado caused high mortality of larvae and adults at field rates (and Actara at 10% field rates) but had no effect on eggs. Intrepid, Esteem and Success were not acutely toxic to any stage tested but had sub-lethal effects. Intrepid reduced adult fecundity and egg hatch; Esteem prolonged development of the last larval stage. Success reduced adult fecundity.
- *Colpoclypeus florus*: Actara, Assail, and Provado caused 100% mortality at field rates and 56%, 65% and 25% at a tenth of field rates. Survivors of Actara show full fertility in preliminary trials.
- *Anthocoris nemoralis*: Actara and Provado were acutely toxic at field rate as was Provado at 10% of field rate. Actara caused 40% mortality after 2 weeks but survivors were nearly as fertile as control insects.

- *Forficula auricularia:* Provado, Guthion, and Imidan all showed acute toxicity to immatures at both 1 and 15 days. Success showed acute toxicity to adults after 15 days. Control mortality was highly variable and sub-lethal bioassays require 2-3 months.

Methods:
In lieu of rehashing methods as outlined in the first year proposal we describe the bioassay methods that were developed and used in 2002 as a major part of the results of year 1. These methods will continue to be used in the coming year, with improvements as required**.** Bioassay methods are species-specific and thus we present them by species.

Results and Discussion
Forficula: Earwigs began to hatch in artificial nests that had been maintained in the laboratory over the winter. When sufficient numbers of second and third instar nymphs were available, ten individuals were placed in a petri dish and treated in a Potter spray tower with 2 ml of the test materials. Treatments were replicated five times and the materials tested were: Guthion, Imidan, Intrepid, Esteem, Dimilin, AgriMek, Provado, and two controls—one treated with water and the other unsprayed. The materials were applied at the maximum label field rate and one-tenth the field rate. In the fall, adults were collected from a block where no insecticides had been applied and treated in a similar manner as described above but only five individuals were placed in a petri dish and only the maximum label rate was tested. Males (six replicates) and females (seven replicates) were treated separately. The materials used in the adult test were: Intrepid, Esteem, Dimilin, AgriMek, Provado, Assail, Danitol, Mitac, Success, and a water control. After 15 days ten surviving males and females from each treatment were paired and a single pair was then placed in an artificial nest. At present, ten pairs of individuals from each treatment (excluding the Success treatment where no females survived) are being maintained in artificial nests in the laboratory. Survival and fecundity will be assessed in the spring of 2003. Because of the long life history of earwigs, the sub-lethal studies will be less extensive than used in other taxa. Rolled corrugated domicile appears to be an effective method to assess earwig populations and to collect earwigs from the field and has many advantages over the beating tray which is inefficient for sampling earwigs. Field studies not presented here suggest that earwigs may be a useful biological indicator species for the effects of pesticides in the environment, especially in pears.

Chrysoperla: Experimental insects were purchased from an insectary (Buena Biosystems), as cocooned pupae. Larvae were fed on eggs of *Ephestia kuehniella*, obtained from Beneficial Insectaries, and adults were fed on an artificial . Three life stages, 0.5 day old eggs, 2 day old 1st instars, and 3-4 day old adults were tested and all *C. carnea* stages were kept in an environmental chamber set at 23°C, 70% R.H. and 16 hours of light. All sprays were carried out using a Potter Tower with an air supply set at 10psi and spray nozzle set at 4psi, using an amount of product calibrated to deliver 1.5mg of formulated solution per cm^2 of arena surface.

Eggs were sprayed in groups of 10, and subsequently separated into individual 2oz plastic cups with eggs of *E. kuehniella*. Acute mortality of hatched larvae was assessed 5 and 6 days later. Larvae were sprayed in groups of 10 and immediately transferred to individual 2oz plastic cups with eggs of *E. kuehniella*. Acute mortality was scored after 48h. Adults were sprayed in groups of five, immediately after immobilization with CO_2. They were transferred to individual 2oz plastic cups, provided with artificial diet and water saturated cotton wicks, and acute mortality scored after 48h.

At the full field rate Actara was 100% lethal to both larvae and adults of *C. carnea*, and Provado was 100% lethal to larvae and 73% lethal to adults. Substantial mortality also occurred at the 10% rate for Actara, but only for larval exposure in the case of Provado. Thus Provado proved more lethal to larvae than adults, while the reverse was true for Actara. There was no evidence for topical toxicity to eggs of *C. carnea* from any of the selective products

For studies of sublethal effects, larvae and adults were subjected to simultaneous topical, residue and oral exposure at the higher of the two dose rates in those cases where survival from acute toxicity tests was greater than 25%. In all cases, products were compared to distilled water controls. Larvae were sprayed in groups of 10 and transferred to individual Petri dishes with dry residue. Ephestia eggs, the arenas and *C. carnea* larvae were all sprayed on the same day and sprayed eggs were used over the first 5 days of the assay, followed by unsprayed eggs. Eggs were replaced every 2-3 days and lacewing larvae were provided a piece of cardboard in which to pupate. Pupae were isolated in 2 oz. cups and upon emergence adults were moved to 4 oz. cups with food water and mate. Egg production was monitored for 14 days. Larval assays were based on 30 replicates which were followed through to egg hatch of the subsequent generation to determine immature development time, immature and adult (first 14 days) survivorship, adult size (hind tibia length) and fecundity (first 14 days), and the success of egg hatch.

For adult exposure sublethal assays unmated females and adult male *C. carnea* were sprayed in groups of 6 within Petri dishes after immobilization with CO_2. 15 pairs of adults were transferred to adult arenas with dry residue, artificial diet and a small cotton wool wick in a glass vial. Eggs were collected every second day, and the artificial diet replaced. Two sets of 30 hatching larvae, approximately equal numbers from each surviving pair of adults, were collected on days 3-4 and 11-12 to follow their performance through the subsequent generation. Measurements noted were survivorship, fecundity (first 14 days) and egg hatch of the sprayed adults, and immature development time, survivorship, fecundity (first 6 days) and subsequent egg hatch of the F1 generation.

Chronic sub-lethal effects on population growth rates were observed for the two IGRs. Although there was no apparent effect on development time, growth (adults size) or survivorship to adult, Intrepid showed some reduction in adult fecundity over the first 14 days, and on the success of egg hatch (data not yet fully analyzed). A similar effect may also occur with Success (data not yet fully analyzed). In contrast, Esteem prolonged the 3rd larval instar, but showed no effects on survivorship, fecundity or egg hatch (data not yet fully analyzed).

The combined routes of toxicity for 10% field rate Provado had no effect on adult survivorship over a 14 day period was comparable to the results from topical exposure alone, suggesting that the addition of the oral and residual exposure had no effect on survivorship. No sub-lethal effects of 10% field rate Provado were detected. As in the larval bioassays, Intrepid reduced adult fecundity for both the adults that were directly exposed and the two sets of subsequent F1 adults. However, there was no apparent influence of Intrepid on egg hatch in these assays. Although Esteem prolonged larval development in the larval assays, this effect was not seen in the F1 generation when adults were the life stage sprayed. Considerable effort has been spent on developing projection matrices to estimate/simulate the combined effects of acute and sub-lethal effects of insecticide exposure.

Galendromus: Bioassays were conducted using a cohort of 20 adult female *G. occidentalis*, reared from eggs laid within a 48-h period. The arena used for both producing females for study and for bioassays consisted of lima bean disks 36 mm in diameter, placed lower surface facing up, on a pad of moist cotton in a 90-ml plastic cup. The edge of the leaf was ringed with Tangle Foot to keep the females from running off the leaf. Females were fed with mixed stages of twospotted spider mites *(Tetranychus urticae* Koch) and pollen. Test arenas were sprayed with two concentrations of various pesticides, plus a separate distilled water check for each pesticide using a Potter Spray Tower. There were five replications per concentration (completely randomized design). Pesticide concentrations corresponded to the concentration obtained using the highest rate allowed on the label, applied at 100 gpa. Where the rates were different between the apple and pear label, the higher rate was used. The second concentration was a 1 in 10 dilution of the first concentration. Two ml of each solution was used for each application (disks were treated separately). Females were present on the disks at the time of application, thus the method of exposure was both contact and through residues on the leaf disk.

The disks were evaluated daily through the 7-d test, recording both the number of live females and the number of eggs produced. Eggs were removed each day as they were counted but females were left undisturbed. Females were fed daily with mites and pollen as described above, so that food was not limiting at any time during the test nor was it treated with insecticides. Variables analyzed were the total number of eggs produced during the course of the experiment; female-days=the number of females per day cumulated over the course of the experiment and fecundity=total eggs divided by female-days. Female-days represent residency on the leaf, whether attrition in the numbers were due to mortality or runoff. The total number of eggs laid integrates both residency and fecundity.

The two chloronicotinyls,Actara and Provado, showed one or more effects on *T. occidentalis* in one or more tests. Two of the four tests with Actara showed a reduction (66%) in total egg production (lab colony) or residency (29%) (CRO). The inconsistency among populations tested may have been due to prior exposure to chemicals. The effect of Provado was more pronounced; in one test (Skeele population) there was a 99% reduction in overall egg production at the field rate, with corresponding reductions in residency and fecundity. There was also a 24% reduction in residency after exposure to 10% of the field rate, indicating that the effect might continue well beyond the time of application. The rate tested was the high label rate for pear, which at 20 fl oz was substantially higher than the high rate for apple (8 fl oz). On the latter crop, it is frequently used at a lower rate, but these results suggest that there may be an effect as low as 2 fl oz/acre.

The IGRs Intrepid and Esteem were tested against three populations each, and there was a detectable effect in one of the variables in one of the three tests. For Esteem, the Bench Rd. populations experienced a 52% reduction in total egg production, and a 35% reduction in female residency. For Intrepid, there was a 29% reduction in total egg production in the lab colony. The two miticides, Acramite (bifenazate) and Secure (etoxazole) had no effect on fecundity or residency of *T. occidentalis* at the high rate (year 2 priority materials).

Anthocoris: Virgin female *Anthocoris nemoralis* (2-4 days old) were treated with 2 ml of solution in small petri dishes using a Potter Sray tower. Females were immediately moved to small, psylla-infested pear seedlings in small, vented cages. An untreated male was added to each cage. Pairs were moved to new seedlings every 3-4 days until 2 weeks following treatment, at which time bugs were discarded. Mortality was checked each time the pair was moved. Eggs deposited into the seedlings were allowed to hatch, and the nymphs were counted on each plant. Both acute toxicity and sublethal studies are ongoing, but here we report results for Actara and Provado at 10% field rates.

Provado caused 100% mortality of adult females within 3 days of treatment. Mortality rates of Actara-treated bugs was lower than that caused by Provado (40% by 2 weeks), but was higher than that in control bugs. Production of nymphs by surviving females was slightly higher in control bugs (15 nymphs per female by 2 weeks) than Actara-treated bugs (13 nymphs per female by 2 weeks). Because eggs were not counted, results are highly conservative estimates of 2-week fecundity.

Colpoclypeus florus: For acute toxicity screens, five 3-5 day old female wasps greater than 0.4 mg wt, immobilized by chilling over ice, were placed in a small arena (4.5 cm Petri dish) with a damp piece of filter paper on the bottom. Pesticides were sprayed in a potters spray tower and insects were given 15 minutes to dry and the plate was covered. Mortality was scored at 24 and 48 hr. Actara, Assail, and Provado caused 100% mortality at field rates and 56%, 65% and 25% at a tenth of field rates. IGRs have not yet been tested.

For sublethal exposures adults were treated as above and , on the same day, leafroller retreats (apple leaves folded by and containing a 4^{th} instar leafroller larvae) and a streak of honey on wax paper were treated in the spray tower. After 2 hr drying time, a leafroller retreat, some of the sprayed honey, and a single surviving wasp were confined together in a clean petri plate. After 3 days, a second leafroller retreat (unsprayed) replaced the first retreat, and the wasp was confined for 4 additional days. Adult

survival after this 7 days was recorded and the wasp was retained for measurement of hind-tibial length (=size; used to estimate potential fecundity). Number of offspring emerging from each exposed host, and percentage of hosts parasitized (as estimated from silken webbing from both the first and second retreat were tallied after 14 days. Sublethal biassays are just getting started.
Survivors of Actara show full fertility in preliminary trials.

Proposed schedule of accomplishments:
Objectives (2003) 1 and 2 will be completed for all test insects by December 2003 for the first[t] 13 pesticides listed. Objective 3 has begun for all but one test species and should be completed for the first 5 insecticides in 12 months. Objective 4, has begun with the lacewings (Nick Mills) and model development should be completed in 12 months.

TITLE: Effects of New Insecticides on Natural enemies: Acute toxicity and sub-lethal effects
CO-PIS Tom Unruh, Dave Horton, Elizabeth. Beers, Richard Hilton, Helmut Reidl. Nick Mills
Current year request 2003: $50,000
Project total (3 years) $117,220
Budget:

Item	2001	2002[1]	**2003[1]**
Salaries		45,000	**45,000**
benefits		5,000	**5,000**
IFAFS Matching		86,000	**86,000**
Total (WRFRC)	17,220	50,000	**50,000***

[1] $10,000 per location; and funds should be sent to 5 locations as in 2002.

Pear supported project at $15,000 in 2002

Table 1. Pesticides tested or to be tested in program. Materials 1-5 have or will be tested in first round (2002), and materials 6-13 in the second round (2003; highlighted) with some variation among species being tested. (For example, miticides will get higher priority in the *Galendromus* work).

Priority	Compound/Form	Chem. Name	Class
1	Provado 1.6F	imidacloprid	Chloronicotinyl
2	Actara 25WDG	thiamethoxam	Chloronicotinyl
3	Intrepid 2F	methoxyfenozide	IGR: Molt accelerator
4	Esteem 0.86EC	pyriproxifen	IGR: JH analog
5	Success 2SC	spinosad	fermentation product
6	Assail 70WP	acetamiprid	Chloronicotinyl
7	Calypso 480SC	thiacloprid	Chloronicotinyl
8	Aza-Direct 0.0987	azadirachtin	Plant derived
9	Acramite 50W	bifenazate	
10	Savey 50DF	hexythiazox	
11	Secure 72WDG	etoxazole	
13	Surround WP (95%)	kaolin clay	
	Pyramite 60W	pyridaben	
new	Mesa	milbamectin	fermentation product
new	Piton	acequinocyl	
		Chlorthianidin	
	Agri-Mek 0.15EC	abamectin	fermentation product
	Apollo 4SC	clofentezine	
	Avaunt 30W	indoxacarb	
	Envidor 240SC	spirodoclofen	
	Orchex 796	petroleum oil	
	Guthion 50W	azinphosmethyl	organophosphate
	Imidan 70W		organophosphate
new		flonicamid	
new		buprofezin	

CONTINUING PROJECT REPORT **YEAR 2/3**

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

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CO-PI Nina M. Bárcenas, Colegio de Postgraduados, Texcoco, Mexico
Postdoctoral Research Associate, Washington State University

Collaborator Lisa G. Neven, USDA-ARS, Wapato

OBJECTIVES (2003):
1) Complete RT-PCR protocol optimization for apple attacking Lepidoptera of the USA and seek its acceptance by pest identification services of Mexico, Canada, Japan, and Taiwan.
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)
3) Complete collection of mite pests of tree fruits, collect DNA sequences, identify diagnostic primers, and develop pilot protocols for identification.

Significant Findings:

- Extraction procedures were developed to allow retrieval and amplification of mtDNA from single legs of decades-old museum specimens of moths.
- Sequences for the COI gene of the mtDNA was collected, aligned, and analyzed for most of the North American and European tortricid Lepidoptera attacking apple fruit.
- A single hot spot in COI showed significant differences between all species and diagnostic primers for the internal fruit feeding species were designed and tested.
- A procedure was developed for these primers using a real-time PCR instrument that allows unambiguous species identifications in < 4 hrs.

Methods for 2003:
1.RT-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.
2.To expand our diagnostic array to key world pests of temperate tree fruits requires requesting specimens from scientists around the globe. Already many of the more important pests have been sequenced in the CO1 gene (the most studied mtDNA in insects). But, important species are needed (from Europe: the plum and pear codling moths, *Cydia funebrana* and *Cydia pyrivora*; from Asia: the Asian codling moth and a dozen more). Acquisition of sequence for these species will be simplified by our ability to amplify DNA from museum specimens.
3.For mites the same general procedures apply as used for any organism. Steps are: acquire specimens, extract/release DNA, amplify part(s) of genome using universal primers, sequence amplification product, align library of sequences, discover diagnostic sites, develop and test diagnostic primers/protocols. We are collecting mite specimens from collaborators and have collected and organized the *Tetrancychus, Eutetranychus*, and related mite sequences from GenBank.

Results and Discussion: Based on 2002 objectives (in bold):

1) Develop species-specific primers for key problematic lepidopteran pests.

DNA sequences were produced, collated, and analyzed for the CO1 gene of the mitochondrial genome of codling moth, lesser apple worm, oriental fruit moth, and cherry fruitworm (the fruit boring complex) and from oblique-banded leafroller, omnivorous leafroller, *Pandemis* leafroller, and *Lacanobia* fruitworm (for non-boring species). Additional sequences for these and others species were retrieved from the public databases (GenBank) and also aligned and analyzed. Diagnostic primers were designed to differentiate all species from one another and have been synthesized and tested for the more critical fruit-boring species. Table 1 identifies the tortricid lepidoptera (codling moth, fruitworms, leafrollers) in North America, Europe, New Zealand and Australia; for those highlighted we have acquired and organized their DNA sequences. Table 2 shows the DNA sequence of codling moth at the diagnostic region and the base pair similarities (dots) and differences (A, T, G, or C) between 12 populations of codling moth as well as several species of tortricid pests that are likely to be found in apple fruits around the world. The right column shows the number of base pair differences at the codling moth diagnostic primer (underlined). Table 2 shows that all species show distinct sequence differences at the hotspot in CO1 and that within species there is little or no variation (no differences between 12 codling moth populations from Europe and USA, none between the USA and Europe populations of OFM, and one difference between two populations of BHL). This diagnostic region does not show much relationship to the evolutionary relatedness of the various species. For example, the most similar to codling moth is OBLR, even though these are very distinct evolutionary lineages, while the *Grapholita* species show differences of 3-7 bp from codling moth.

2) Develop and test procedures suitable for any diagnostic lab using RT-PCR.

Currently RT-PCR temperature profiles are being optimized for a real-time PCR instrument (SmartCycler) that the laboratory purchased to support this and related efforts. Preliminary results indicate we have a highly robust method to differentiate species within 1 day of receipt
These optimizations will be complete in time for the research review and will be presented graphically there.

Even the small, 2-base pair, difference between codling moth and OBLR pose no problem for this technology as single nucleotide differences are now being routinely used in molecular biology studies (especially in disease detection in human). Figure 1 shows the amplification profile (increase in florescence as DNA amplifies) for codling moth and OBLR using codling moth primers. At 20 cycles codling moth exceeds the threshold for positive amplification while OBLR does not exceed the threshold in 40 cycles. Subsequent melt analysis of the amplified DNA shows a different melting temperature for bands of different sizes demonstrating that non-target DNA was very modestly amplified on OBLR (primer-dimer amplification; not shown)

3) Develop sequence information for mites, mealybug complex, or other pests

We have made no attempt to pursue this objective except to begin collecting verified mite specimens from various collaborators. Instead of pursuing a new group we have expanded our interests to other lepidopteran pests of apple that do not occur in North America. We would like to continue to collect these lepidopteran species and have a protocol that is globally diagnostic for the fruit boring and non-boring lepidoptera on apple (new objective 2). We have found that we can often, but not always, amplify the DNA from museum specimens (ie. pinned insects) and this greatly facilitates acquiring sequences. We think it would be valuable for the industry to not only have rapid identification of species intercepted in our exports, but also to be able to identify unambiguously immature specimens intercepted as imports into North America. The latter would help protect our growers from the importation of new, exotic pests. Specifically, these data and protocols would lead to exact information on the species intercepted in interceptions on Chinese apples and pears. **We request input from the commission on priority species groups and this priority shift.**

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)

Current year request $36,200

Project total (3 years): $100,400

Budget:[3]

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

[1]Salary represents part of support for Nina Bárcenas, a visiting professor. The effort will be fully supported by a 50% technician on base funds.
[2]Salary represents 50% of a postdoctoral candidate.
[3]This proposal is also being submitted to the Foreign Agricultural Service Technical Research Fund. If funded, requested support from WTFRC will be reduced.

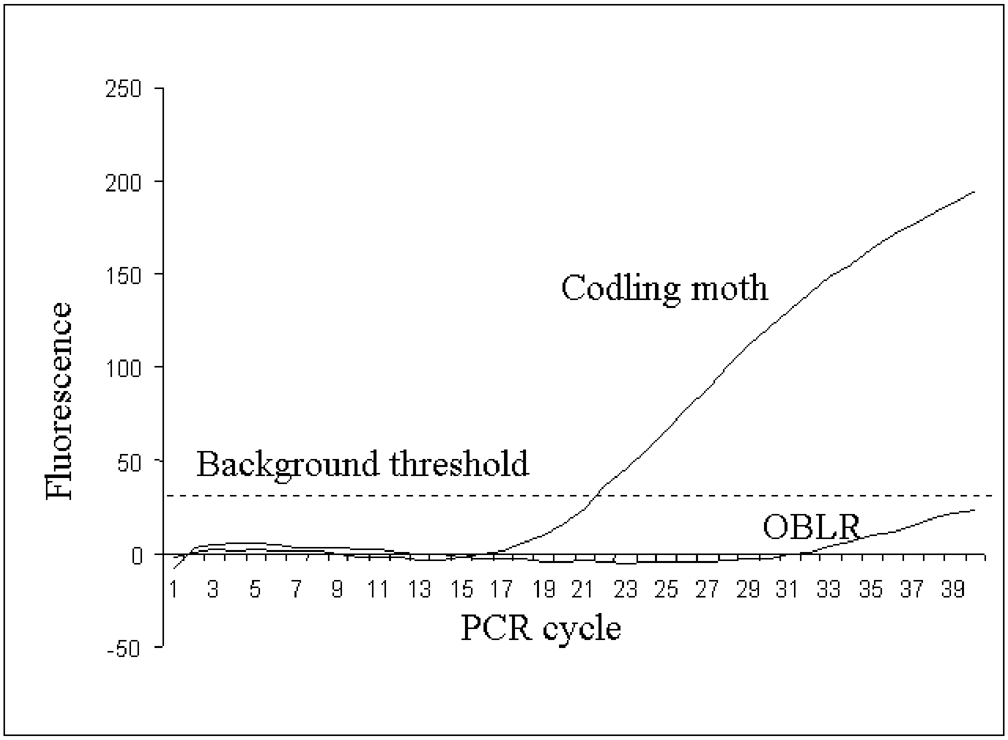
Table 1.**Tortricidae pests likely to be found in apple fruits from North America, Europe, New Zealand and Australia.**

Species	**Common name**	**ID**	**Origin, Distribution**
	Internal Fruit Feeders		
Cydia pomonella	Codling moth	CM	Eurasian, World
Cydia pyrivora	Pear moth, Pear tortrix	PET	Eurasian
Grapholita janthinana	Hawthorn berry moth, Hawthorn leafroller	HBM	Eurasian
Grapholita lobarzewskii	Apple seed moth	ASM	Europe
Grapholita molesta	Oriental fruit moth	OFM	Eurasian, NA
Grapholita packardi	Cherry fruit worm	CFW	North America
Grapholita prunivora	Lesser apple worm	LAW	North America
	Leafrollers - External Fruit Feeders		
Archips argyrospilla	Western fruit tree leafroller	WFT	North America
Archips fuscocupreanus	Apple tortrix	APT	Japan, NA
Archips rosana	European leafroller	ELR	Eurasian, USA
Archips semiferana	Oak leafroller	KLR	North America
Argyrotaenia citrana	Orange tortrix, Apple skin worm	ASW	North America
Argyrotaenia velutinana	Red banded leafroller	RBL	North America
Cacoecimorpha pronubana	European carnation tortrix	ECT	Eurasian, USA
Choristoneura rosaceana	Oblique banded leafroller	OBLR	North America
Ctenopseustis herana	Light brown apple moth	LBA	NZ- Australia
Ctenoseustis obliquana	brown headed leafroller	BHL	NZ- Australia
Epiphyas postvittana	light brown apple moth	LBM	NZ-Australia, Eurasia,
Hedya pruniana	Plum tortrix	PLT	Eurasian
Pammene rhediella	Fruitlet mining tortrix	FMT	Eurasian
Pandemis cerasana	Barred fruit tree tortrix	BFT	Eurasian, USA
Pandemis heparana	Dark fruit tree tortrix	DFT	Eurasia, WA
Pandemis pyrusana	Pandemis leaf roller	PLR/3LL	North America
Planotortrix excessana	green headed leaf roller	GHE	New Zealand
Planotortrix octo	green headed leaf roller	GHO	NZ-Australia, Europe,
Platynota flavedana	Variegated leafroller	VLR	North America
Platynota idaeusalis	Tufted apple budmoth	TAB	North America
Platynota stultana	Omnivorous leafroller	OLR	North America
Sparganothis sulfureana	Sparganothis fruitworm	SFW	North America
Spilonata ocellana	Eyespotted budmoth	EBM	Eurasian, NA

Table 2. DNA sequence of codling moth at the diagnostic region. The right column shows the number of base pair differences between the diagnostic primer (underlined) for codling moth and 12 populations of codling moth as well as several species of tortricid pests around the world that are likely to be found in apple fruit (see Table 1 for species I. D.).

Species I.D.	DNA sequence	# of bp differences
CM-Pop1	: GCTCTTTTACTTCTTTTATCATTACCAG :	0
CM-Pop2	: .... :	0
CM-Pop3	: .... :	0
CM-Pop4	: .... :	0
CM-Pop5	: .... :	0
CM-Pop6	: .... :	0
CM-Pop7	: .... :	0
CM-Pop8	: .... :	0
CM-Pop9	: .... :	0
CM-Pop10	: .... :	0
CM-Pop11	: .... :	0
CM-Pop12	: .... :	0
OFM-Pop1	: .........T.A..AC.T...C...... :	6
OFM-Pop2	: .........T.A..AC.T...C...... :	6
CFW	: .........T.A..............C. :	3
LAW	: .....CC.....T.AC.....C....T. :	7
OBLR	: ....................T.....T. :	2
PLR	: ..C.........T.A.....T.....T. :	4
DFT	: ............T.A.....T.....T. :	4
WFT	: ...T.A......T.A.....T....... :	5
ASW	: ..A......T.AT.A...........T. :	5
OLR	: ..C..A.........C.T........T. :	4
LBA	: ..AT.A...T.AT.AC.....C....C. :	8
BHL-Pop1	: ..AT.A...T.AT.AC.....C....T. :	8
BHL-Pop2	: ..AT.A...T.AT.AC.....C....T. :	8
BHL-Pop3	: ..AT.A...T.A..AC.....C....T. :	7
LBM	: ..C........AT..C.C.......... :	4
GHE	: ..C..A......T.A...........T. :	4
GHO	: ..C..A......T.A...........T. :	4
LFW (Noctuidae)	: ..AT.....T.AT.A.....T.....T. :	6

Figure 1. Real-time monitoring of double-stranded DNA accumulation in samples of Codling moth and OBLR amplified with Codling moth diagnostic primers in the presence of SYBR-green I fluorochrome. The minor amount of DNA that begins to accumulate in OBLR is non-specific and shows a different melting temperature than the target DNA region (not shown). Melt curve analysis is a simple form of verification that can be conducted after the PCR reaction and requires only 5 additional minutes. The whole process reported here requires ½ hour.



CONTINUING PROJECT REPORT **YEAR 2/3**
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, 1100 N. Western Ave., Wenatchee, WA; (509) 663-8181 ext 234; ebeers@wsu.edu

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

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. Of the newer cultivars evaluated, 'Gala,' 'Fuji,' 'Cameo,' and 'Granny Smith' showed some damage by campylomma feeding. Only 'Braeburn' did not show any damage symptoms under the experiment conditions.
2. The susceptibility of the cultivars 'Gala,' 'Granny Smith,' 'Fuji,' and 'Cameo' was similar to that of 'Delicious,' and lower than that of 'Golden Delicious.'
3. 'Golden Delicious,' the most susceptible cultivar tested, had a visibly lower density of trichomes on the surface of the fruitlet during bloom compared with the other six cultivars.
4. Feeding damage became visible as early as petal fall.
5. Delta traps and Pherocon II traps caught significantly fewer campylomma than did 1C traps. Efficiency did not correlate exactly with the amount of sticky surface. The efficiency of the delta trap was approximately half that of the 1C. The total capture in the Pherocon IIB trap was not significantly different from that of the 1C.

Methods for 2002 research:
1. Apple cultivar susceptibility to campylomma feeding.
Seven orchards with the cultivars 'Golden Delicious,' 'Delicious,' 'Braeburn,' 'Cameo,' 'Fuji,' 'Granny Smith,' and 'Gala' were selected in Brewster WA. A second site with the cultivar 'Fuji' was located in Manson, WA. At king bloom, campylomma nymphs were collected in an orchard in Orondo. Second instar nymphs were placed in 15 x 20 cm sleeve cages placed over flower clusters at each site in Brewster. Flowers in the cages were pruned to a single king bloom and two leaves. Each cultivar had 25 cages containing a single campylomma nymph and a corresponding number of check (empty) cages. Flowers of each cultivar were collected and photographed to investigate if morphological differences might be correlated to susceptibility. Fruit injury was assessed at petal fall and the presence of campylomma nymphs recorded. Damaged fruits were photographed from petal fall until the fruit aborted.

2. Development of an economic threshold for 'Gala.'
Three orchards with a history of campylomma damage were selected in Brewster, WA for sampling campylomma during bloom. Campylomma tap samples were taken every few days from pink through bloom. This experiment was terminated because no campylomma nymphs were found in these blocks in 2002.

3. Comparison of pheromone trap designs for monitoring campylomma.
Beginning in August, two 1C traps and two delta traps loaded with campylomma pheromone were placed in four 10-acre blocks (replicates) in Brewster, WA. Traps were checked weekly. Each trap station was at the center of a 2.5-acre quadrant. Individual traps were re-randomized among the trap stations after each weekly count. When the recommended life of the pheromone ended (five weeks), the traps were collected. Data analysis consisted of an AOV with an LSD mean separation for the total number of males captured per trap over the course of the study.

Four trap designs (1C, delta, Pherocon II and Pherocon IIB) were compared in an Orondo orchard with a high population of campylomma adults. Four replicate blocks of the four trap types were placed in a single row of the orchard. Traps within replicates were placed every other tree, and replicates were separated by 10 trees. Traps were collected after one week. The mean number of males caught in the delta, Pherocon II and Pherocon IIB traps was compared to the mean caught in the 1C traps. Differences were tested for significance with Dunnett's test.

4. Pheromone traps to determine risk level for spring sampling of campylomma.

Ten 'Golden Delicious' orchards and 10 'Delicious' orchards were selected from Quincy to Brewster, WA. Four delta traps, loaded with campylomma pheromone lures, were placed in each 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.

Results and discussion:
1. Apple cultivar susceptibility to campylomma feeding.
Usually nymphs were discovered with their heads in the nectaries, apparently feeding on nectar. Nectaries were full because no bees had emptied them during bloom. Fruits were periodically photographed to record the damage progress. By June, most of the fruits had aborted. Photographs of mature fruits of 'Delicious,' 'Golden Delicious' and 'Fuji' were taken.

Brewster site: Damage was immediately visible on 'Golden Delicious' and, as in previous reports, susceptibility to injury was higher for this cultivar. Of the fruits caged with campylomma, 'Golden Delicious' had 75% damage. The next most susceptible was 'Gala' (12%), followed by 'Delicious' (9.5%) and 'Fuji' (4.8%), 'Granny Smith' (4.3%) and 'Cameo' (3%). 'Braeburn' fruit showed no damage. No damage was found in any of the check cages. The percentage of fruit damaged by campylomma of cultivars 'Gala,' 'Fuji' (both trials), 'Granny Smith,' and 'Cameo' was significantly lower than that of 'Golden Delicious' (2X2 χ^2, α=0.005) but not significantly lower than that of 'Delicious' (2X2 χ^2, α=0.05).

Manson site: Fruit damage on 'Fuji' at this site was 15% compared to 3% for 'Cameo.'

Fruit morphology: Fruitlets of 'Golden Delicious,' the most susceptible cultivar, had the lowest visible density of trichomes. There was no visible difference among the other cultivars. Dense trichomes are associated with partial host plant resistance in many crop species, with a possible mechanism of presenting a physical barrier to stylet penetration or nonpreference for probing.

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

The experiment was terminated because no campylomma were present.

3. Comparison of pheromone trap designs for monitoring campylomma.

In the original research on pheromone trapping as a monitoring method, Reding (2000) used the 1C trap. This trap is used less often today in favor of various forms of the delta trap. Two experiments were completed in 2002 to compare the performance of the 1C trap with the delta and other traps.

Brewster: 1C traps caught an average of 26.25 males per trap, whereas the delta traps caught an average of 13.5. The catch of the delta trap was significantly lower (about half) than that of the 1C trap (LSD=8.46, df=3, α=0.05).

Orondo: In the second experiment, the average catch in the 1C traps was 100 males/trap, whereas the average caught in the Pherocon II, Pherocon IIB and delta traps was 36.0, 83.0 and 54.8, respectively. Pherocon II and delta trap catches were each significantly lower than that of the 1C, whereas the catch of the Pherocon IIB traps was not significantly different from that of the 1C (Dunnett's test, df=3,9, α=0.05).

In both trials, the catch of males in the delta traps was about one-half the catch in the 1C traps. However, this does not invalidate the use of the delta trap which is easier to handle and maintain. Lowering the threshold for a delta trap by one-half should give approximately the same risk categories proposed by Reding (2000). Thus, the delta trap thresholds would be 125 males/trap for 'Golden Delicious' and 175 for 'Delicious.'

4. Pheromone traps to determine risk level for spring sampling of campylomma.

The trapping period in this study corresponded to the 'Long Fall' described in Reding (2000). Based on the trap catches from each site, two orchards of 'Delicious' were ranked high risk and eight were ranked low risk. Eight orchards of 'Golden Delicious' were ranked high risk and two were ranked low risk. These orchards will be sampled for nymphs and damage in the spring of 2003 to validate the thresholds.

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 (publisher), Yakima, WA, 276 pp.

Reding, M. E. 2000. Biology, monitoring, and management of *Campylomma verbasci* (Meyer) (Hemiptera: Miridae) in Washington apple orchards. Ph.D. Washington State University, Pullman.

Budget:

Title: Feeding behavior, thresholds and pheromone trapping of *Campylomma verbasci.*

PI: Elizabeth H. Beers, Entomologist

Project duration: 2002 through 2004

Current year: 2003

Project total (3 years): $76,052

Current year request: $24,156

Year	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Total	27,385	**24,156**	24,511

Current year breakdown:

Item	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Salary (0.40 FTE, 12 mo)[1]		**14,200**	14,768
Benefits (33%)		**4,686**	4,873
Wages[2]		**1,440**	1,440
Benefits (16%)		**230**	230
Equipment[3]		**400**	-
Supplies[4]		**1,200**	1,200
Travel		**2,000**	2,000
Miscellaneous		**-**	
Total	27, 385	**24,156**	24,511

[1] Salary for Steve Cockfield.

[2] Time-slip wages.

[3] Computer (40%).

[4] Telecommunication charges are allowed on this project.

Fig. 1. ‘Delicious’ with campylomma damage, early May

Fig. 4. ‘Golden Delicious’ with campylomma damage, early May

Fig. 2. ‘Delicious’ with campylomma damage, July

Fig. 5. ‘Golden Delicious’ with campylomma damage, July

Fig. 3. ‘Delicious’ with campylomma damage, August

Fig. 6. ‘Golden Delicious’ with campylomma damage, August

Fig. 7. 'Gala' with campylomma damage, early May

Fig. 8. 'Cameo' with campylomma damage, early May

Fig. 9. 'Granny Smith' with campylomma damage, early May

Fig. 10. 'Fuji' with campylomma damage, mid May

Fig. 11. 'Fuji' with campylomma damage, July

Fig. 12. 'Fuji' with campylomma damage, late August

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project # AE-02-222 **WSU Project # 13C-3643-4094**

Project title: Stink bug behavior and control in orchards.

PI: Jay F. Brunner, WSU Tree Fruit Research and Extension Center
Co-PIs: 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 – 2002:
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. De-Vac collections from the orchard failed to indicate that stink bugs were present in the orchard, and this was further backed up by fruit injury patterns occurring 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 were provided by commercial companies for testing. Two of the lures showed promise, equaling or exceeding attraction achieved by the WSU lure.

Methods:
Orchard cover crops as hosts: To evaluate whether plants commonly found in orchards have potential to support stink bug populations, we reared stink bugs from the egg stage upon five broadleaf weeds commonly found in orchards, as well as orchard grass. Development of the stink bugs was followed on 12 of each kind of plant until death was noted or the adult stage was reached. This test was repeated two times.

In addition, we conducted D-Vac or vacuum samples of orchard ground cover throughout the summer to determine whether stink bug nymphs were present in-orchard. In each of three orchards one-meter areas were vacuumed in three rows including border and interior rows. D-Vac samples were taken to the laboratory and the number and stage of stink bugs counted.

Orchard ground cover treatments: Two treatments were applied to the orchard ground cover in an attempt to reduce the in-orchard populations of stink bugs. Danitol, 2,4-D or nothing (control) was applied to ground cover in an attempt to either kill stink bugs or eliminate possible host plants. Treatments were applied to one-acre plots each replicated three times. Mite counts were conducted in apple trees following these treatments by taking three 25-leaf samples (five leaves from five trees) in each treatment area and will continue in 2003.

Aggregation pheromone studies: Prototype lures designed to have a release rate of the *Euschistus* aggregation pheromone approximate to that of the polyethylene cap-lure developed by WSU were supplied by three companies. In all cases, lures were placed on mullein (*Verbascum thapsus*) plants located on the borders of orchards in areas known to support high stink bug populations. All lures were affixed to mullein using a plastic twist tie. Depending on the physical characteristics of the lure, the tie was either wrapped around the lure or threaded through a hole in the non-reservoir portion of the lure.

Experiment 1 - fresh lures: All lures were kept in a freezer at -20°C until 16 h before use. They were removed from the freezer and allowed to come to room temperature gradually while being held in impermeable containers. At no time were lures allowed to touch one another or any other source of pheromone.

Lures were placed on mullein plants that had all stink bugs removed prior to lure placement. Each lure was placed on a plant for 72 hours, then removed, at which time all stink bugs present on that plant were counted and removed. Each plant was used only once during the course of the study. Each treatment (lure type) was replicated three times in each test period (a total of 15 baited mullein plants). The test protocol was repeated five time during the summer (May 20-June 28, 2002), when stink bugs were at their highest pheromonal response activity. There was a one-week delay between each independent test period. Treatments included the three commercial lures (A, B and C) contributed by industry, the WSU lure, and an untreated control.

All data are expressed as mean # bugs/plant/72-hour period ±SE and were analyzed using Fisher's LSD.

Experiment 2 - aged lures: To estimate field longevity, lures were field-aged for periods of three and six weeks. Lures were aged by attaching them to the limbs of apple trees using twist ties. Lures were hanging free within the shaded canopy of the trees and were never in direct sunlight. Six-week lures were aged beginning July 5, and three-week lures were aged beginning July 24, 2002. Protocols used to field-test the aged lures were similar to those given above. However, only two commercial lures were tested in this experiment; the third was not included due to its poor performance during Experiment 1. Ten plants were used/observation period, with one untreated plant and one of each lure type: (WSU fresh, WSU 3-week, WSU 6-week, Lure B fresh, Lure B 3-week, Lure B 6-week, Lure C fresh, Lure C 3-week, Lure C 6-week). As in the previous experiment, plants were checked 72 hours after placement and stink bugs counted and removed. This experiment was conducted during mid-late August (August 15 to 30), when bugs are no longer reproductively active but remain responsive to pheromone. Eight replicates were used in this experiment (i.e., eight observations/treatment). All data are expressed as mean # bugs/plant/72 hours ± SE and were analyzed using Fisher's LSD.

Border management: In 2002 damage counts were conducted throughout the season on the border and interior of a single orchard to determine the pattern of damage timing and to develop a picture of damage distribution within the orchard. In this same orchard an experiment was conducted comparing treatments of Danitol to borders alone vs. treatments of the entire orchard and an unsprayed check. There were three replicates of each treatment. Danitol was applied at label rates (16 fl. oz./acre) in late August after the onset of damage. The effect of the treatments on stink bugs in each plot was evaluated at harvest by counting the number of damaged fruits on 10 trees in the border and two interior rows in each plot.

Trap crops: We did not pursue this objective further in 2002.

Candidate insecticide evaluation: There were no suitable options for stink bug management presented in 2002.

Results and discussion:
We found no evidence to support the concept that stink bug populations are reproducing and building within orchards. D-Vac samples taken from orchard ground cover yielded very few stink bug nymphs compared with border samples (Table 1), and damage counts conducted in the orchard once again revealed a trend of decreasing damage away from border rows (Fig. 1).

Table 1. Average number of stink bugs of in-orchard vs. border D-Vac samples of ground cover vegetation, 2002.

DATE	SITE	# BUGS/SAMPLE	INSTAR
06/27/02	In-orchard Border vegetation	0.11 1.00	2nd 2nd
07/09/02	In-orchard Border vegetation	0 0.55	N/A 2nd-4th
08/01/02	In-orchard Border vegetation	0 0.5	N/A 4th-adult
08/14/02	In-orchard Border vegetation	0 0.88	N/A 5th-adult
08/31/02	In-orchard Border vegetation	0 0.33	N/A 4th

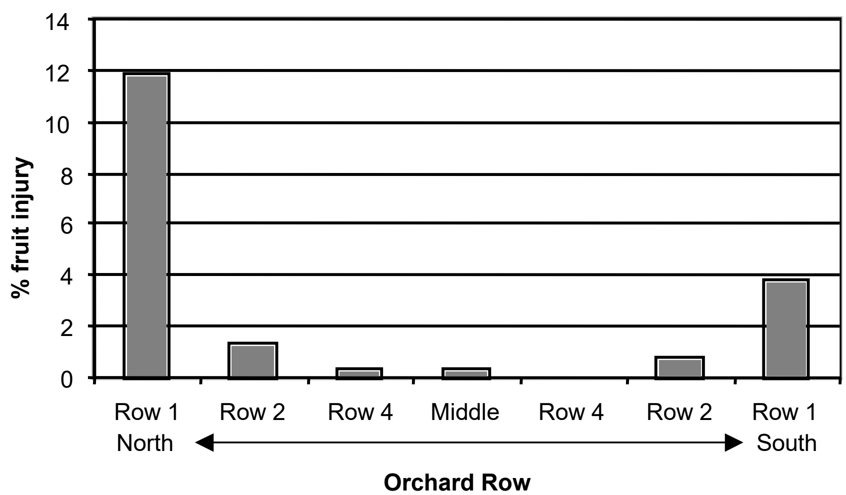


Figure 1. By-row distribution of stink bug injury at harvest, data represent pooled results of 2 orchards surveyed.

Results of rearing experiments conducted with a variety of host plants indicate that stink bugs are able to develop from egg to adult on common mallow, mullein and white clover only (Table 2). These plants could be managed with effective broadleaf weed control. Since previous experiments have shown that stink bugs are unable to develop upon apple, this may represent an ideal way to restrict stink bug populations to areas outside orchard borders.

Table 2. Percentage of stink bugs reaching the adult stage and weight of adults reared on different ground cover plants.

PLANT	% reaching adult	Mean wt. males	Mean wt. females
Common mallow	13.91	0.057	0.064
Dandelion	0	-	-
White clover	1.83	N/A	0.093
Mullein	7.27	0.079	0.079
Lamb's quarters	0	-	-
Orchard grass	0	-	-
Field-collected		0.083	0.096

We conducted experiments to compare three in-orchard strategies for stink bug management: 1) application of a broadleaf herbicide (2,4-D) to orchard ground cover to remove potential stink bug host material; 2) application of Danitol to ground cover to kill developing nymph populations; 3) no ground cover treatment (check). Combined with results of previous experiments that indicate that stink bugs are unable to develop upon apple, this indicates that effective control of broadleaf weeds in the orchard may remove any potential hosts for stink bug nymphal development. However, in view of the lack of stink bug nymphs found inside orchards in any of the plots (Table 3), the emphasis of management efforts may be better confined to orchard borders.

Table 3. Average number of stink bugs from in-orchard and border vegetation D-Vac samples of ground cover taken before (June) and after applications of 2,4-D and Danitol.

DATE	TREATMENT	# NYMPHS/SAMPLE
06/27	Orchard pre-2,4-D	0.11
	Orchard pre-Danitol	0
	Orchard pre-Check	0
	Border vegetation	0.66
07/09	Orchard 2,4-D	0
	Orchard Danitol	0
	Orchard check	0
	Border vegetation	0.55
08/01	Orchard 2,4-D	0
	Orchard Danitol	0
	Orchard check	0
	Border vegetation	0.33
08/14	Orchard 2,4-D	0.11
	Orchard Danitol	0
	Orchard check	0
	Border vegetation	0.88
08/31	Orchard 2,4-D	0
	Orchard Danitol	0
	Orchard check	0.11
	Border vegetation	0.22

We tested three pheromone dispenser types in 2002, and two of these performed satisfactorily in field attraction of stink bugs (Figure 2). Both the Pherotech lure and the IPM Technologies lures attracted significant numbers of stink bugs in the field. Lure effectiveness declines over time as the reservoir is depleted, but this may be remedied by adding more pheromone. We expect one or both of these companies to market a commercial lure in the near future.

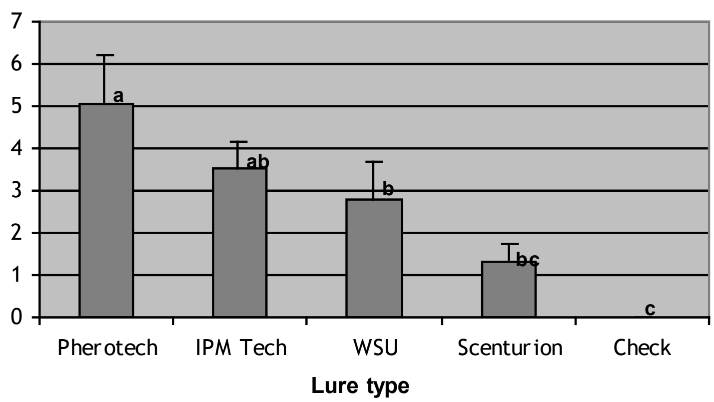


Figure 2. Comparison of field attractiveness of different lure types placed on mullein plants.

Applications of Danitol in 2001 had a marked negative effect upon mite populations. The short-term effects are a reduction in all populations of mites. The long-term effects of these spray applications were more serious with levels of pest mite species approaching threshold levels, with few or no predator mites present (Table 4). These orchards were sprayed with miticide on July 31, 2002, to prevent economic loss due to these heavy mite infestations. This disruption of integrated mite control is a serious downfall of Danitol as an in-orchard stink bug control and has led us to evaluate alternative methods of employing this compound as a management tool.

Table 4. Average mites per leaf in Danitol treated orchards compared to orchards treated with Phosphamidon or left untreated.

		ERM per leaf		Pred./leaf	ERM per leaf		
		2001			2002		
Orchard	Treatment	Aug. 13	Nov. 11	Nov. 11	May 13	June 10	July 15
Gala 1	Danitol	5.10	2.30	0.00	0.27	0.13	32.80
	Untreated	6.00	11.50	1.70	0.80	0.40	6.80
Gala 2	Danitol	2.50	2.50	0.00	0.13	0.40	23.70
	Phosphamidon	0.20	6.40	0.30	0.27	0.40	18.70
Golden	Danitol	0.00	0.07	0.00	0.00	0.67	1.20
	Phosphamidon	0.13	0.13	0.13	0.53	0.00	7.80
Fuji	Danitol(1)	1.70	0.13	0.00	1.60	1.87	7.07
	Danitol(2)	1.90	2.50	0.27	7.07	1.73	8.47
Red	Danitol	0.00	0.00	0.00	0.00	0.00	0.07
	Untreated	0.27	0.00	0.20	0.00	0.27	0.00

Counts of spider mites represent totals of European red mite and twospotted spider mites; counts of beneficials represent totals of Typhlodromus + Zetzellia spp., as the dominant species varied by locations.

Damage timing was investigated in detail, and it was found that the onset of damage occurred at the end of July and continued until harvest (Figure 3). These data demonstrate that there is not a discrete period of stink bug injury that growers could target for spray applications. This is of interest in light of our other work showing that Danitol is extremely disruptive after 1-2 applications, meaning that in-orchard prophylactic treatments may not be a viable option.

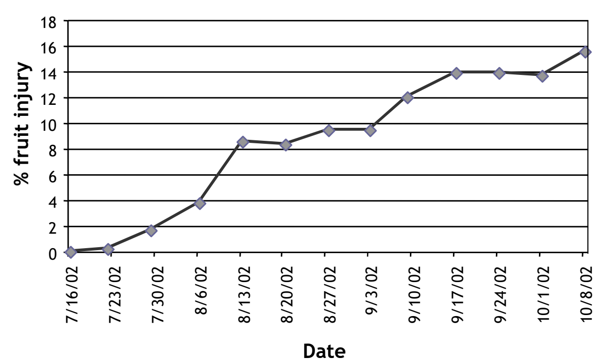


Figure 3. Cumulative stink bug fruit damage on orchard borders, counts are conducted weekly on flagged limbs, and are confined to border rows only.

Applications of Danitol to entire orchards were significantly more effective at reducing stink bug injury at harvest than applications to the border rows only (Figure 4). However, we will continue to evaluate the long-term differences between these modes of applying Danitol by assessing mite populations in treated areas.

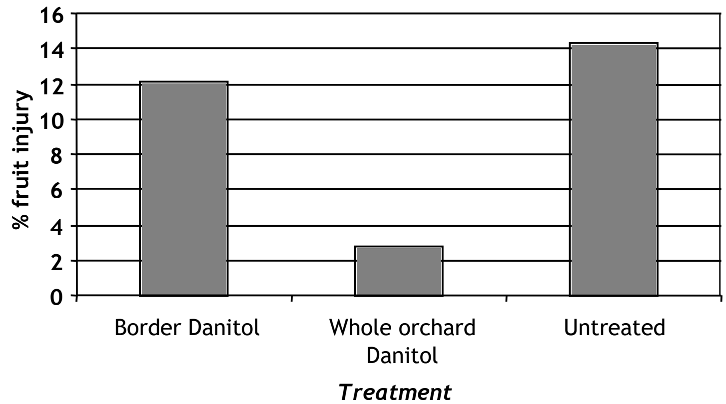


Figure 4. Damage counts in orchard, minimum of 200 fruit sampled. Treatments are 2 applications of Danitol at 16 fl. oz./acre on border only, entire orchard block, or no Danitol. Danitol applied on August 6 and August 27, 2002.

Budget:
Project title: Stink bug behavior and control in orchards.
PI: Jay F. Brunner
Proposed project duration: 3 years
Project total (3 years): $81,607
Current year request: $26,687

Year	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Total	28,197	**26,687**	26,723

Current year breakdown

Item	Year 1 (2002)	**Year 2 (2003)**	**Year 3 (2004)**
Salaries [1] (Krupke)	12,609	**11,995**	12,475
Benefits	1,308	**1,412**	1,468
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
Miscellaneous	0	**0**	0
Total	28,197	**26,687**	26,723

[1] C. Krupke is starting a Ph.D. program. He will be taking classes in the winter semester of 2002 in Pullman. The request is for a 50% nine-month salary with benefits but NOT including tuition. He will work full time on this project from May through October. His research, while related to this project, involves different objectives.
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. Telecommunication charges are allowed on this grant.
[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.

Supplemental funding from IFAFS/RAMP projects ($15,000), CAHE graduate student assistantship program ($15,000), and Washington Commission on Pesticide Registration (in years 2 and 3).

CONTINUING PROJECT REPORT **YEAR 2/3**
WTFRC Project #AE-02-221 **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 North Western Avenue, Wenatchee, WA; phone: 509-663-8181; FAX: 509-662-8714; jfb@wsu.edu

Co-PIs and affiliations: Betsy 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 and leafroller.
2. Determine the potential of a fiber pheromone formulation as a behavioral control for leafrollers and codling moth.
3. Refine the attract & kill technology for leafrollers based on a Last Call technology and implement an optimized formulation against leafrollers.
4. Develop a bait & kill system for control of lacanobia fruitworm and assess the impact on other noctuids in orchards.

Significant findings – 2002:

1. Sprayable pheromone for CM control did not perform well in orchards with moderate to high pressure. This technology might only be applicable to very low-pressure orchards where it could be incorporated as part of a spray management program.
2. Sprayable pheromone as a control for leafrollers was not encouraging but could provide some suppression if incorporated into a regular spray management program for other activities.
3. The Scentry fiber pheromone showed promise in preliminary studies for longevity of attraction, retention on foliage and suppression of moth activity when applied with a prototype ground applicator.
4. An attracticide formulation for OBLR and PLR was developed and optimized. Field studies indicate sufficient longevity, and preliminary small plot field trials were promising enough to propose large plot trials in 2003.

Methods:

Sprayable pheromone

3M sprayable codling moth pheromone – Orondo: 3M sprayable CM pheromone (3M-CM) was applied to an unreplicated block (4 acres) at a rate of 10 g ai/acre on April 29 and repeated on May 14. On June 5, the block was divided into two 2-acre treatments of 20 g ai/acre and 40 g ai/acre. All applications included Nu-Film 17 at 8 oz/acre. The orchard was monitored with 1X pheromone lures, SuperLures (Pherotech) and DA kairomone lures in delta-style traps. A 4-acre conventionally managed (no pheromones) block adjacent to the pheromone treated block was monitored in the same manner with 1X lure-baited traps in the first generation and 1X and SuperLure-baited traps in the second generation. Damage during the first generation was assessed on June 6 and July 8. In the second flight, one of the 2-acre blocks was treated with 3M-CM at 40 g ai/acre plus Nu-Film 17 and compared with the other 2-acre block treated with Isomate C+ hand-applied dispensers at 400 dispensers per acre. Monitoring remained the same in the sprayable treated areas, but the untreated block had two 1X lures and two SuperLures for comparison.

3M sprayable codling moth pheromone – Pateros: 3M-CM pheromone was applied to two unreplicated large blocks (10 acres) at rates of 5 g ai/acre and 10 g ai/acre. All pheromone treatments included Nu-Film 17 at 8 oz./acre. All treatments were applied in 100 gallons of water per acre. Treatment blocks were monitored with SuperLures and DA kairomone lures in delta-style traps. There were two 10-acre untreated blocks adjacent to the pheromone blocks, and they were monitored in the same manner excluding the SuperLures. Pheromone applications were made on May 1, June 1, July 1, July 29 and August 17. Fruit injury was evaluated at the end of first and second generations.

Suterra sprayable codling moth pheromone – Wapato: In the first generation, Suterra CM-F was applied to 40 acres in four 5-acre replicated blocks at rates of 5 g ai/acre and 10 g ai/acre alternated with 3-acre untreated blocks as comparison. Treatment blocks were monitored with delta-style traps loaded with Suterra BioLure, SuperLure or DA kairomone lures. The alternating untreated blocks were monitored with standard 1X lures and DA lures. In the second generation the pheromone rates were increased to 10 g ai/acre and 20 g ai/acre. Monitoring remained the same. Pheromone applications were made by Suterra at 2 gpa without Nu-Film 17 on April 25, May 15, June 7, June 11 (after rain event), July 11 and August 12. Fruit injury was evaluated at the end of each generation.

Suterra sprayable codling moth pheromone – Pateros: Sprayable pheromone, Suterra CM-F, or Isomate C+ was applied to large blocks of approximately 8 acres each replicated three times. The first application of sprayable pheromone was made on May 1 at a rate of 20 g ai/acre plus Nu-Film 17 at 8 oz./acre. Subsequent applications were made June 1, July 4, July 29 and August 19 at a rate of 10 g ai/acre plus Nu-Film 17 at 8 oz./acre. The Isomate C+ was applied by hand prior to biofix at a rate of 200 dispensers per acre. Monitoring consisted of the delta-style trap baited with SuperLures or DA kairomone lures. Fruit injury was evaluated at the end of each generation.

Suterra sprayable codling moth pheromone – Mattawa: Two large blocks (14 acres each) received treatments of Suterra sprayable pheromone at a rate of 5 g ai/acre and 10 g ai/acre. A 14-acre adjacent block was treated with Isomate C+ hand-applied dispensers at a rate of 200 dispensers per acre for comparison. Monitoring consisted of delta-style traps baited with SuperLure or DA kairomone lures. Sprayable pheromone was applied on April 25, May 23, June 21 and July 21. Isomate C+ was applied by hand 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 all blocks.

Suterra OBLR sprayable pheromone – Mattawa: This was a large trial of approximately 98 acres divided into 4-acre treatment areas with alternating untreated (no pheromone) 4-acre blocks. There were three replicates of three sprayable pheromone treatments consisting of 2.5 g ai/acre, 10 g ai/acre and 20 g ai/acre. Monitoring in each 4-acre section consisted of delta-style traps loaded with either 10 mg lure, standard 1 mg lure or acetic acid bait-lures to assess male and female activity. Larval densities were evaluated by examining 200 buds per treatment at delayed dormant and 1000 shoots per treatment in spring and summer. Fruit injury evaluations were done prior to harvest by examining 25 fruits per tree from 30 trees in all areas. Treatments were applied by WSU-TFREC on June 3, July 2 and August 5 with Nu-Film 17 at 8 oz./acre using an airblast sprayer at 25 gpa.

Suterra OBLR sprayable pheromone – Royal Slope: This trial consisted of 30 acres divided into four 5-acre treatments and one 10-acre untreated block. Treatments consisted of 1 g ai/acre plus Nu-Film 17, 1 g ai/acre plus Reguard, 10 g ai/acre plus Nu-Film 17 and 10 g ai/acre plus Reguard. Each block was monitored with delta-style traps baited with either a high load (10 mg) or standard load (1 mg) or acetic acid bait-lure. There were two of each of the pheromone lures and five acetic acid lures to evaluate male and female activity in each treated block. The untreated area was monitored in the same manner. Treatments were made on June 3, July 2 and August 5. Larval densities were evaluated using the same methods as in the Mattawa trial (above). Fruit injury was evaluated prior to harvest by examining 20 fruits per tree from 50 trees per treated area and 100 trees in the untreated area.

Scentry fiber pheromone system **–** Scentry Biologicals contracted with Blue Line Manufacturing to develop a prototype fiber applicator. This applicator was field tested for fiber distribution and to apply treatments against codling moth and leafrollers late in 2002.

Attractancy of pheromone fibers – Fibers loaded with CM or OBLR pheromone were provided by Scentry Biologicals. Individual fibers or a grouping of five fibers, with or without a BioTac coating, were placed on a card attached to the side of large delta-type traps. Traps were placed in an orchard along with a trap containing a standard (1 mg) CM lure or a 1 mg or 0.01 mg OBLR lure (Scenturion). Traps were examined three times per week, moths counted and removed, and then the location of traps rotated. After each rotation was completed new CM lures were placed in traps, but the fibers were aged throughout the duration of the test. In the OBLR test the lure was not changed after a rotation but was always the same age as the fibers. There were five replicates of each treatment. The same test was repeated starting with fresh fibers in the second generation of CM and OBLR.

Field-aged fibers – Fibers in the above tests were never exposed to the environment, being protected from sun by the trap. Therefore, fibers containing CM pheromone were coated with BioTac and placed on the upper surface of leaves in an orchard. At regular weekly intervals, fibers were removed from leaves, placed in foil packets and then placed in cold storage. Aged fibers were removed from the cold storage on the same date and placed in traps as groups of five fibers (age of fibers tested was 0, 7, 14, 21, 28, 35 and 42 days). Five replicates of each fiber age class were evaluated along with a fresh 1 mg lure. Traps were placed in the field for 7 days, after which the number of moths captured was counted.

Retention of fibers on trees – Scentry pheromone fibers were coated with BioTac in the laboratory, then taken to an orchard where they were placed on a leaf or limb. In May, fibers were placed on the upper surface of the leaf near the midrib or on the top of a limb measuring at least one inch in diameter. Ten fibers were placed on leaves or bark on the east and west side of three trees. Fiber locations were marked by flagging tape and permanent marker arrows pointing to their location. Fibers were checked weekly and the number remaining on the leaves or bark recorded.

Retention of fibers applied by the prototype applicator was evaluated by running the applicator for two minutes adjacent to four different trees. The fibers that could be located on the trees were tagged. The fibers were checked at regular intervals and the number remaining recorded.

Distribution of fibers by prototype applicator – The prototype fiber applicator was operated for a period of two minutes in a stationary position. Fibers found on one square foot targets around the applicator were counted.

Fiber field trails – Scentry CM fibers were applied against CM at four locations and OBLR at two locations. The exact number of fibers applied was difficult to evaluate, but the target was 100 grams of fibers per acre. CM treated orchards were monitored with 1 mg lures and DA lures. OBLR treated orchards were monitored with 1 mg lures. Traps were checked for 35 days after treatment in the CM treated plot and 52 days in the OBLR treated plots.

Fibers as Attract & Kill – Scentry fibers were mixed with permethrin at a concentration of 6% w:w. The fibers were coated with the BioTac/permethrin mixture and placed on top of leaves. The fibers were collected weekly and stored at 0˚C until all were collected (28 days). Adult OBLR (approximately 25 moths/fiber age) were allowed to touch aged fibers. Moth mortality was assessed at 2, 24 and 48 hours after exposure. This research is currently underway.

Attract & Kill – The Last Call-CM formulation was evaluated at two locations. One site had high pressure and the other moderate pressure. At the Orondo location two rates, 1200 and 600 drops per acre, were applied to large plots (1-4 acres) replicated two times. The Last Call was applied four times, May 1, June 5, July 9 and August 14. Monitoring was done with large delta traps baited with 1 mg lures and DA lures, two traps per replicate. Fruit injury was evaluated three times in the first generation and at harvest. Supplemental sprays were applied. In Tonasket, Last Call-CM was applied at a rate of 1200 drops per acre to an unreplicated 5-acre block. A conventionally treated adjacent block of 7 acres served as a comparison. Monitoring was the same as described above. Last Call was applied three times, May 7, June 7 and July 20. Guthion supplemental sprays were also applied, twice to the Last Call treatment areas and three times to the control areas.

The development of an attracticide formulation for leafrollers was done in conjunction with IPM Technologies. IPM Technologies supplied the base matrix and pheromone components for the attracticide, as well as the insecticides and blends used in the studies. First tests were conducted in an indoor wind tunnel. The attracticide was formulated with different concentrations of pheromone blends for OBLR and PLR. Moths of known ages were flown in the wind tunnel to sources of the attracticides, and success was based on source contact. Thirty moths of each species were tested at each pheromone concentration, and the results were compared with responses to a calling female.

In the field the attracticide with different concentrations of pheromone was evaluated for attraction of moths of both species. A small drop of the attracticide (50 micro-liters) was placed on foil in an enclosed but ventilated container in a delta trap. Traps were placed in orchards, checked daily and rotated after each examination. The capture of moths in traps baited with the attracticide formulation was compared with traps baited with commercial pheromone lures.

To evaluate the possibility of blending the OBLR and PLR pheromones into one attracticide formulation, the pheromones of both species were mixed in the base matrix at proper proportions, then placed in traps as described above. Traps were examined daily to determine the relative attractiveness of the OBLR/PLR blends compared to the same attracticide formulation with only one of the leafroller pheromones.

The attracticide formulation was evaluated with and without an insecticide to evaluate the possibility that the insecticide would be repellent. The attracticide formulation for each species was tested at three different pheromone concentrations with and without insecticide. The formulation was put in traps as described above and placed in orchards.

Results and discussion:

Sprayable Pheromone

3M sprayable codling moth pheromone – Orondo: During the first flight there was an 80-90% reduction in moth capture in the pheromone treated area relative to the untreated area. However, the level of fruit damage evaluated at the end of the first generation was 9% for the 40 g ai/acre rate and 14% for the 20 g ai/acre rate and not different from the untreated area that had 12% fruit injury. In the second flight, 3M sprayable pheromone did not reduce pheromone trap catch relative to the untreated area. The application of Isomate C+ did reduce moth catch (97% - 1X lure) compared to the untreated block, but moth pressure remained very high and there was no reduction of fruit injury associated with either pheromone treatment.

3M sprayable codling moth pheromone – Pateros: This was a very low-pressure site with few moths being captured in the first generation. This fact made it difficult to assess longevity and efficacy of the product. During the second flight, moth activity increased, and 3M-CM reduced catch by 30% in the 5 g ai/acre area and 91% in the 10 g ai/acre area relative to the untreated areas. Fruit injury was very low in all plots and was restricted to the orchard border.

Suterra codling moth sprayable pheromone – Pateros: The first flight showed a reduction in moth capture of 76% in the sprayable pheromone area compared to Isomate C+. This was a low-pressure site, and fruit damage at the end of the first generation was restricted to border rows. Isomate C+ reduced moth capture relative to Suterra CM sprayable pheromone by 43% during the second flight. At harvest, fruit injury remained very low, with Isomate C+ blocks averaging 0.47% and Suterra sprayable 0.85%; again injury was primarily on orchard borders.

Suterra codling moth sprayable pheromone – Wapato: The Pherotech SuperLure trap catch was reduced by 86% (5 g ai) and 95% (10 g ai), whereas the Suterra BioLure trap catch was reduced by 55% (5 g ai) and 77% (10 g ai) in the first generation. After the first generation there was no significant difference between the sprayable pheromone blocks and the untreated blocks. This orchard was under moderate to high pressure from an adjacent block that had been pulled out the previous year. In the second generation, moth activity varied between the SuperLure and the BioLure. Trap capture in the SuperLure was reduced by 95% (10 g ai) and 90% (20 g ai) and in the BioLure by 59% (10 g ai) and 54% (20 g ai) relative to the untreated areas. Fruit injury was not significantly different between treated and untreated areas. At harvest there was no difference in fruit injury in blocks with or without pheromone treatment.

Suterra codling moth sprayable pheromone – Mattawa: This was a very low-pressure site with low moth activity in the first flight. There was zero fruit injury in the sprayable pheromone and Isomate C+ blocks at the end of the first generation. Moth activity increased during the second flight, and Isomate C+ had fewer moths captured compared to the sprayable pheromone treated areas. Fruit injury remained unchanged in the second generation in all areas.

Suterra OBLR sprayable pheromone – Mattawa: Leafroller moth activity was slightly reduced in the first generation by the use of sprayable pheromone relative to untreated areas. Larval densities for first generation remained low in all areas. The summer larval population increased slightly but was not significant. Sprayable pheromone decreased the pheromone trap catch in all treatments during the second generation, with the 20 g ai/acre rate being most effective with a 47% reduction. Fruit evaluation prior to harvest remained low at 0.1% damaged fruit in all areas.

Suterra OBLR sprayable pheromone – Royal Slope: Sprayable pheromone reduced leafroller trap catch 86-100% in treated areas during the first flight. Larval densities in spring and summer were very low. Flight increased during the second generation and sprayable pheromone did not perform as well, reducing trap catch in the 10 g ai/acre treatments only by 9%.

Scentry fiber pheromone system – The prototype fiber pheromone applicator is shown to the right. Fibers and BioTac are placed in the cylinder; the mixture is pushed into a spinning cone that throws them up and away. Some modifications are planned for 2003.

Attractancy of pheromone fibers – Fibers loaded with CM pheromone were attractive for up to 60 days in both generations. Fibers were only slightly less attractive than a fresh 1 mg lure. A very similar pattern was observed with fibers loaded with OBLR pheromone. These were not as attractive as a fresh 1 mg lure but were as or more attractive than a fresh 0.01 mg lure.

Field-aged fibers – There was no difference in the capture of CM in traps baited with five fibers aged in the field from 0 to 42 days. These fibers also captured as many moths as a fresh 1 mg lure. These data indicate that the fibers provide protection of CM pheromone from environmental degradation.

Retention of fibers on trees – Fibers were retained on the foliage much better than on bark. The thicker grade of BioTac (100 vs. 25/100) retained fibers better. After 49 days 70% of fibers were still retained on leaves. Most fibers applied by the prototype applicator land on leaves. The ones that land on the upper surface of the leaf are retained for a long period, but those that land in other positions drop from the leaf. Some of those, no doubt, land in favorable positions and others do not.

Distribution of fibers by prototype applicator – Fibers are flung off the spinning cone and land 10 to 15 feet away. If the tractor was headed north the fibers tended to be clustered in a southwestern and northeastern direction.

Fiber field trials – Preliminary field trials were conducted in the late summer. There was good suppression of moth captures in most plots and that the effect lasted for up to 35 days. The same kind of reduction in OBLR captures was noted, but the effect seemed to last even longer, up to 52 days.

Attract & Kill – Last Call-CM failed to provide any suppression of CM flight or damage in the high-pressure site in Orondo. At the Tonasket site three applications of Last Call-CM suppressed moth capture, but there was an excessive amount of damage at harvest even though it had received two supplemental azinphosmethyl sprays. The conventional control had no damage with three azinphosmethyl sprays.

Both OBLR and PLR males responded to attracticide formulations when the pheromone concentration was above 0.16%, and there was increasing success in reaching the source of pheromone with increasing concentration. OBLR and PLR moths were captured in traps baited with the attracticide formulation when pheromone concentrations were above 0.16%. More moths were captured at a pheromone concentration of 16% but not statistically more moths than the 1.6% pheromone concentration. There was no effect of adding the insecticide on the attraction of moths. Blends of the two leafroller pheromones in the same attracticide formulation suppressed attraction of OBLR but not PLR. These data indicate that an attracticide formulation containing between 1.6% and 16% pheromone could be a potentially valuable tool in management of these pests.

Budget:

Project title:	Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm.
PI:	Jay F. Brunner
Project duration:	3 years
Project total (3 years):	$105,139
Current year request:	$33,652

Year	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Total	37,280	**33,652**	34,207

Current year breakdown

Item	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Salaries [1] (0.5 Betsy Valdez)	15,000	**15,600**	16,224
Benefits (37%)	4,500	**5,772**	6,003
Wages [1]	8,000	**8,000**	8,000
Benefits (%)	1,280	**1,280**	1,280
Equipment [4]	5,000	**0**	0
Supplies [2]	1,000	**1,000**	700
Travel [3]	2,500	**2,000**	2,000
Miscellaneous	0	**0**	0
Total	37,280	**33,652**	34,207

1 50% of salary for Associate in Research and temporary labor for summer activities.
2 Pays for traps, lures, bait, gloves, vials, etc. Telecommunication charges are allowed on this grant.
3 One vehicle for six 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.

Additional funding supporting this project in the amount of $40,000 per year is provided through the IFAFS/RAMP grants.

CONTINUING PROJECT REPORT **YEAR 3/3**
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:
1. All OP and NON-OP apple blocks maintained low pest populations and had very low fruit damage levels at harvest, in most cases lower than in 2001.
2. The average number of pesticide applications and the cost per acre also declined, significantly so for the NON-OP apple blocks.
3. Monitoring of codling moth, leafrollers and lacanobia fruitworm provided growers with information needed to respond with well-timed control measures where needed. Codling moth captures in the 10X and DA lure-baited traps were similar.
4. There were no surprises relative to secondary pests or their natural enemies in any orchards.
5. Good control was obtained of most pests in the soft pear blocks, including pear psylla, spider mites, codling moth and leafrollers.

Methods:
The 15 apple and six pear sites established in Washington used the same monitoring methods established in year 1 (2001). Each site consists 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 (NON-OP supplements). Controls for other pests followed a "traditional conventional" or a "selective alternatives" approach. With the exception of certain NON-OP products that might be donated by the registrant, pest control costs will be borne by the grower.

***Monitoring activities*:** Standardized monitoring activities developed under CAMP will be used to assess pest insects in cooperation with crop consultants working at the sites. Pheromone trapping was used for CM and leafroller. Non-pheromone monitoring systems were used for leafrollers and 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 in apple, and pear psylla/general predators-parasitoids in pear.

***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 and cost of 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:

APPLES

Trap data

Codling moth: A wide range of CM populations was again found within the 15 AWII apple sites, 0.0 to 10.5 moths per trap in the first generation and 0.0 to 12.7 in the second generation. Codling moth catches were lower in the second generation in most orchards. There was no significant difference in codling moth populations between the OP and NON-OP treatment blocks in 2002. The average seasonal CM catch in pheromone traps in 2002 declined 70% from 2001 levels (7.2 moths/trap to 2.1 moths/trap).

The DA lures attract both sexes of CM. Capture of moths in the DA lure-baited traps was low. In the first generation, captures in the OP and NON-OP blocks averaged 72% and 70% males, respectively, and in the second generation males accounted for 67% and 63%, respectively. The percent mated females was slightly lower in the OP compared to the NON-OP blocks in the first generation, 32% versus 50%, respectively; and percent mated females increased in the second generation to 80% and 78%, respectively. Average catch in DA lure-baited traps in the first generation was similar to the pheromone lure-baited traps. The average second-generation catch in DA lure-baited traps (although less than the first generation) was slightly greater than the catch in the pheromone lure-baited traps.

Leafroller: A wide range of leafroller densities was detected in the AWII blocks in 2002. OBLR was the main species captured in the Columbia Basin, Quincy and Okanogan orchards. PLR tended to be the dominant species in north-central Washington and the Yakima Valley. There was no significant difference in the average capture of moths in standard lure-baited traps between the OP and NON-OP treatments for either leafroller species. In low load lure-baited pheromone traps, moth capture trends were similar to those observed in standard load lure-baited pheromone traps and captured 27 to 30% fewer moths than the standard load traps.

Captures of OBLR in standard load pheromone traps were similar in 2002 to those in 2001 but tended to be higher in the low load traps in 2002. Capture of PLR moths was significantly higher in both standard and low load pheromone traps in 2002 compared to 2001.

There was nearly a 50:50 ratio of males and females captured in the AA-baited traps. So few moths were captured that dissection of females was not done. Total moth captures in AA traps were less than 2% of the standard lure-baited traps.

Lacanobia fruitworm: This relatively new pest was monitored with a pheromone lure. There was a wide range of moth captures detected in pheromone traps, 0 to 2000+ moths/trap/generation. There was no significant difference in moth capture between the OP and NON-OP treatments in either generation.

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 surveys revealed very low levels of fruit damage in July and preharvest surveys showed only slightly more damage. In no block did damage exceed 1.0%, and in only one block was damage greater than 0.5%. CM damage was largely confined to block edges. There was no significant difference in CM damaged fruit between treatments. **Leafroller** surveys in May detected shoot damage in only 5 of 30 treatment-blocks and damage was only slightly higher in the August counts, with damage found in 14 treatment-blocks. There was no significant difference between treatments. Shoot feeding damage by **lacanobia** or other cutworms was found in 23 of the 30 treatment-blocks in the July survey. Damage levels never exceeded 3.2% of shoots with damage.

Fruit and shoot damage levels in field surveys showed several changes between 2001 and 2002. **Codling moth** damage to fruit was significantly lower in the pre-harvest samples in 2002 when compared with 2001 in both the OP and NON-OP treatments. **Leafroller** shoot damage in 2001 had become significantly less in the NON-OP treatment-blocks by the August sample. This difference was again apparent in the May 2002 samples, but by August there was no difference found between treatments. Shoot feeding levels by **lacanobia** (cutworms) in July were lower in 2002.

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.

Harvest fruit evaluation

During harvest, fruit was checked for damage from major lepidopteran pests and other secondary pests. **Codling moth** damage was detected in only 9 of the possible 30 treatment-blocks. The level of fruit damage was low and exceeded 0.2% in only five blocks. Overall, there was no difference in the average percent CM damage between treatments. **Leafroller** feeding on fruit was detected in 13 of the 30 treatment-blocks, and in only five blocks did the amount of damaged fruit exceed 0.2%. There was no significant difference between treatments. Fruit feeding by **cutworms** was detected in eight blocks but only in one did it exceed 0.2%, and there was no difference in damage levels between treatments.

Damage by other pests was sporadic and rare. **Stink bug** damage was found in only one block and was associated with the border area. **Lygus** damage was reported from four blocks and never exceeded 0.2%. 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. **Thrips** damage was found in only one orchard of Granny Smith (0.2% fruit with marking in the OP block, 0.3% in the NON-OP). One orchard of Golden Delicious had **grape mealybug** on the fruit at harvest (1.4% infested in the OP block, 0.8% in the NON-OP). Fruit damage levels in 2002 were quite low and similar to the levels found in 2001 (Table 1). Only in one case (leafroller damage in OP blocks) was there a significant decline in damage.

Table 1. Fruit damage by lepidopterous pests from bin samples during harvest in AWII orchards, 2001 and 2002.

	CODLING MOTH		LEAFROLLER		CUTWORM	
Year	**OP**	**NON-OP**	**OP**	**NON-OP**	**OP**	**NON-OP**
2001	0.11%	0.05%	0.28%	0.09%	0.07%	0.05%
2002	0.07%	0.07%	0.09%	0.08%	0.04%	0.05%

Pesticide use

All AWII apple blocks used CM mating disruption, generally at rates close to 200 dispensers/acre. CM mating disruption is included as a single foliar pesticide application with cost based on the number of dispensers per acre. Applications of carbaryl (Sevin) for chemical thinning are also included as foliar pesticides. The main organophosphate (OP) insecticides used in the OP treatment blocks were chlorpyrifos (Lorsban) [12 of 15 blocks] and azinphosmethyl (Guthion) [six blocks]. For the control of lepidopteran pests the NON-OP blocks relied upon methoxyfenozide (Intrepid) [11 of 15 blocks] with lesser use of pyriproxifen (Esteem) [two blocks]. The use of the more selective "soft" insecticides was not limited to the NON-OP blocks; six OP blocks also received methoxyfenozide, generally applied soon after bloom for leafroller control. Spinosad (Success) was used mostly in the OP blocks for leafroller control (five OP blocks, one NON-OP block). Chloronicotinyl insecticides were used in both treatment blocks but to a greater extent in the OP blocks: imidacloprid (Provado) in eight OP and six NON-OP blocks, thiamethoxam (Actara) in two OP and one NON-OP block, and acetimiprid (Assail) in two OP and two NON-OP blocks.

The number and cost of pesticide applications were not significantly different between the OP and NON-OP treatment programs in 2002, although the NON-OP blocks tended to be lower in both areas. The total number of sprays varied with the cultivar (e.g., mildew-susceptible varieties received more fungicide applications) and the pest pressure at the site. For example, from zero to four codling moth sprays were applied depending upon trap counts and history.

In 2002, the total numbers of sprays and the cost per acre for both treatment types were reduced from the levels of 2001 (Table 2). This drop was most pronounced in the NON-OP blocks in which the reduction in sprays and costs was significant. In 2001, on average the NON-OP treatment-blocks applied more sprays and at greater cost than the OP blocks; in 2002, this order was reversed.

Table 2. Average number of foliar pesticide applications and costs for AWII apple orchards, 2001 and 2002.

	Codling moth		**Leafroller**		**CM+LR**		**Other**		**Total**		**Cost**	
Year	OP	NON-OP	OP	NON-OP	OP	NON-OP	OP	NON-OP	OP	NON-OP	OP	NON-OP
2001	2.3	2.3	1.4	0.9	0	0.5	5.7	6.2	9.3	9.9	$219	$250
2002	1.9	1.5	1.6	0.9	0	0.1	4.7	4.8	8.2	7.3	$208	$189

PEARS

Sample data

Pear psylla: Psylla is the major pest for most pear growers and was controlled well at all AWII sites. Psylla populations varied considerably and were consistently higher in the north-central Washington (NCW) sites (P4-P6) than in the Yakima sites (P1-P3). Two of the NCW orchards (P5 and P6) had consistently lower psylla populations post-bloom in the SOFT treatment-blocks.

Spider mites: Twospotted spider mite was the most common mite species found but occurred only at low levels. Counts were above 0.5 mites/leaf in only five of the 98 samples examined and never exceeded 1.0/leaf.

Grape mealybug: This pest was found in all three NCW pear orchards but in none of the Yakima orchards. Mealybug was found in tray samples and August-timed tree searches in all 12 treatment-blocks in NCW. There was a trend for lower counts in the SOFT treatment-blocks.

Pear rust mite: Rust mites were rarely detected in leaf samples, but fruit russeting caused by this pest was found in two blocks (P1 and P3). Nearly 9% of the fruit had rust mite damage in the SOFT treatment of P3; this block had pear rust mites counted in leaf samples several times in 2001, and additional controls will be needed in 2003 to prevent further damage.

Codling moth: There was a wide range in codling moth (CM) populations among the six sites. The total catch in pheromone traps for the season at sites P1 and P2 averaged two moths per trap or less, while sites P3, P4 and P6 averaged over 10 moths/trap/season. Second-generation CM catches increased significantly in P3 and P4 and fell dramatically in P6. DA lure-baited traps caught few moths in these pear orchards, never exceeding an average of 2.0 moths/trap/season. The CM catches in DA traps showed little correlation with the catch in pheromone traps in these orchards.

Leafrollers: Pandemis leafroller was caught in all orchards and was the dominant species in five. Obliquebanded leafroller was caught in all orchards in NCW but was the dominant species only in P6. Catches of both species of leafrollers were higher in almost every orchard in 2002 compared to 2001 catch totals, the sole exception being OBLR in P6. Total pandemis catches on average were nearly four times greater in 2002; OBLR catch totals were double the 2001 amounts.

Natural enemies: Ten types of predators and parasites were counted in this project. The most common, and most significant in terms of potential biological control of pear psylla, were deraeocoris, campylomma, lacewings, *Trechnites sp.* and spiders. Natural enemy counts were higher on average in the NCW orchards. This may be a result of more suitable nearby habitats, more food (psylla) to attract and retain them and, in several blocks, less use of disruptive insecticides. There were few differences in natural enemy counts between treatments in the Yakima orchards, but the NCW orchards had consistently more natural enemies in the soft treatment blocks.

Fruit damage

All treatment-blocks had 2500 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 was a particular concern in orchard P4, especially in the SOFT treatment block. **Codling moth** damage was low; the damaged fruit found was mostly on block edges. **Leafroller** damage was quite low, if it was found 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.

Grower	Treatment	Psylla	GMB	CM	LR	Fruitworm cutworm	Box elder stink bug	Lygus	SJS	PRM
Avg.	CONV	0.23%	3.63%	0.17%	0.07%	0.03%	0.01%	0.03%	0.01%	0.07%
	SOFT	0.36%	6.01%	0.07%	0.05%	0.01%	0.02%	0.01%	0.00%	1.54%

Pesticide use

Two Yakima growers have failed to submit spray data. We will endeavor to obtain these data by the research review date.

SUMMARY

Apples: All OP and NON-OP blocks maintained low pest populations and had very low fruit damage levels at harvest, in most cases lower than in 2001. The average number of pesticide applications and the cost per acre also declined, significantly so for the NON-OP blocks. The reduction in sprays probably stems from increased confidence in the efficacy of the new insecticides, primarily methoxyfenozide (Intrepid) and pyriproxifen (Esteem), used for codling moth and leafroller control in the NON-OP blocks.

The thorough monitoring of codling moth, leafrollers and lacanobia fruitworm provided growers with the information needed to respond with well-timed control measures where needed. Leafroller monitoring with standard and low-load pheromone lures showed similar population trends. The AA lure attracted very few leafrollers and will probably not be used in the AWII orchards next year. There were no surprises relative to secondary pests or their natural enemies in any orchards

Pears: Effecting changes in pest and natural enemy populations, by shifting to a selective, less disruptive pest control program, can take one, two or more years until the new populations are established. The year 2002 can be considered Year 1 in this process, as new treatment protocols were adopted. The NCW pear orchards show reduced psylla numbers and increased natural enemy numbers in the SOFT blocks; no such trend is evident in the Yakima orchards. Good control was obtained of most pests in the SOFT blocks, including codling moth and leafrollers. However, potential pest problems are posed by grape mealybug and pear rust mite, particularly in SOFT blocks, and leafrollers, based on greatly increased catches in pheromone traps. The AWII pear orchards should be followed for at least two more years to clearly establish changes in pest and natural enemy populations with the use of selective insecticides.

Budget:
Project title: New pest management programs for apple and pear.
PI: Jay F. Brunner
Project duration: 3 years
Current year: Year 3 (2003)
Project total (3 years): $291,059
Current budget request: $94,978

Year	Year 1 (2001)	Year 2 (2002)	**Year 3 (2003)**
Total	89,760	106,321	**94,978**

Current year breakdown

Item	Year 1 (2001)	Year 2 (2002)	**Year 3 (2003)**
Salaries[1]	24,000	37,440	**17,378**
Benefits (29%)	9,360	11,981	**5,040**
Wages[2]	40,000	40,000	**53,500**
Benefits (16%)	6,400	6,400	**8,560**
Equipment	0	0	**0**
Supplies[2]	8,000	8,000	**8,000**
Travel[3]	2,000	2,500	**2,500**
Miscellaneous	0	0	**0**
Total	89,760	106,321	**94,978**

[1] Mike Doerr's salary for 4 months. Mike will manage the project along with assistance of Nana Simone in view of Ted Alway's departure. The request is for a portion of Mike's salary. One-third of Mike's salary is already paid from grant dollars.
[2] Consultant compensation plus Nana Simone.
[2] Traps, lures, vials, solvents, etc. Telecommunication charges are allowed under this grant.
[3] Reimbursement for travel and cost of one vehicle for six months plus fuel and maintenance.

CONTINUING PROJECT REPORT **YEAR 2/2**
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: Jay F. Brunner, Entomologist & Director, WSU TFREC, Wenatchee, WA
Vincent Hebert, Assistant Entomologist, WSU FEQL, Richland, WA

Cooperators: Rick Hilton, OSU

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 where MD problems cannot be traced to poor timing, low rates or other obvious factors and compare it to data taken in Objective 2.

Deviations from objectives:

No significant deviations from the original plan have been made.

Significant findings:

- Residual analysis of pheromone dispensers showed all types tested have a significant loss of pheromone immediately after opening the factory package.
- The data on residual analysis collected this year agree with patterns that were seen in 2001, suggesting that some problems in MD may be related to dispenser performance problems.
- Data gained during field analysis of delayed mating was greatly improved by the development of new mating tubes that reduce time and cost of determining delay in mating. These tubes work well with all three species and should allow us to monitor several orchards at the same time.
- Lab studies on the delay in mating of OBLR show that at least two factors contribute to the observed reduction in population growth. First, older females tend not to mate and are more likely to be completely infertile. Second, delayed mating reduces egg production, increases the generation time and increases the time required for the population to double in size.

Methods:

Objective 1. To insure our data, we implemented a chain of custody so that there was complete tracking and control of dispensers, from manufacturer to experimental site and to FEQL where final analysis was performed. Manufacturers sent the dispensers to directly to FEQL, where an initial day-0 sample was removed. The dispensers were then placed in Mylar bags, sealed and sent to TFREC (Wenatchee) where a second day-0 sample was removed. From Wenatchee, dispenser packages were also sent to Rick Hilton in Medford, OR, who ran studies in that location to replicate the Wenatchee experiments. After the aging process, all samples were returned to FEQL for final analysis.

Dispensers were aged by placing them on apple tree branches in a manner similar to how they would normally be used. Enough dispensers were placed on trees to allow collection of 20 of each kind per sample date (every 28 days in Wenatchee for a total of 140 days and every ≈30 days in Medford for a total of 162 days). Dispensers being shipped were packaged in Mylar bags and kept in a –12°C freezer until shipped to FEQL via one-day express mail. At FEQL, dispensers were stored in a –80°C freezer until analysis. All dispensers were labeled with date of collection, age and location. To date, only the data from the residual analysis are analyzed. This winter, work will focus on getting the volatile capture method on-line.

At FEQL, each dispenser was analyzed for amount of codlemone remaining in the dispenser (residual analysis). The extraction procedures for the different pheromone dispensers were requested from the manufacturers and, where provided, were followed. Essentially, all the pheromone in a dispenser was removed by use of appropriate solvents or digestion of the dispenser. The amount of actual pheromone present in the dispenser was then determined using a gas chromatograph. This system was 97-100% efficient in recovery of target chemicals based on internal standards used during analysis.

Fig. 1. Original mating table design modified from McBrien and Judd.

Objective 2. Mating studies in the field began with a modification of the mating tables developed by Heather McBrien and Gary Judd (Fig. 1). Initial experiments were carried out in a non-MD orchard and were primarily aimed at perfecting the techniques used. The fore tarsi of females were removed and one of the forewings clipped to prevent escape. Mating tables were placed in the orchard and spaced so that a minimum of 50 feet separated each mating table. The moths placed in the field were treated as a cohort and followed nightly for several hours after dusk until females were no longer calling. Any mating was recorded, and jars with mating pairs were removed from the field and the females dissected to determine mating status.

Objective 3: A group of pupae from the OBLR colony maintained at TFREC was segregated by sex and placed into separate 550 ml emergence cages. When adult females emerged they were individually placed into smaller mating arenas equipped with nectar dispensers and creased waxed-paper sheets to serve as oviposition surfaces. A single adult male, which was less than three days old, was introduced to each female in the mating arenas 0, 2, 4, or 6 days after female emergence. Approximately 25% of each cohort were designated as 0 delay mating pairs to serve as controls. The mating arenas were checked daily for egg masses and adult mortality. Males that died before females were replaced with a fresh male. Egg masses were either removed on the day they were discovered or were circled with a colored marker to indicate the date on which they were deposited, allowing them to be removed later. Egg masses collected from the mating arenas from each day were placed into labeled Petri dishes and stored in an incubator with approximately 60% humidity. A 14:10 (L:D) h photoperiod and ambient temperature of 22°C was maintained in both the incubator and the room where mating arenas were stored.

Results and discussion:

Objective 1: In 2002, a pattern of residual pheromone remaining in dispensers was observed (similar to work performed in 2001 which was not on a Commission-sponsored grant). It is interesting that all dispensers lost a significant amount of pheromone from the day they were received at the FEQL and the day they were opened in Wenatchee or Medford. This might be explained by the amount of pheromone on the outside of dispensers, as most manufacturers charge the dispenser to make sure pheromone will be ready to be released when the dispensers are placed in the field. The amount of codlemone residual in the Isomate C+ dispenser declined gradually with the exception of the period from day-84 and day-112 when very little change in pheromone content was observed (Table 1). This same pattern was noted for day-60 and day-90 in Medford (Table1). The residual pheromone in Isomate C+ dispensers was more variable in 2002 than in 2001. The variability from sample date to sample date could account for the pattern of residual pheromone observed. It is possible that twisting of the Isomate C+ dispenser around a twig causes cracks to occur that would allow for faster than expected release of pheromone. We are looking into this as a possible explanation of our data.

The Isomate CTT dispenser showed a gradual decline in residual codlemone at both Wenatchee and Medford. This dispenser does not have the potential problem of being damaged when applied because the twin tubing is separated and slipped over a twig or branch.

The NoMate dispenser had the proper amount of codlemone load in 2002. It also showed a similar pattern of residual decline as in 2001. There was a rapid decline in the residual codlemone in the middle of the season, and the pattern was observed at both Wenatchee and Medford (Table 1). For example, after 112 days (Wenatchee) or 90 days (Medford) this dispenser had lost 90% of its codlemone, whereas the other dispensers had lost only 55-70%.

As in 2001, the CheckMate dispenser had a very low release rate as seen in the slow decline in residual codlemone. By the end of the season, the CheckMate dispenser had only lost about 64% of its codlemone in Medford and only 38% in Wenatchee. Most of the other dispensers had lost between 97% and 76%, indicating that this dispenser is the least efficient in terms of releasing nearly all its pheromone at the correct rate over the course of the entire season (Table 1).

Many factors influence the release of pheromone from a dispenser. The hand-applied dispensers are all passive release devices, and the rate of release of pheromone is governed to a great degree by temperatures the dispensers experience. In general, the higher the temperatures the faster the release rate of pheromone from a dispenser. There is a balance therefore between making a dispenser that will release enough pheromone to provide effective mating disruption and having one that lasts the entire season, which seems to be the goal of all current hand-applied dispenser manufacturers.

This report summarizes information only on the behavior of different hand-applied dispensers in releasing codlemone. In and of itself, the release of codlemone from a dispenser does not state whether it will be efficacious or not. At best, we understand that the efficacy of codling moth mating disruption is based on having some threshold amount of pheromone present in the orchard air that limits the ability of males to find females, thus reducing the potential of the population in that orchard to increase. The information presented on different dispensers must be taken into consideration with the recommended number of dispensers used per acre as a full rate. In this way, one can fairly assess the relative amounts of pheromone being released per acre by different aged dispensers and gain some understanding of the potential of each dispenser to negatively impact the target population.

***Objective 2**:* After several trials it became apparent that the original mating tables were relatively inefficient – that is, we had a fair number of females escape (probably because of either wind or males helping the females escape during the mating process) and, upon dissection, it was revealed that some of the females that had never been observed mating at night were mated. In addition, the heat in the middle of the summer increased mortality, particularly with the trap allowing sunlight to hit the jar at the bottom of the trap. We modified this trap several ways but finally changed the design in mid-August to a plastic tube with screen funnels at each end (Fig. 2). This trap design worked

Table 1. Average amount of codlemone remaining in dispensers field aged in Wenatchee or Medford.

	Average Amount of Codlemone Remaining (mg.)				
Age in days	**Isomate C+**	**Isomate CTT**	**NoMate**	**CheckMate**	**Disrupt CM**
Manufacturer day 0	139.1	295.5	137.2	310	-
Wenatchee day 0	123.2	276.4	126.4	290.9	-
28	99.6	246.5	117.3	262.9	-
56	78.3	191.2	64.3	246.6	-
84	54	167	33.1	236.1	-
112	51.8	124.6	14.3	211.4	-
140	33.9	53.9	10.1	192.3	-
% of Total Released	76	82	93	38	-
Medford					
0	140.4	292.8	110.6	289.2	176.3
35	111.5	243.7	54.1	243.1	148.8
56	96.7	220.5	38.2	226.9	139.2
84	82.3	171.5	15.1	194.6	106.6
112	46.6	128.3	5.3	184.9	109.7
148	35.5	97.3	2.3	124	87.5
162	24.4	88.3	1.7	108.8	81.1
% of Total Released	83	70	98	62	54

much better with no escapes of 40 moths tested, it required no mutilation of the females, and it allowed us to just put the trap in the orchard and check it daily (since males were retained in the trap). When males were found, the trap was removed from the field and the female was dissected to determine mating status.

Using the modified mating table with OBLR, we were able to get mating in 60% of our tubes over a 5-day period in a non-mating disruption orchard. The average cumulative percent mating was 30, 58, 62, 92, and 100% captures for days 1-5, respectively.

To date, these tubes have been tested in the field on OBLR, PLR and CM with only minor modification necessary to switch between the species. The new design should allow us to work several orchards simultaneously because no modification of the females are necessary and the mating tubes are inexpensive and easy to produce. In addition, checking the traps every few minutes does not have to be done throughout the night to determine if mating occurred because presence of the male in the tube indicates that potentially mating did occur and the females can be dissected to check mating status. The new mating tube should be an excellent tool as long as the colonies of OBLR, PLR and CM females are representative of wild type females.

Objective 3: To date, only studies with OBLR have reached the point that any analysis is possible. With OBLR, we find no difference in mortality between females where mating is delayed 0, 2, 4, or 6 days. However, the mean production of female offspring is dramatically affected by the delay in mating (Table 2). For example, females experiencing a 2-, 4- or 6-day delay have only 67, 50, or 13% the net reproductive rate of females mated on the day of emergence and population doubling time increased 12.3, 27.1 and 106%, respectively. Further, while our experiments started with newly emerged virgin females to simulate field conditions, we used 1- to 3-day old males that were replaced with another 1- to 3-day old male if any male mortality occurred. As such, males should not have been the limiting factor in female fertility. However, we found that no egg masses laid after females were 11 days old were fertile in any of the treatments, even though they continued to lay new egg masses.

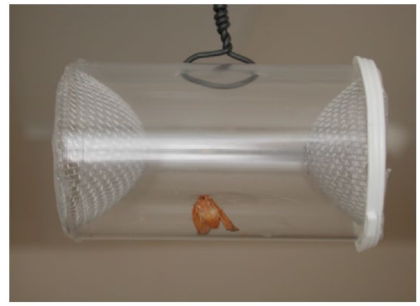


Fig. 2. New Mating Tube design with OBLR female (top), complete trap with mating tube inside a delta trap.(bottom).

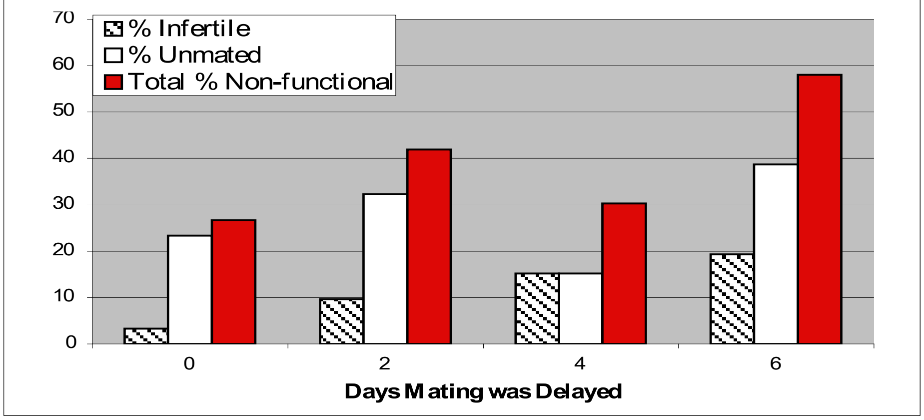
Table 2. Effect of delayed mating on population growth of OBLR

	Number of Days Mating was Delayed				Percent of Control Value		
Population Parameter	0	2	4	6	2	4	6
Net fertility rate[1]	163.8	109.9	81.6	21.2	67	50	13
Fertile eggs/female/day	16.6	11.5	8.9	2.2	69	54	13
Net Reproductive Rate[2]	81.9	54.9	40.8	10.6	67	50	13
Population Double Time (days)	6.8	7.6	8.6	14.0	112	127	206
Generation Time (days)	43.1	44.0	46.1	47.6	102	107	111
Without infertile and non-mating females							
Net fertility rate	223.3	189.2	129.1	54.8	85	58	25
Fertile eggs/female/day	23.6	21.4	13.7	5.9	91	58	25
Net Reproductive Rate	111.7	94.6	64.6	27.4	85	58	25
Population Double Time (days)	6.3	6.7	7.7	10.0	106	121	157

[1]Net fertility rate is the number of eggs a female would be expected to lay during her life span given the observed mortality rate.
[2]Net reproductive rate is the number of daughters a female would be expected to lay during her life span given the observed mortality rate.

Examination of the individual female performance in each treatment showed that the number of females not laying any eggs or that laid eggs that were all infertile (i.e., female did not mate) generally increased as the delay in mating increased (Fig. 3). The data thus suggest that part of the effect of delayed mating is that females are no longer interested in mating even when capable males are present. To determine what effect the delay in mating has on actual life table parameters independent of the tendency to mate or not, we removed all the data for females that either never laid eggs or those that never laid any fertile eggs. When this analysis was performed, the effect of the delay in mating was less but still considerable (Table 2). For example, the net reproductive rate was 85, 58, and 25% of the control and the population and doubling time was 5.9, 21, and 57% longer than the control.

Figure 3. Effect of a delay in mating on female OBLR mating



Implications for organic IPM:
Mating disruption of codling moth is key to organic fruit production in most areas. The selection of dispensers may have a marked impact on the success of mating disruption. Dispensers that run out too early or do not release enough throughout the key parts of the season will result in unacceptable damage that will be difficult to control.

Budget:
Project title: Examining the mechanisms of mating disruption in codling moth and leafrollers
PI: Vincent P. Jones
Project duration: 2002-2003
Current year: 2003
Project total (2 years): $98,312
Current year request: $49,702

Item	Year 1 (2002)	**Year 2 (2003)**
Salaries (Associate in Research)[1]	17,500	**18,200**
Benefits (31%)	5,250	**5,642**
Wages	11,000	**11,000**
Benefits (16%)	1,760	**1,760**
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	**49,702**

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

CONTINUING PROJECT REPORT **YEAR 3/3**
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 and Director, WSU TFREC, Wenatchee, WA

Cooperator: Tom Unruh, USDA-ARS, Wapato, WA

Objectives:
1. Develop sampling plans for PLR and OBLR that minimize the number of samples per acre, maximize repeatability of the estimate, and reduce the time and cost.
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 the natural enemies with minimized sampling.

Significant findings:
- Geostatistical analysis of PLR and OBLR populations showed that damage begins in small pockets and each pocket expand outwards until they join.
  - Spatial variation accounted for an average of 77% (range of 39-99%) of the total observed variation in mean population level.
  - Application of Bt in our plots resulted in a distribution of LR that was fragmented into a large number of pockets, probably related to the spray coverage in the field.
- Lab studies on phenology at 15, 20 and 25°C have been completed. We need to run studies at 10°C to help determine whether the lower thresholds currently used are reasonable, as our lab studies suggest OBLR thresholds should be higher.
- Studies of Bt on the developmental rate of PLR and OBLR are in progress. We found that PLR was much more sensitive to Bt effects than OBLR.
  - The instar after the treated one had the slowest developmental rate, and the negative effects were reduced with each successive molt.
  - Larvae fed the low dose of Bt for two days or those fed at the fourth instar had a slower developmental rate than those fed one day or at the third instar.
- Parasitism in our phenology study blocks varied between 13 and 34%, with between two and three species of parasitoids being common.
  - In PLR orchards, *Transonema*, *Apanteles* or *C. florus* was the dominant species.
  - In the OBLR orchards, *Macrocentrus* or the tachinids *Nilea* or *Nemorilla* were the most common species.

Methods in 2003:
Objectives 1&3: Data on the distribution of leafrollers are taken by collecting bud samples early in the season and leaf samples later in the season. We visually inspect 25 sampling units per tree noting the date, degree-days and tree phenology at the time of sampling. The different tree samples are collected using GPS-coupled Palm Pilots to determine the exact location of every sample. The

information required for sampling parasitoids of leafrollers will be taken concurrently with the leafroller sample. All other methods are the same as described in last year's progress report.

Data will be taken on LR distribution primarily to validate the sampling programs and distribution studies of the past two years.

Objective 2: Sampling for the phenology of leafrollers will continue this next year as in the past two years, with emphasis on resolving any problems in the current data set (see the results below). Lab studies on developmental rate will examine 10°C for both PLR and OBLR to clarify concerns over the current lower thresholds for development. Laboratory studies with Bt will be finished this winter, and we will begin work on the effects of Intrepid (rather than Confirm).

Results and discussion:
Objective 1: We collected 12 orchard samples for the spatial distribution of LR: two OBLR orchards, seven PLR and three orchards with a mixture of both species. Over all plots, 1228 samples were collected, each sample consisting of 25 buds or branch tips (total of >30,000 sampling units). This year we were able to interface GPS receivers with our Palm Pilots and obtain location data accurate to 1-2 m. Our geostatistical analysis showed that spatial variance accounted for an average of 77% (range 39-99%) of total variability in an orchard. There did not seem to be any difference between large plots and small plots in the importance of spatial variation. Within limits, the intensity of sampling (i.e., samples per acre) appears to be related to the effective range (distance over which samples are related). Similar to what we noted last year, damage begins in a rather small area and expands outwards. It may be nearly a perfect circle (Fig. 1) or it may be more irregular (Fig. 2). Interestingly, when we sampled in one of our orchards in both the first and second generation after Bt sprays (Fig. 3), it appeared the Bt fragmented the population into a large number of small pockets (probably related to uneven spray coverage).

Fig. 1. Distribution of PLR damage showing expansion of damage from a two central points

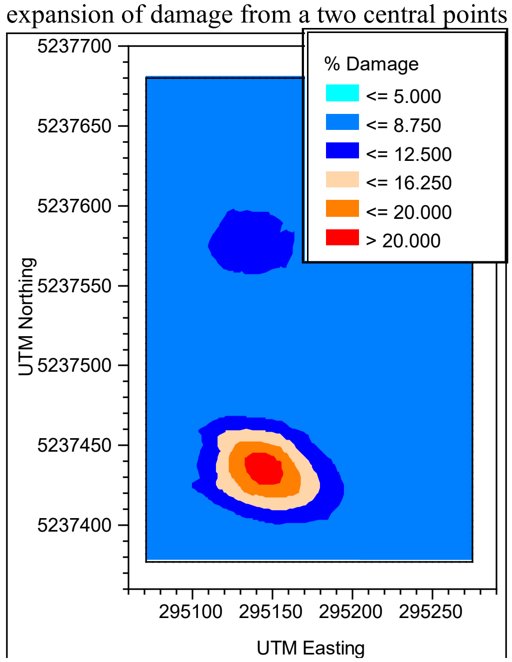


Fig. 2. PLR distribution showing more irregular growth of population by end of the overwintering generation.

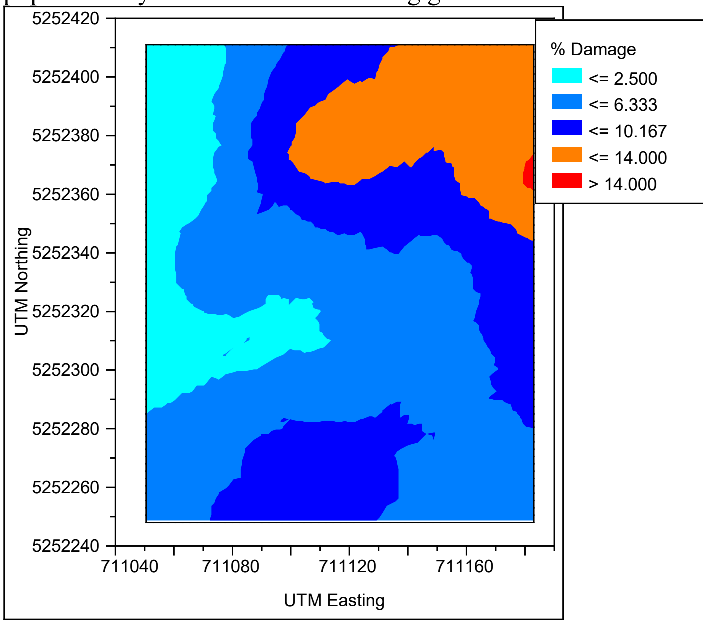
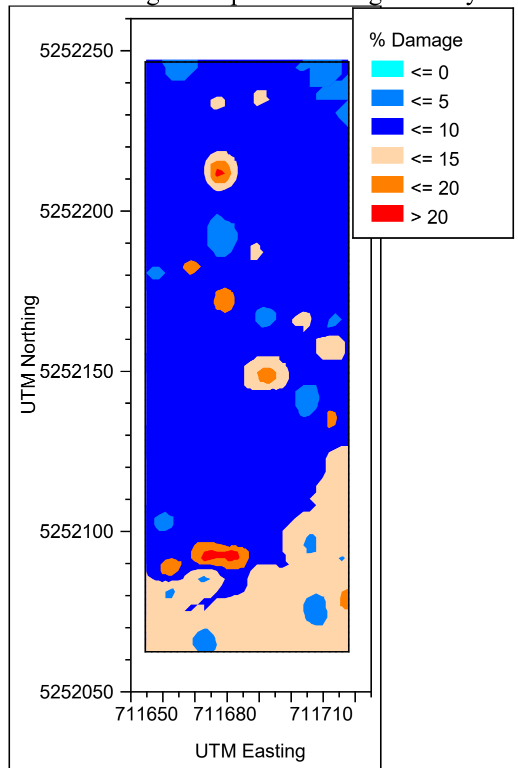


Fig. 3. Distribution of PLR following a spray of Bt showing small pockets of high density.



Objective 2: Lab studies on phenology. Laboratory studies of phenology have been completed at three temperatures (15, 20 and 25°C) and suggest that the 5°C lower threshold for development used for PLR is probably a good compromise when trying to model all different stages, although 6°C might be slightly better. However, we did notice that the threshold for development of the stages varies tremendously, with the second and third instars (the overwintering stages) having a very low developmental threshold. This helps to explain why we noticed that early in the season larvae collected in buds and placed in the refrigerator were still active. It also shows why we have some later instars present almost immediately in the spring when we begin sampling.

The OBLR data suggest that the current lower threshold for development (6.1°C) is too low for all stages except the first instar. The lower threshold for OBLR would probably be better at about 11°C. Before making the changes to either OBLR or PLR, at least one additional lower temperature needs to be run (probably 10°C) because uncertainty in the lower threshold can affect all model calculations. *For this report, all the model calculations for PLR are at a base of 5°C and OBLR at 6.1°C.*

Effect of Bt on developmental rates: The effect of Bt on developmental rates is important in developing models because Bt has been shown to affect developmental time in leafrollers. As Bt is still one of the effective materials against leafrollers when timed properly (particularly in organic orchards), the phenology change has been reported to change the current model predictions. This could then affect both any model-based activity (spraying, survey for parasitoids, etc.). We examined this effect of Bt on both OBLR and PLR (although more replications are still necessary for both species, particularly OBLR). We fed the leafrollers artificial diet spiked with fixed amounts of Bt for either one or two days, transferred them to diet with no Bt and measured the time required to emerge. We exposed the larvae as either third or fourth instars and determined the time required for emergence to each of the remaining stages. For example, larvae exposed as third instars would have developmental time to fourth, fifth, sixth, pupae and adult. Mortality was measured at each stage as well. The doses of Bt used were quite low (1/100th field rate for PLR, 1/25th, 1/50th, and 1/100th field rate for OBLR).

Table 1. Effect of Bt on developmental rate of OBLR.

Instar fed Bt	% Field rate	% of time control insects required to complete development to a given stage			
		4th Instar	5th Instar	Pupae	Moth
3	4	123.5	110.2	103.6	108.5
3	2	130.5	112.1	103.9	102.7
3	1	130.3	110.7	95.4	99.1
4	4	-	150.0	132.3	118.9
4	2	-	150.6	131.3	122.7

The overall mortality to the low doses of Bt was much higher for PLR than OBLR in the same situations (dose). PLR fed two days had a higher mortality, and the effects were greater than those fed Bt for only1 day (Table 1). The developmental rate was slowest in the instar after the insect was treated, and the effect decreased with each molt.

Table 2. Effect of Bt on developmental rate of PLR. Rate used is 1% field rate.

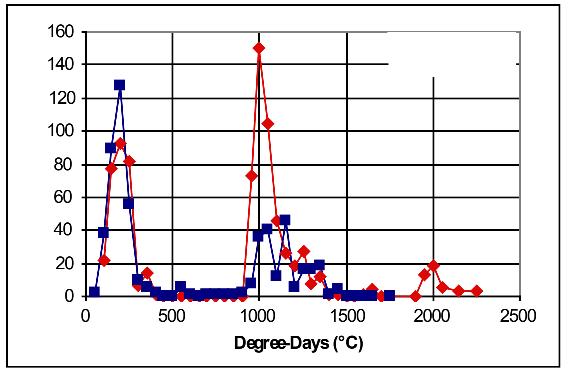
		% of time control insects required to complete development to a given stage			
Length of time fed (d)	Instar treated	4th Instar	5th Instar	Pupae	Moth
1	3	182.6	141.3	146.4	123.1
2	3	191.9	197.8	144.1	129.7
1	4	-	138.8	109.2	106.8
2	4	-	210.0	153.6	136.1

For OBLR, the increase in developmental time was much lower and, overall, the time required to complete development was relatively minor for insects fed Bt in the third instar (Table 2). However, the effects were greater for the group fed Bt in the fourth instar, probably because of the greater amounts eaten by the older caterpillars. For OBLR, the time of moth flight would be off by about 18%.

Field phenology of OBLR and PLR: When PLR occurred in the orchards over the past two years it was very similar when plotted on a degree-day basis (Fig. 4). However, preliminary models were reliable only for the second and third instars. Instars 4-6 occur at similar times but have a different rate of emergence. We have not yet received all the spray records from our collaborators, but in at least one of the orchards, an Intrepid (methoxyfenozide) spray was applied and appeared to spread out the emergence drastically to the point that it appeared that there was no break between the generations. We will investigate this in the lab this winter and will need next year's validation to determine whether a full phenology model will be possible or whether we will just be able to a range in degree-days when sampling for parasitoids should occur.

Analysis of OBLR has only begun but, as with PLR, the timing of emergence across the two years of data is similar. In 2001, we did not start collecting larvae in OLBR orchards early enough and missed the first generation. However, the second generations appear quantitatively similar in their intensity and rate of emergence.

Figure 4. Collection curves for phenology of PLR over 2001-2002.



Objective 3:
Parasitoids collected: In 2001, 15 parasitoid species were collected and 11 were collected in 2002. In general, the difference was a few very rare species, but the major species were the same in both years. As occurred last year, *C. florus* was not collected until the second generation, even though some PLR do overwinter as early third instars.

In 2001, we attempted to use yellow sticky panels to monitor *Apanteles* and *C. florus*. Despite rather high parasitism at several sites, we only collected a few parasitoids on the cards, suggesting that they were not easily monitored using this method.

Field parasitism rates: Parasitism varied between locations sampled and the primary LR species present (Table 3). OBLR parasitism was composed primarily of the Tachinid flies *Nilea* and *Nemorilla*, and *Macrocentrus* or *Meteorus*. At the sites dominated by the tachinids, parasitism was relatively low (≈10%), but the two sites dominated by *Macrocentrus* had about 2.5-3x higher parasitism.

For PLR, *Transonema* dominated at two sites and *Apanteles* dominated at two others. Overall, *Apanteles* was the most common parasitoid, being one of the three most important at all of the PLR sites.

Table 3. Parasitism of OBLR and PLR and key parasitoids

Location	Major LR	Total % parasitism	Number parasitoid sp.	Dominant parasitoid	Parasitoid 2	Parasitoid 3
TFREC5	OBLR	33.3	5	*Macrocentrus*	*Nilea, Meteorus*	
DD2	OBLR	33.9	6	*Nemorilla*	*Macrocentrus*	*Nilea*
CFA	OBLR	9.8	2	*Nilea*	un id Tachinid	
Bev	OBLR	10.7	3	*Nilea*	*Nemorilla*	
STEO	OBLR (90%)	24	6	*Macrocentrus*	*C. florus*	
WVCO	PLR	13.9	3	*Apanteles*		
WVC14	PLR	26	7	*Apanteles*	*Nemorilla*	*C.florus*
STE2	PLR	14.1	4	*C. florus*	*Nemorilla*	*Apanteles*
TFREC24	PLR	19.3	5	*Nilea*	*Apanteles*	
CV19	PLR	13.1	6	*Transonema*	*Apanteles*	*Meteorus*
DDW	PLR (66%)	25.9	10	*C. florus*	*Nemorilla*	*Apanteles*
TFREC26	PLR (85%)	25.5	6	*Transonema*	*Apanteles*	*Meteorus*

Budget:
Project title: Developing sampling plans for leafrollers and their natural enemies
PI: Vincent P. Jones
Project duration: 2001-2003
Current year: 2003
Project total (3 years): $133,211
Current year request: $41,737

Year	Year 1 (2001)	Year 2 (2002)	**Year 3 (2003)**
Total	49,740	41,734	**41,737**

Current year breakdown:

Item	Year 1 (2001)	Year 2 (2002)	**Year 3 (2003)**
Salaries	8,750	14,560	**13,865**
Benefits[1] (30%)	2,800	4,368	**5,823**
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	**41,737**

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

The budget is increased $6,352 over that originally approved to reflect the cost of collecting and rearing the insects (i.e., supplies, travel and wages for time-slip personnel). The data collected next year for the phenology of PLR and OBLR will have to be greater than initially predicted to resolve differences observed between 2001 and 2002.

CONTINUING PROJECT REPORT **YEAR 3/3**
WTFRC Project # AE-01-32

TITLE: Development of operational strategies for practical control of codling moth neonate larvae and prepupae using codling moth granulovirus (*Cp*GV) and *Steinernema feltiae* (Nematoda).

PI: Lawrence A. Lacey

ORGANIZATION: USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA

OBJECTIVES:

1. Continue evaluation the effects of the frequency and timing of application of the Carpovirusine *Cp*GV formulation on insecticidal efficacy, fruit damage and density of subsequent generations. Increase treated acreage in 2003.

2. Continue research on the improvement of nematode and viruses through formulation

3. Investigate several sprinkler types for automated post application wetting to enhance nematode efficacy.

4. Evaluate the combined effect of application of *Steinernema spp.* on overwintering populations of CM nematode and *Cp*GV application after biofix for control of neonate larvae on insecticidal efficacy, fruit damage and density of overwintering population

SPECIFIC FINDINGS:

1. Two commercial formulations of *Cp*GV provided significant reduction of deep entries by codling moth and reductions in numbers of live larvae following six weekly applications during the first flight codling moth (June 5-July 8).

2. Studies to determine the decrease in virus activity due to solar inactivation revealed a steady decrease in activity over the 3 days following application, but significant control was still observed 3 days after application.

3. Combination of virus with adjuvants for solar protection did not extend the life of *Cp*GV on the leaf but did enhance control.

4. Commercial formulation of nematodes of *Steinernema feltiae*) for control of codling moth using an airblast sprayer provided fair control under sub-optimal conditions.

5. Treatment of fruit bins with nematodes (*S. feltiae*) in low and high humidity with and without the addition of a humectant or wetting agent showed that nematode efficacy could be improved with the addition of adjuvants.

METHODS:

Source of virus. The Carpovirusine *Cp*GV formulation will be supplied by Sumitomo, Corp. and the Cyd-X formulation will be supplied by Certis, Corp.

Sources of nematodes: *Steinernema feltiae* will be supplied by Becker Underwood and *Steinernema carpocapsae* will be supplied by Certis, Inc. For small scale testing, nematodes will be produced at YARL using procedures outlines by Kaya and Stock (1997).

Field application and evaluation:

Large plot field applications of the CM granulovirus and entomopathogenic nematodes will be made at the Moxee farm and in commercial orchards using protocols described by Lacey et al. (2000) and Unruh and Lacey (2001).

Efficacy assessment of granulovirus against codling moth larvae. Treatment and control plots will be arranged in completely randomized blocks to account for variation found within the orchard. Plot sizes will be 1/10 acre in research Farm and an acre or more in grower blocks.

Dosage, timing of applications and frequency of applications. We will use the operational dosage of 10^{13} capsules/ha (one liter of Carpovirusine formulation/ha) applied with an air blast sprayer. Initial applications will be made shortly after initial flights of moths in the spring when neonate larvae are beginning to emerge (200 DD). Depending on experimental goals, 2 to 6 applications at weekly intervals will be made during the spring and early summer.

Assessment of efficacy. *Fruit damage.* Fruit damage will be assessed within two weeks of the first virus application and late spring and at harvest. Fruit damage will be made *in situ* in late spring. Sample sizes will be at least 200 fruit per plot during the growing season and all harvested fruit. The number and condition of fruit that has fallen prior to harvest will also provide an indication of codling moth activity. The number of "stings" and severity of damage will be recorded based on the following criteria: minute stings: 1 mm across and 2 mm deep or 2 mm across and 1 mm deep; likely to cause downgrading but not cullage; medium stings: 2-4 mm across and up to 3 mm deep; large stings: > 4 mm across and 5 mm deep.

Numbers of larvae. Monitoring of the larval population outside of fruit will be accomplished using cardboard bands (B flute, 2-9 cm wide) at the end of the growing season. Bands will be placed around the trunks and larger branches. During the course of our experiments we will monitor environmental conditions including: temperature, rainfall, irrigation, and solar radiation.

Use of entomopathogenic nematodes for codling moth control. *Experimental design*. A randomized block design will be utilized to determined the effects of spring and/or fall applications of *S. feltiae* on larval mortality, fruit damage and numbers of overwintering cocooned larvae. The combined treatment of nematode and granulovirus applications will be explored in this year.

Equipment. The same equipment used for application of virus will be used for application of *S. feltiae*. The major difference will be the size of droplets and volume of water. The fan will be turned off and nozzles changed to ones that permit coarser droplets (*e.g.*, solid cone nozzles). The object will be to apply the IJs to the trunk and scaffold branches of the tree, not to foliage.

Dosage and timing of applications. An application rate of 2×10^6 IJs of *S. feltiae*/tree will be used for spring and/or fall experiments. Applications will be timed to correspond to wet, warm weather.

Pre- and post-treatment sampling. The codling moth stages that will be targeted for control using entomopathogenic nematodes are cocooned larvae. Because mature larvae seek cryptic habitats for spinning their cocoons, natural populations may be difficult to monitor, especially when population density is low. Infested sentinel logs will be used in experiments to monitor mortality in cocooned larvae in the manner prescribed by Unruh and Lacey (2001). The logs will be attached to the trees with wire just before application of nematodes. In addition to sentinel logs on trees, infested cardboard strips can also be placed in other habitats likely to be used by larvae for hibernacula (leaf litter, cracks in soil, etc.). Sentinel logs and cardboard strips will be removed from the orchard 24 h after treatment and incubated under conditions that will allow field infected individuals to show patent signs of infection (25 for 3-4 days). This prevents excessive mortality due to predators and allows rapid differentiation of nematode-killed larvae versus larvae that have died of other causes. Nematodes die when the substrates become dry, so exposure of sentinel prepupae to IJs ends before logs are removed from the orchard. Banding of trees with cardboard strips will also be employed to monitor overwintering larvae at the end of the season.

RESULTS AND DISCUSSION:
Orchard trials of the Carpovirusine (Sumitomo) and Cyd-X (Certis) formulations (thousand fold dilutions) of the codling moth virus conducted over the first six weeks of the first flight of codling moth yielded encouraging results. The solar effects were not as pronounced as anticipated. Although the number of codling moth entries was similar between treatments and controls, the number of live larvae recovered the week following application was significantly lower in virus treatments (Table 1). Combinations of the Carpovirusine formulation and adjuvants Raynox or NuFilm did not appear to provide UV protection. The Cyd-X formulation appeared to be twice as effective as the Carpovirusine over the 3 day exposure period. The data shown in Table 1 are the average of three separate tests conducted in the same orchard on three separate dates (June 5, June 18, and July 1, 2002). At the end of the first flight 100 fruit per treatment and control were harvested and assessed for level of damage and presence of codling moth larvae (Table 2). The number of deep entries and number of larvae per 100 was highest in the control trees. The lowest numbers of deep entries were observed in the Cdy-X treatment and in Carpovirusine treatments that were applied at half rate twice a week or those combined with oil, Raynox, or NuFilm. The number of live larvae per 100 fruit was comparable between virus and virus combination treatments. Oil alone and Guthione provided comparable reduction of larvae.

Large plot nematode trials in two orchards for codling moth control were conducted in Parker Heights and Quincy. Both tests used conventional spray equipment (airblast sprayer with minimum or no fan) and the nematodes were applied while irrigation was running. Irrigation was continued for 8 hours following application. In Quincy, the Certis granular formulation of S. *carpocapsae* was used under fairly good conditions (70-80 F, moderate winds). Control was measured with sentinel larvae in logs and strips of cardboard. Mortality of cocooned larvae in treated plots ranged from 15-23% in cardboard strips and 24% mortality in logs. Quality control measurement of field collected samples of nematodes directly from the sprayer yielded on 37% mortality indicating a problem with the product. This granular formulation is stabilized infective stage nematodes in a clay matrix. Other researchers have noted delayed activity due to gradual revival of the infective stages. Because cocooned larvae are in microhabitats that are subject to drying, this problem must be overcome. I am working with Certis to investigate the possibility of using different formulations or freshly produced nematodes that supplied on short notice. The trials conducted in Parker Heights were with *S. feltiae* supplied by Becker Underwood and were applied in sub-optimal conditions (60+ F, high winds). Mortality ranged from 53-69%. Despite less than complete control, these results were encouraging. As we have seen from previous studies, post treatment wetting of surfaces sprayed with nematodes is vital so the infective stages can penetrate the hoist cocoon before drying. Maintenance of moisture with have to be balanced with avoiding the washing of infectives from the treated surfaces. This will be the subject of future studies. During this reporting period we also investigated the potential using adjuvants to increase efficacy for control of cocooned codling moth larvae in fruit bins. Control (95%) was highest in both high and low humidity when bins were immersed in a suspension of nematodes (10 infective stages/ml of tank water) that contained a humectant and surfactant.

The use of nematodes and codling moth virus offers potential for early season control of codling moth with minimal environmental impact. These agents could also be used in combination with mating disruption for selective control of codling moth without the need for initial sprays with broad spectrum insecticides.

ESTIMATED DURATION:

The project will last 3 years. Determination of timing and frequency of viral applications on the efficacy of *Cp*GV and *S. feltiae* will take place in the first year. The effect of individual and combined treatments on fruit damage and overwintering population density will be studied in years 2 and 3.

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BUDGET

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).
Principal Investigator:	Lawrence A. Lacey
Project duration:	3 years
Current year:	2003
Project total (3 years):	$86,685
Current year request:	$30,000

	2001	2002	**2003**
Salaries and wages (includes benefits)			
Technician, partial support for GS-5		20,000	**$25,000**
Summer help, GS-3, 1 FTE (3 mos.)		5,185	**3,500**
Subtotal		$25,185	**$28,500**
chemicals, plasticware, misc. materials		1,500	**1,500**
Subtotal		1,500	**1,500**
	25,000	$26,685	**$30,000**

Table 1. The average number of codling moth entries per fruit and the number of live larvae resulting from bioassays[a] conducted on apples picked soon after application of the codling moth granulovirus and 6, 30 and 54 hours after application. Data are the average of three separate tests conducted in the same orchard on three separate dates (June 5, June 18, and July 1, 2002).

Treatment	Stings/fruit sampled at four intervals after application			
	0	6	30	54
control	1.85	2.06	2.03	1.70
Carpovirusine	1.80	1.64	1.65	1.68
Carpovirusine +NuFilm	1.91	1.84	1.68	1.62
Carpovirusine + Raynox	1.87	1.77	1.58	1.66
Cyd-X	1.65	1.58	1.59	1.51
	live larvae/fruit sampled at four intervals after application			
control	1.37	1.61	1.73	1.42
Carpovirusine	0.39	0.62	0.79	1.00
Carpovirusine +NuFilm	0.31	0.67	0.65	0.86
Carpovirusine + Raynox	0.32	0.62	0.76	0.84
Cyd-X	0.12	0.31	0.38	0.48

each apple was infested with 3 neonate larvae in the laboratory within 2 hours of removal from the orchard

Table 2. Depth of entries and presence of live larvae after six successive weekly treatments with virus and/or oil. Guthione was applied twice at 21 day intervals.

	% of entries			
Treatment	minute	shallow	deep	number of live larvae /100 fruit
Control Block 1	1.8	2	5.2	2.3
Carposvirusine	7.5	2.7	3.2	0.1
Carpovirusine + Oil[1]	1.8	0.4	1.6	0.1
Oil[1]	2.1	0.6	1.4	0.1
Guthione	0.4	0.5	2.4	0.1
Control Block 2	3.4	2.0	6.9	4.3
Carposvirusine	7.6	0.8	4.0	0.2
Carpovirusine half rate	7.5	1.2	1.1	0
Carpovirusine + NuFilm	5.9	1.3	2.2	0.3
Carpovirusine + Raynox	4.5	0.4	1.9	0.1
Cyd-X	8.9	1.8	2.0	0

CONTINUING PROJECT REPORT **YEAR 3/3**

Project Title: Insect Responses to Induced Defensive Chemistry of Apple and Pear
PI: Peter J. Landolt
Organization: USDA, ARS, Yakima Agricultural Research Laboratory, Wapato,
Cooperator(s): 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.

2002 Objectives:
1. Quantify defensive chemistry in apple leaves.
2. Determine effects of apple foliage quality on Lacanobia movement.
3. Determine effects of infested fruit on codling moth oviposition.

Significant findings:
1. Development of Lacanobia fruitworm larvae on apple foliage was again better in spring compared to summer, with heavier pupae, faster development and greater survival.
2. In a comparison of apple varieties, Lacanobia fruitworm larvae did better on red delicious leaves compared to Fuji, Golden Delicious, Braeburn, and Gala.
3. Apple foliage treated with methyl jasmonate to induce production of defensive chemicals resulted in more rapid weight gain by Lacanobia fruitworm larvae.

Methods:

Data were obtained on the suitability of foliage as food for Lacanobia larvae using two methods. To determine effects on lifelong development, newly hatched larvae were placed in small wax-coated paper cups with apple foliage and were fed new foliage. Notes were made daily until they were mature. One inch of potting soil was then placed in the bottom of the cup for the larva to pupate in. The second method was designed to determine weight gain of larvae and involved feeding 3rd instar larvae apple foliage for 7 days and weighing the larvae on day 0 and on day 7. In this manner, we determined weight gained in the 7 days.

Experiments were conducted to compare apple varieties, to compare time of the season, to determine effects of hormonal treatment of leaves, and to determine effects of larval damage to leaves on host suitability. Apple varieties were evaluated with the lifelong assay. Larvae were fed daily until they died or pupated, with data on pupal weight, development time, and survival/mortality. This experiment compared 5 apple varieties and was conducted in late May. The second experiment, a comparison of season, was done using the 7 day weight gain assay. Larvae were placed on apple leaves in late May, mid July and mid August. The third experiment evaluated effects of methyl jasmonate treatments to apple foliage on Lacanobia growth, using the 7 day weight gain assay. A 0.5 mM/L solution of methyl jasmonate was sprayed onto foliage of apple trees and branches were cut 48 hours later. The fourth experiment also used the 7 day weight gain assay to evaluate effects of damage to foliage on the suitability of that foliage as food for other larvae. Apple branches with and without larvae were enclosed with netting to obtain feeding damage to leaves. Leaves were harvested for assays 48 hours later.

Samples of apple leaves were also obtained for chemical analyses at the same time that leaves were obtained for assays. This was accomplished as seasonal samples, varietal comparisons, and following treatment with methyl jasmonate. Leaf samples were placed in an ultralow freezer for storage until processing for analyses. A spectrophotometric method was adopted for quantification of protease inhibitors in apple leaves. Methods for analysis of terpenoids in foliage include organic solvent extraction, followed by LC purification and GC-MS analysis for terpene and sesquiterpene compounds.

Codling moths were tested for oviposition in a flight tunnel in response to the odor of infested pear fruit. Pear fruit were placed on a ring stand at the upwind end of the tunnel and moths were placed in screened plastic cyllinders at the center of the downwind end of the tunnel. Ten moths were placed in each tube which was collected 4 hours later to count numbers of eggs laid in the tube. In this manner, oviposition in the presence of pear, infested pear, and no fruit were compared.

Results and Discussion:

Lacanobia fruitworm larvae developed similarly on leaves from Braeburn, Fuji, Gala, and Golden Delicious apple varieties, with similar pupal weights, development times, and survival to adult. Larvae on leaves of Red Delicious apple however, grew heavier.

Development of Lacanobia fruitworm larvae on foliage of different apple varieties, n - 10.

Variety	pupal weight	development time	survival
Braeburn	369 mg	37.6 days	50%
Fuji	368 mg	37.3 days	60%
Gala	315 mg	35.3 days	70%
Golden Delicious	312 mg	32.1 days	70%
Red Delicious	422 mg	41.6 days	70%%

Lacanobia fruitworm larvae fed Fuji apple foliage gained weight much faster during the spring, compared to mid summer, and particularly compared to late summer. Weight gained by larvae in 7 days of feeding was almost 5 times more in May, compared to Aguust.

Table 2. Mean weight gained in a week by 3rd instar Lacanobia fruitworm larvae on Fuji apple foliage at 5-6 week intervals through the summer.

Start date	mg gained
28 May	29.1 $\pm$ 2.5
12 July	18.8 $\pm$ 3.5
15 August	6.5 $\pm$ 1.1

Lacanobia fruitworm larvae gained significantly more weight when fed Red Delicious apple leaves that had been treated with methyl jasmonate (61.8 $\pm$ 8.4 mg) compared to untreated leaves (26.5 $\pm$ 3.8 mg) ($t = 3.77$, $p = 0.0007$).

There was no difference in mean weight gain of Lacanobia fruitworm larvae fed Red Delicious apple foliage damaged by other larvae (6.1 $\pm$ 2.3 mg) compared to undamaged foliage (8.4 $\pm$ 2.3 mg) ($t = 1.03$, $p = 0.33$).

Quantitative analysis of protease inhibitors and terpenoid compounds in apple leaves will likely not be finished until spring. Thus, reporting a summary of results would be premature. Because this is strictly laboratory analyses of field material processed in summer, it is best scheduled as a winter activity.

Codling moths in a flight tunnel layed many eggs during the four hour test period regardless of treatment, with no differences in eggs laid between treatments.

These results support the idea proposed earlier that apple leaves deteriorate in quality as food for fruitworms through the season. A series of tests to determine the cause of this change in quality have not yet been successful, but several hypotheses have been eliminated. The positive influence of the methyl jasmonate treatment to leaves on weight gain by larvae was a surprise and may indicate chemical changes to leaves that larvae are adapted to take advantage of and add to leaf nutrition. These findings are of significance to apple production because 1) they indicate that development rates vary with the season (which impacts day degree models), 2) they may provide an explanation for fruitworm damage to apple, instead of strictly damage to foliage, 3) they may provide a treatment to reduce leaf quality and reproduction of fruitworm in apple orchards, when the proximate cause is identified.

Budget:

Project Title:	Insect Responses to induced defensive chemistry of apple and pear.
PI:	Peter J. Landolt
Project Duration:	2001-2003.
Current Year:	2003.
Project Total:	$64,400
Current Year Request:	

Year	2001	2002	**2003**
Total	$23,000	$24,400	**$17,000**

Current Year Breakdown	Year 1	Year 2	**Year 3**
Item			
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**

CONTINUING PROJECT REPORT **YEAR 3/3**
Project # AE-01-41

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:
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 ochards.

2002 Significant Findings:
•All rates of 28% ammonia release, based on release from vials with 0.05-(about 3-4 mg ammonia/hour), 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, but….
•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 3-4 mg/h, as rates higher than this did not increase captures.
•The 28% ammonium hydroxide lures significantly outlasted the commercial supercharger and Pherocon AM lures over 3-week tests.
•Increasing volumes (10-60 ml/trap) of ammonia using multiple vials did not increase attractiveness; only 10 ml was needed.

Methods for 2003:
1. The longevity of experimental ammonium hydroxide lures will be compared with commercial lures for the entire 3-month season, from mid June through mid September 2003, using similar designs and same sites as in 2002. Commercial lures will be superchargers with 2 and 10 g ammonium carbonate. The Pherocon AM trap will also be compared.
2. Ammonia lures will be 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. will be tested: these volatiles will be butyl hexanoate or a 5-component blend that includes butyl hexanoate and has been isolated from apples. For both trap types, treatments will be 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 will be conducted in Puyallup and the Vancouver areas and will also last the entire 3 months of the season.
3. The effect of tree size on use of ammonia/apple volatile cues or visual cues will be tested in orchards in Puyallup and Vancouver. Tall trees and short trees within the same orchards or

within the same general areas will be 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 and red spheres will be baited with ammonia, the 5-component blend, or the two together, along with controls, for 8 treatments. Each test will be run for 4 days.

Results and Discussion

In 2002, as in 2001, ammonia lures used were 15-ml polyethylene vials filled with 10 ml of the 100% ammonium hydroxide solutions saturated in 0.75 g cotton. Vials with 0.025, 0.05, and 0.64 cm holes or different numbers of vials 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 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, 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, there were few differences between spheres and panels, and control –baited trap differences were larger (Fig. 1). Traps with more than one vial 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, particularly the sizes of trees in them, may determine the numbers detected using spheres or panels by affecting the visibility of the traps.

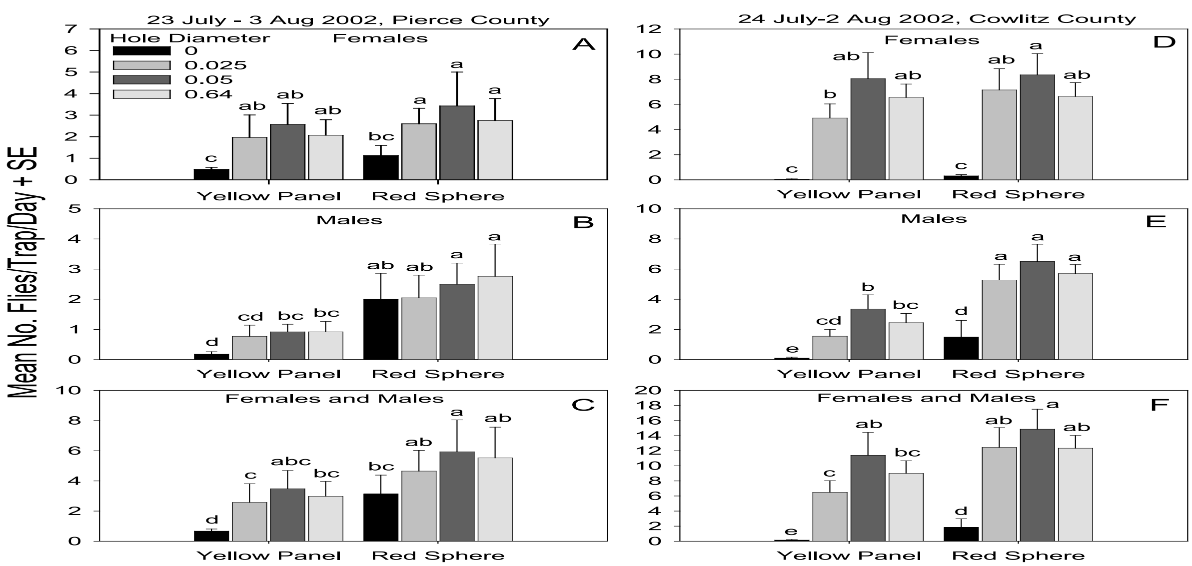

Fig. 1. Effects of trap type and hole size in ammonia-baited vials on fly captures in Pierce and Clark Co., WA, 2002.

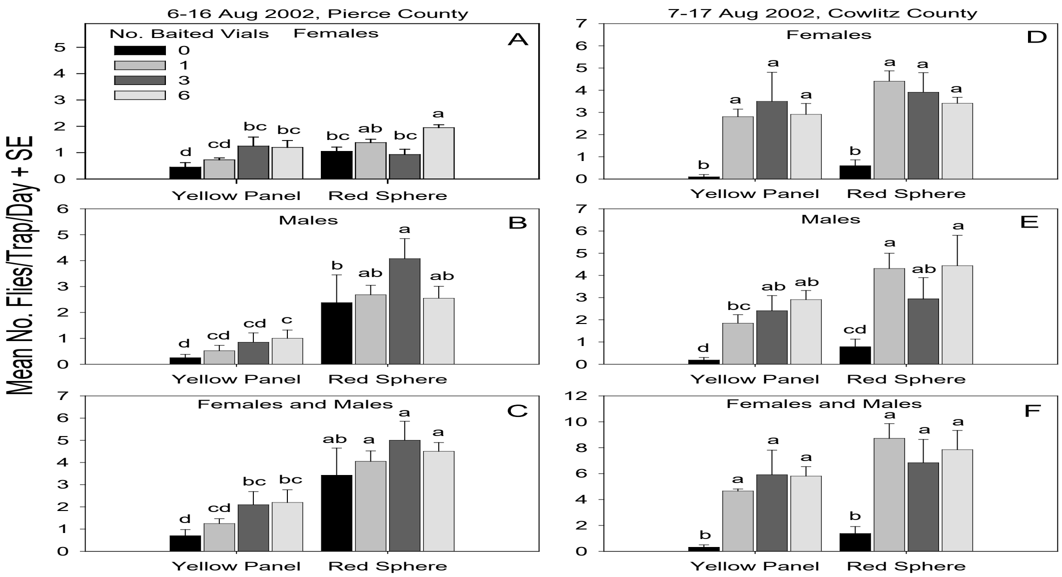

Fig. 2. Effects of trap type and numbers of vials filled with ammonia on fly captures in Pierce and Cowlitz Co., WA, 2002.

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, in 2001 and 2002. Ammonia was released from a 0.05 cm diameter hole in each vial. 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 (Figs. 3 and 4). The wide range of ammonia concentrations attracted females with similar egg loads (but data are not complete, currently being processed). Based on these and previous results and results from laboratory determinations, flies responded maximally to ammonia released from traps at 3-4 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 (Figs. 5 and 6). A modified supercharger (MS) containing 8.46 g AC, 4 times the amount in SC, was also more effective than the Pherocon AM 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 3-4 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. 7). Results indicate that the simple AH lure or MS used in this study can be a major improvement over 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.

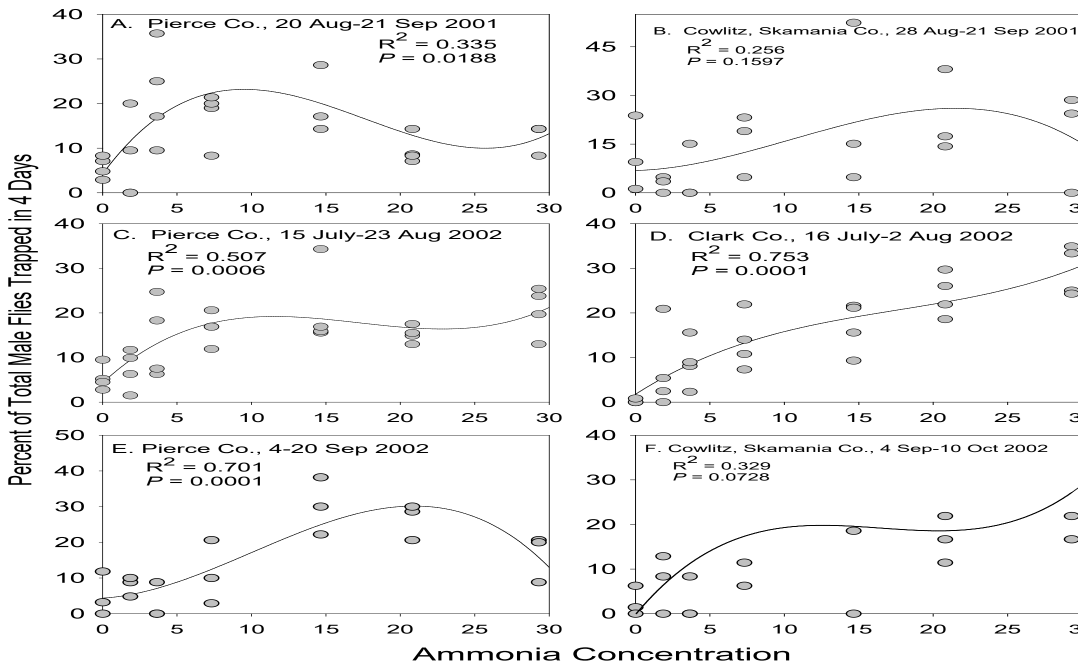

Fig. 3. Effects of ammonia concentrations on male reponses to yellow traps in Pierce, Cowlitz, Clark, and Skamania Co., WA, in 2001 and 2002.

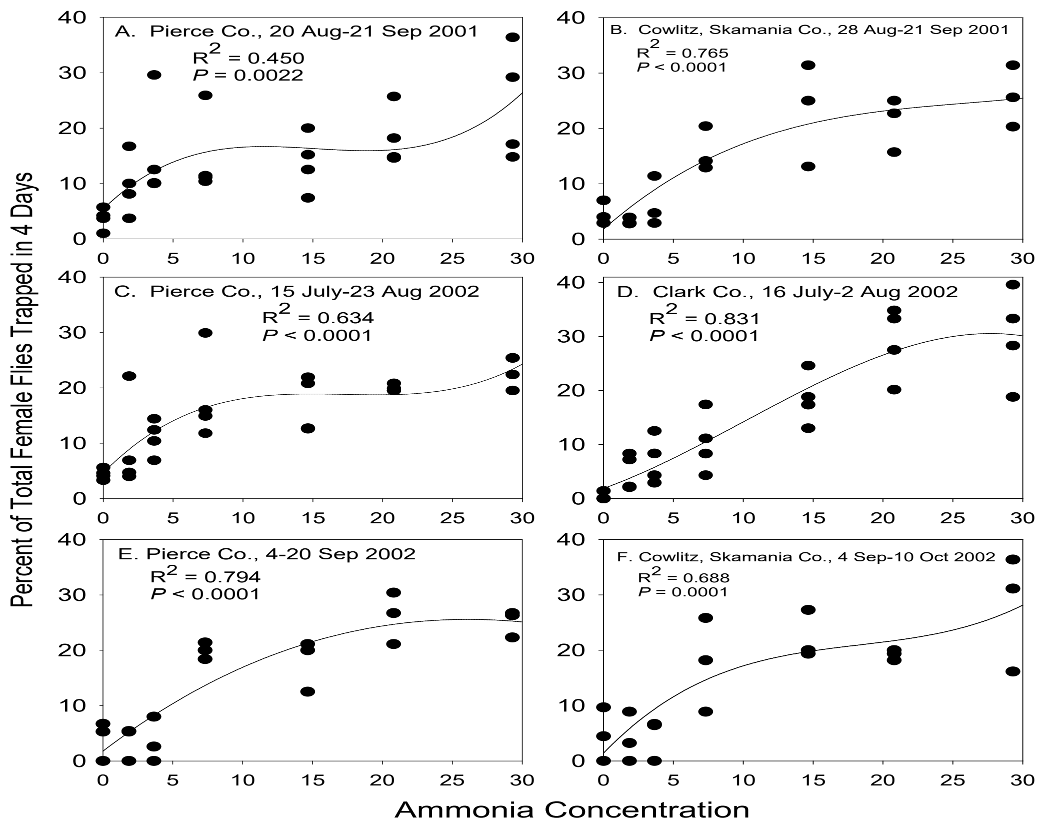

Fig. 4. Effects of ammonia concentrations on female fly responses to yellow traps in Pierce, Cowlitz, and Skamania Co., WA, in 2001 and 2002.

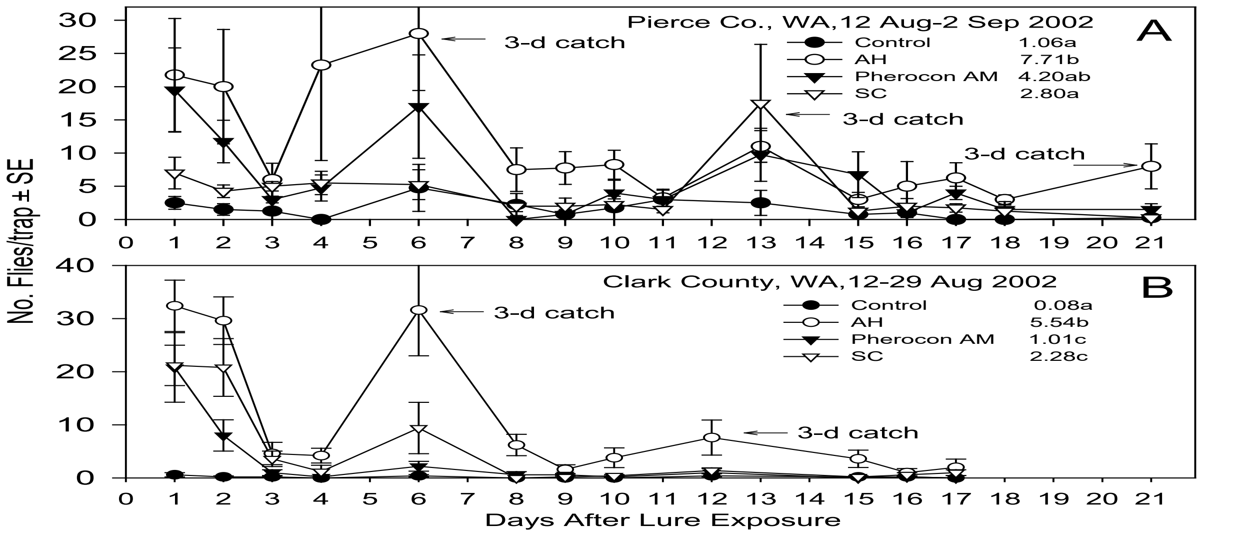

Fig. 5. Effects of experimental ammonia and commercial lures on captures of apple maggot in Pierce and Clark Co., 2002. Means with same letters are not significantly different (*P* > 0.05).

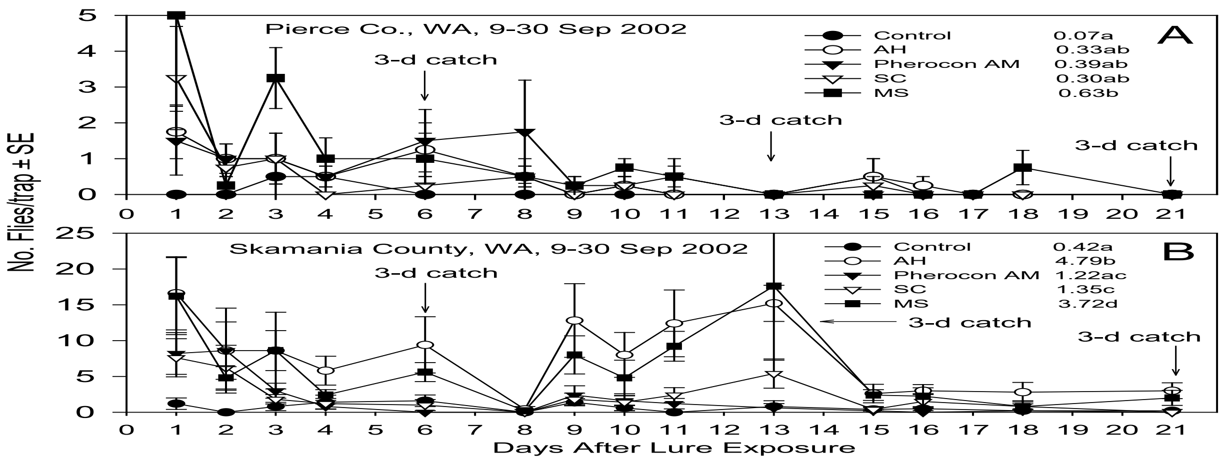

Fig. 6. Effects of experimental ammonia and commercial lures on captures of apple maggot in Pierce and Clark Co., 2002. Means followed by same letters are not significantly different (*P* > 0.05).

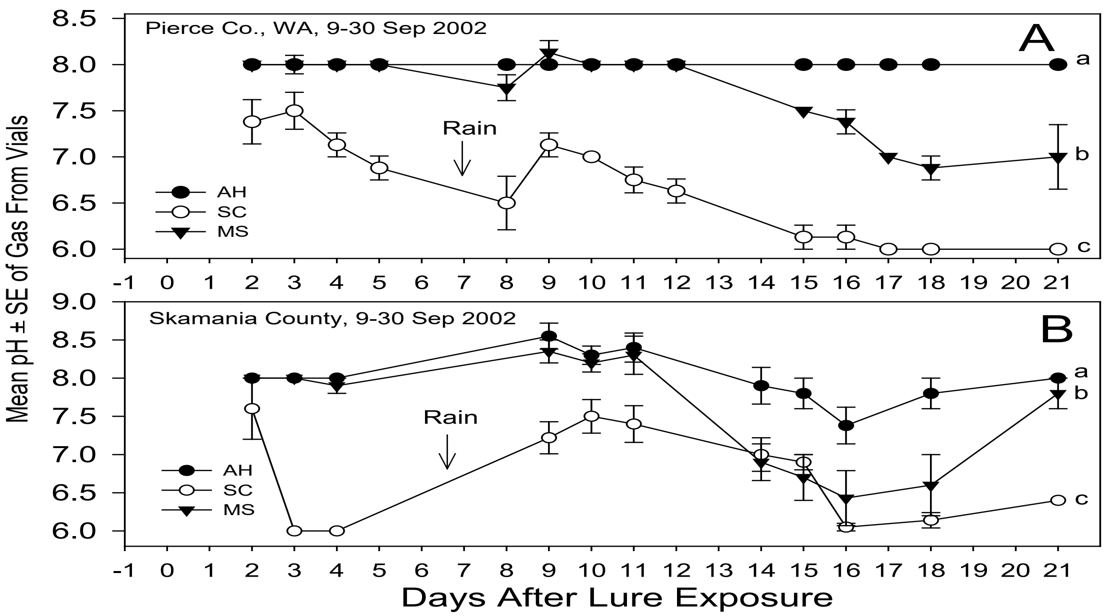

Fig. 7. Longevity of ammonium hydroxide and commercial lures as determined by pH of volatiles emitted from lures over 3 weeks in Pierce and Skamania Co., 2002. Lines followed by the same letters are not significantly different (*P* > 0.05).

Budget:

Project title:	Optimizing Ammonia with Traps to Manage Apple Maggot in Washington
PI:	Wee Yee
Co-PI:	Pete Landolt
Project duration:	2001-2003
Current year:	2003
Project total (3 years):	**$111,000**
Current year requested:	**$ 40,000**

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

CONTINUING PROJECT REPORT **YEAR 2/2**
Project # AE-02-217

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

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.
●Asia 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; pupae are being kept in the cold and rearing the adults out in the winter and spring of 2003 will confirm whether these are apple maggots – studies are still in progress.

Methods:
1. As in 2001 (preliminary studies) and 2002, sticky yellow traps will be placed in Asian pear, pear, rose, cascara, plum, and other hosts in Cowlitz, Clark, and Skamania Counties from July through September 2003. Cultivated and native wild cherries will also be trapped. Traps will also be placed in apple, hawthorn, and crabapple trees, the known hosts in Washington, for comparative host use purposes. Several sites 60 miles apart will be surveyed to establish that infestations on non-apple hosts are not limited to just a few trees or localized sites. Flies will be removed from traps every 1 to 3 weeks and identified.
2. Representative fruit from all trapped trees will be collected and brought into the laboratory. Larvae will drop from the fruit into a moist vermiculite/peat moss/sand mixture. After 1-2 weeks at room temperature, the pupae will be placed in a 3-4 °C refrigerator where they will be held for 4.5 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 caught on traps placed in different trees in 2001 and 2002, including a new host, Asian pear, *Pyrus serotina* L., in Cowlitz County (Fig. 1). Because the Asian pear was isolated from apples and hawthorns, comparisons with these hosts were not made to assess relative abundance among hosts.

Fly pupae were collected from many hosts in 2001 (Table 1). However, only apple, black hawthorn, ornamental hawthorn, crab apple, and Asian pear produced apple maggots, as confirmed by wing banding patterns and ovipositor lengths. The 3 female and 4 male flies that developed in Asian pear in 2001 were as large as flies from apples (Table 1), although the longevity and ability of females to lay as many eggs as apple and hawthorn flies were not determined. Most pupae from other hosts, including pear and rose, failed to emerge. In 2002, pupae were again collected from Asian pear, pear, and plum (Table 2) and are being held in the cold room for emergence in 2003. The infestation rate in Asian pear was relatively high (Table 2). In 2002, Asian pears at two sites 60 miles apart (Cowlitz and Skamania Counties) also yielded infested fruit, indicating infestation by flies was not confined to a single location. The fact that apple maggots were collected in 2001 and 2002 from Asian pear indicates that it is not an incidental host, but may be a commonly used host, at least locally.

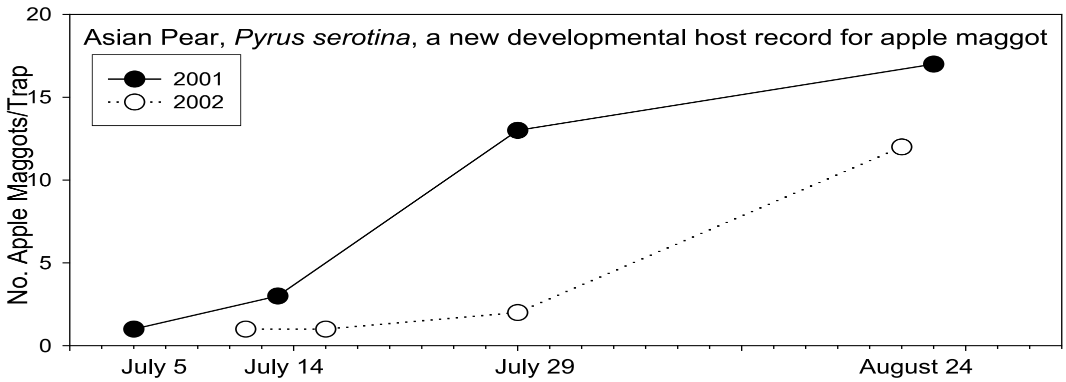

FIG. 1. Numbers of adult apple maggots collected on unbaited yellow sticky traps placed in an Asian pear tree in 2001 and 2002 Cowlitz County, WA.

Table 1. Numbers and body lengths (mean ± SE) of apple maggots reared from various fruit collected in southwestern Washington in 2001.

	28 August to 28 September 2001 Collections (Preliminary Collections)					
		No.	No. adult AM reared from pupae			
Developmental Host	Site	Pupae	Females	BL, OL	Males	BL
Apple	S,L	575	10	5.44 ± 0.04, 0.85-1.09	3	4.37 ± 0.05
Black Hawthorn	S	100	3	4.95 ± 0.30, 0.84	3	4.37 ± 0.06
Ornamental Hawthorn	S, B	665	1	4.35	2	4.21 ± 0.14
Crab Apple	S, E	187	4	------, 0.73	0	------
Asian Pear	L	161	3	5.08 ± 0.34, 0.91-1.11	4	4.18 ± 0.10
Pear	L	24	0	------	0	------
Rose	S	95	0	------	0	------
Quince	L	1	0	------	0	------
Snowberry	S, W	293	0	------[a]	0	------
Bitter Cherry	A	5	0	------	0	------
Cascara	S	7	0	------	0	------

BL, bodylength (mm), OL, ovipositor lengths (mm).
S, St. Cloud (Skamania Co); B, Belle Center (Skamania Co.); W, Sam's Walker (Skamania Co); F, Fort Vancouver (Clark Co); A, Amboy (Clark Co); L, Lewis River (Cowlitz Co).
[a]All flies that emerged were snowberry maggots, *Rhagoletis zephyria*.

Table 2. Numbers of fly pupae (not yet confirmed to be apple maggots) collected from various fruit species in southwestern Washington in 2002. Pupae currently (December 2002) held at 3 °C.

		August to October 2002 Collections		
Developmental Host	Site	No. Fruit	No. pupae	Infestation Rate
Apple	D	44	33	0.75 0
Black Hawthorn	S	2,095	371	0.177
Crab Apple	A	88	100	1.136
Asian Pear	L, S	153	68	0.444
Common Pear	S,W,D	332	36	0.108
Red Plum	D	28	6	0.214
Italian Plum	D	80	0	0.000
Plum	S	30	0	0.00 0
Blue Berry	T	87	0	0.000
Quince	W, L	111	1	0.009
Rose	S	394	5	0.013
Bitter Cherry	A,S,L	2,807	201[a]	0.072
Cotoneaster	T	160	1	0.006
Red Huckleberry	C	120	1	0.008

S, St. Cloud (Skamania Co); B, Belle Center (Skamania Co.); W, Sam's Walker (Skamania Co); F, Fort Vancouver (Clark Co); A, Amboy (Clark Co); L, Lewis River (Cowlitz Co), D, Donaldson Farm (Cowlitz Co.); T, Straton Farm (Clark Co.); C, Carlson Farm (Clark Co.).
[a]Mostly western cherry fruit fly, but some may be apple maggots.

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 and the Spokane area, but sporadic catches have occurred in Yakima, Ellensburg (21 findings in 2002), and the 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 the presence of apple maggot. If an apple maggot is found, 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, and perhaps even rose in or close to areas with previous apple maggot finds.

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 this is not the case in 2001 and 2002. 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 possible apple maggot usage of pear is especially 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 (Table 2). Nevertheless, if apple maggots use pear or any previously unrecorded hosts in Washington, then trapping efforts by the state to delimit the fly's spread may need to be modified to include them.

Further work is ongoing and will determine if flies from the Asian pear and other possible new hosts survive as long and lay as many eggs as flies from apples, crabapples, and hawthorn. Studies in winter 2003 will concentrate on these parameters as a way of assessing the true suitability of these hosts and their importance in the ecology and management of apple maggot in Washington.

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Budget:

Project title: Re-evaluation of host usage by apple maggot in Washington state
PI: Wee Yee
Project Duration: 2002-2003
Current year: 2003
Project total (3 years): $10,000
Current year requested: $5,000

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.

CONTINUING PROJECT REPORT **YEAR 2/3**
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, Department 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 (Washington State University, Department of Entomology; vhebert@tricity.wsu.edu)

Cooperator(s): Shawn McNeill (Washington Tree Fruit Research Commission)
Jay Brunner (Washington State University, Department of Entomology)
Linda Finch (USDA-Wapato)
David Putnam (Stemilt)

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.

We have hypothesized that new sprayer technology can allow reduced application rates in reduced volumes of water. Based on our first year of study, we also hypothesize that the characteristics of spray delivery from the Proptech sprayer may allow efficacious application by reducing spraying to every other row. The following objectives define the kinds of experiments we are conducting to test the hypotheses relevant to sprayer technology. The objectives will remain the same during the second year of the project.

1. Determine residue deposition from two or more reduced volume alternative sprayers (for example, the Proptec Tower) using reduced AI application rates.
2. Determine efficacy of reduced application rate residues deposited by the alternative sprayers and the conventional Rears airblast sprayers.
3. Determine the residue decline rate of reduced application rates using chemical and biological assays.
4. Improve the accuracy of estimating worker exposure by determining the rate in decline of dislodgeable foliar residues after application of reduced active ingredient rates.
5. Determine the drift reduction potential of alternative sprayers.

Significant findings (Crop Year 2002):

- A leaf disk bioassay was developed for measuring bioactivity of insecticides against neonate codling moth (CM). A simple entry-hole bioassay was developed for measuring bioactivity on whole apples.
- Dose-response relationships (LC50 and LC95) were developed for neonate CM exposed to leaf surface residues of azinphos-methyl (Guthion) and methoxyfenozide (Intrepid).
- Bioactivity of Guthion and Intrepid as determined by the leaf disk and apple assay was not significantly affected by sprayer type (Rears airblast vs. Proptech).

- Bioactivity of Guthion and Intrepid did not differ between leaves collected from mid canopy and leaves collected from the top of the canopy.
- Half-rates of Guthion and Intrepid were as efficacious as full rates in both the leaf disk and apple bioassay.
- Bioactivity of Guthion persisted for about one month (i.e., bioactivity generally reached ~90% mortality) on treated foliage but only persisted for about two weeks on treated apples.
- Intrepid bioactivity was lower than Guthion throughout the whole test. Persistence of bioactivity (as evidenced by <50% mortality within 24 hours of larval exposure) lasted for less than three weeks. Cessation of feeding activity, rather than lethality, should be used as the appropriate IPM-relevant toxicological endpoint for Intrepid.
- Persistence of Guthion bioactivity generally paralleled persistence of residues. As leaf residues dropped below the LC95 after 30 days following the first spray treatment and after 13 days following the second spray treatment, percentage mortality of neonates dropped significantly.
- Persistence of Guthion residues on foliage seemed shorter after the second spray than the first spray. This observation suggests that foliar residues may dissipate faster following applications in mid-summer than applications in early summer.

Methods (Planned for Crop Year 2003):

1. Objective 1 (Determine Residue Deposition from Alternative Sprayers)

Deposition of Guthion and two reduced risk insecticides, Intrepid and Assail (acetamiprid), will be monitored by bioassay and chemical assay after delivery from the conventional airblast sprayer and the Proptech sprayer. Two spray cycles will be tested (late May; mid July). In addition to the Proptech, we will make arrangements to test a novel but commercial air-assisted sprayer called the Dagani, which is marketed by Blue Line Manufacturing (Moxee). This sprayer has a flexible boom design that may have some advantages for closed canopies.

Last season we used adjacent plots of 10 trees by five rows arranged in a stratified block design with three replications (Figure 1). To avoid confounding results from drift across rows, we plan to expand each plot to six trees wide and avoid spraying directly the outer rows of each plot. Based on field observations of spray drift, we predict that the outer rows will still be protected enough to meet commercial interests

To visualize deposition, fluorescent tracer will be added to the tank. We will increase the rate of dye that we use by about 25% because last year we found that the manufacturer had changed the formulation and the strength we had used was too low to obtain good pictures of distribution

2. Objective 2 & 3 (Determine efficacy of reduced application rates)

Three rates will be used: 0X, 0.5X, 1X based on the product label instructions and typical grower practices. Foliage and apple samples will be collected after application and 15, 30, and 40 days following application. Samples will be bioassayed using neonate CM larvae and also subjected to surface extractions for residue quantification of dislodgeable foliar residues (DFRs). We hypothesize that the DFRs are the biologically active residues and there is no need to macerate whole leaves (or apples) to obtain efficacy-relevant residues.

This season we plant to supplement bioactivity determinations by deploying a field-based bioassay. After spraying, sections of branches at mid canopy and at the top of the canopy will be enclosed in mesh sleeves. Each cage will be inoculated with either CM eggs or neonate larvae. After holding the cages in the field for about five days, branches will be clipped and examined for evidence of feeding as well as the presence of live larvae.

3. Objective 4 (Improve the accuracy of estimating worker exposure)

We will re-examine the standard method for determining DFRs that are used to estimate post-application worker exposure. The EPA-recommended method employs a soap solution and two cycles of vigorous shaking of leaf punches. We believe this method give excessively high residues that are not truly available for rub-off and therefore results in unrealistically high estimates of what a worker is likely to pick up from a treated surface. We will compare the standard method to a solvent-based shaking method and a surface wipe method. DFRs for purposes of this objective will be determined on frequent intervals (0, 3, 5, 7, 10, 14, 21, 28, 35 days) to correspond to standard worker exposure studies.

5. Objective 5 (Determine the drift reduction potential of alternative sprayers)

Drift will be determined by employing silica gel cards spaced at varying distances from the outside row of the orchard. We will also place cards in the orchard to examine drift across tree rows. Cards will be placed in trees as well as on the ground. Fluorescent tracer dye will be deployed as a detection system. We will test the hypothesis that the Proptech sprayer can be operated by spraying every other row and still result in sufficient bioactivity to control CM feeding damage.

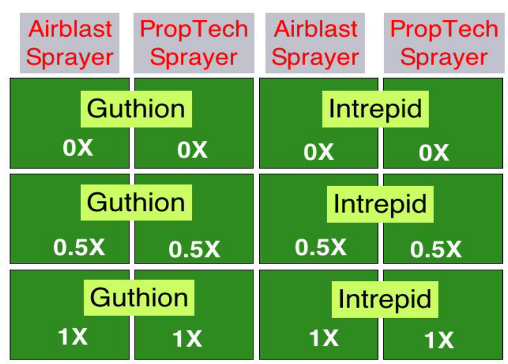
Results and Discussion

Three replicate blocks encompassing over 1800 trees (var. Gala) were delineated at the Stemilt orchard west of Quincy, WA (Figure 1). The experimental treatments were Rears airblast sprayer and the Proptech sprayer, two application rates (0.5X and 1X of label rate) and an untreated control (0X), and two insecticides (Guthion and Intrepid). Plots were sprayed twice (May 31 and July 24). Foliage was periodically collected, and one set of 5-cm^2 diameter leaf punches was bioassayed with neonate CM larvae and another set was extracted for Guthion residues. Apples were also collected and subjected to bioassay and chemical analysis.

Innate Bioactivity of Guthion and Intrepid

The dose-response relationship for analytical grade azinphos-methyl and methoxyfenozide was determined by treating leaf disks with increasing concentrations of insecticide. The 24-h dose-response relationships were deemed most relevant to understanding field efficacy of the insecticides.. The LC50 and LC95 for azinphos-methyl was 0.015 and 0.055 $\mu g/cm^2$, respectively. For methoxyfenozide, the LC50 and LC95 was 0.077 and 1.134 $\mu g/cm^2$, respectively.

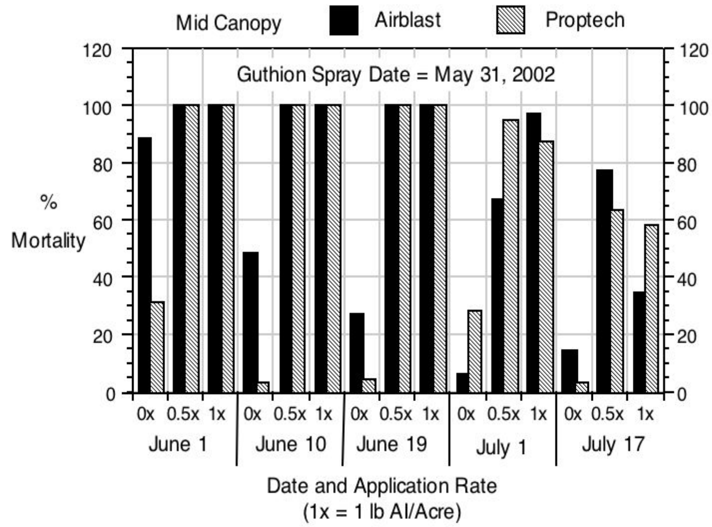
Figure 1. Plot layout and design for testing sprayers and application rates. Each solid square represents five rows of ten trees. This figure shows one of three replicate blocks. Application rates were stratified across the blocks in the same position to minimize drift effects. However, bioassay and residue data indicated cross contamination from Guthion sprays in the orchard.



Effect of Sprayer Type and Application Rate on Bioactivity of Insecticides on Foliage

The unusually high percentage mortality of neonate CM on the control leaves (0X) (Figure 2) was attributed to drift from adjacent experimental plots and perhaps commercial spray treatments. Mortality on subsequent sampling days dropped significantly enabling a clearer distinction between controls and the insecticide-treated plots. Residue results (shown in Figure 3) confirmed that the 0X treatment plots contained Guthion residues.

Figure 2. Percentage mortality of neonate CM on mid canopy apple foliage sprayed with Guthion from either a Rears airblast sprayer or a Proptech sprayer. Bioactivity was not significantly affected by sprayer type nor by location in the canopy. Persistence of bioactivity (~90% mortality) extended for approximately one month after application but dropped after 47 days.

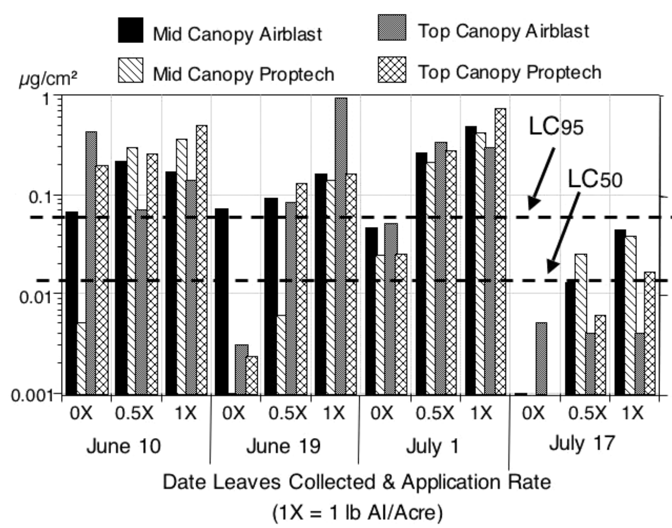


In general, foliage and apples collected from both Guthion and Intrepid sprayer treatments gave similar levels of bioactivity. For both sprayer treatments, bioactivity was similar on foliage and apples collected from mid canopy and the top of the canopy. Bioactivity was similar for the 0.5x and 1x rates of Guthion and Intrepid. Bioactivity of Guthion persisted for about 30 days after application (Figure 2). Foliar and apple bioactivity of Intrepid was much lower than bioactivity observed for Guthion. However, we noted that larval feeding essentially ceased after 24 hours of Intrepid exposure although larvae were still alive.

Distribution and Persistence of Guthion Residues

Guthion residues (µg/cm^2) were of similar magnitude on all leaves collected from the 0.5X and 1X treatments (Figure 3). As long as the magnitude of residues were near or above the LC95 (the dashed line running across the graph in Figure 3), percentage mortality in the leaf disk assay was near or above 90%. However, on 47 days (July 17), when the residues generally were below the LC95, percentage mortality of larvae also dropped off significantly (Figure 2). Residues and consequently bioactivity declined faster after the second spray than after the first spray (data not shown). We concluded that residue analysis was predictive of larval mortality.

Figure 3. Average azinphos-methyl residues recovered (µg/cm^2) from apple foliage collected from mid canopy and at the top of the canopy 10, 20, 30, and 47 days after application (May 31, 2002) of Guthion 50SP by an airblast or a Proptech sprayer. Dashed lines indicated the LC50 and LC95 (µg/cm^2) that was determined in the leaf disk bioassay.



Drift Study

Two drift studies were conducted with each sprayer type. Drift cards were laid in a perpendicular transect to the last three tree rows of the orchard out to a distance of 300 feet. In the first study, the sprayers were operated between the two outer rows of the orchard. In the second drift study, we operated the sprayer between the two outer rows as before, but we also ran a second drift study with operation of the sprayer in between the second and third row from the outside. We had observed excessive drift (documented photographically) when the sprayer was operating in between the first and second rows. We hypothesized, therefore, that drift could be greatly reduced by not treating the outside row. Presently, the analysis of the drift cards is in progress.

Previously Unreported Results

During cropping year 2001, the WTFRC had funded a GLP magnitude of residue study to determine carbaryl (Sevin) residues on apples after use of a thinning spray or as a late season leafhopper control. A complete report that has gone through a quality assurance audit is available ("Sevin: Magnitude of the Residue on Apples", FEQL-0201). Gala apples were treated by airblast sprayer either once or twice for thinning in May 2001 and three days prior to harvest in late August 2001. Average carbaryl residues on apples treated with Sevin as a thinning spray ranged from 0.009 ppm for one thinning spray to 0.007 ppm for two thinning sprays. These values are just above the limit of detection (0.005 ppm) but well below the definitive limit of quantification (0.025 ppm). The average carbaryl residue of 0.74 ppm on late-treated apples was well below the FDA tolerance of 10 ppm and the Codex MRL of 5 ppm. We concluded that thinning sprays do not contribute to dietary risk and are unlikely to raise regulatory concerns regarding the FQPA.

Budget

Project Title: Reduction of Pesticide Inputs, Worker Exposure, and Drift Through Alternative Sprayer Technology

PI: Allan Felsot

Project Duration: 2002-2004 (Three years)

Current Year: 2003

Project total (3 years): $162,033

Current Year Request: $54,253

Year	2002	2003 (Requested)	2004 (Projected)
Total	52,448	54,253	55,332

Current Year Breakdown (Year 2003)

Personnel (for 2003)	Year 1 (2002)	**Year 2 (2003)**	Year 3 (2004)
Salary (0.6 FTE in 2003)	17,730	**21,072**	21,915
Benefits (28%)	4,787	**5,900**	6,136
Wages	6,880	**6,880**	6,880
Benefits (16%)	1,101	**1,101**	1,101
Equipment (Fluorometer)	4,500	**0**	0
Supplies	16,550	**18,403**	18,403
Travel	900	**897**	897
Total	52,448	**54,253**	55,332

The differential between the initially requested budget of $47,655 and the new budget request for year 2 ($54,253) is related to salary needs and supply needs. We employ a classified staff Research Aide II to assist with the experiments and data analysis. Previously, we anticipated only funding a 0.5 FTE, but our experience after the first year indicates we need more personnel to analyze all the samples in a timely manner. The wages are used to fund summer help, which is required to ensure that all the bioassays are conducted in a timely manner. The increase in supplies is needed because of increased costs of materials routinely used in pesticide extractions and maintenance of our aging equipment.

We do plan to request from the Washington State Commission for Pesticide Registration a supplemental grant to defray the costs of a new microscope and additional salary money. The scope of the work plan proposed for crop year 2003 assumes that we will be successful in obtaining supplemental funding.

FINAL REPORT
WTFREC Project #: AE-02-219 **WSU Project #13C-3643-3386**

Project title: Biology, ecology and management of Western flower thrips in apple orchards.

PI: Elizabeth H. Beers, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave., Wenatchee; (509) 663-8181 ext. 234; ebeers@wsu.edu

Co-PIs and affiliations: Stephen D. Cockfield, Associate in Research, WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Rich Zack, Assistant Professor of Entomology, WSU Dept. of Entomology, Pullman, WA; 509-335-3394: zack@mail.wsu.edu

Objectives:
1. Determine host plant and seasonal occurrence of Western flower thrips in intra- and extra-orchard habitats.
2. Determine the species composition of thrips found on apple during bloom.

Significant findings:
1. Apple shoots harbored adult and immature thrips throughout the season until frost.
2. Adult thrips appeared on dandelion flowers before apple flowers developed and first appeared on apple buds at delayed dormant.
3. On the orchard floor, dandelion flowers, as well as other flowers sampled, harbored thrips until frost, sustaining a resident population in the orchard.
4. Thrips fed and reproduced throughout the year in near-orchard habitats by switching hosts that produced new leaves and flowers in different seasons. Some plants, such as arrowleaf balsamroot, *Balsamorhiza sagittata,* bloomed in spring and then were dormant until the next year. Others, such as gray rabbitbrush, *Chrysothamnus nauseosus,* provided growing shoots throughout the spring and summer and flowers in the fall. The dominant climax woody species of the steppe, big sagebrush, *Artemisia tridentata,* attracted great numbers of thrips when in bloom in the fall.
5. Native grass species attracted some thrips when in flower, but in general samples contained very few thrips. All native grass species sampled were dormant in the summer and fall and could not serve as a continuous food source for immature thrips.
6. The great majority of thrips on apple and other host plants in Washington appeared to be Western flower thrips.[1]

Methods:

Four orchards of the cultivar 'Granny Smith' were selected in Vantage, Orondo, Brewster and Bridgeport, Washington. The Vantage site was under organic management; all the other orchards were under conventional pest management. Beginning in early March, after the snow melted, samples were taken from the apple trees, the orchard ground cover, and 50 m into surrounding uncultivated steppe habitat. As soon as the apple buds started to grow, four samples of buds were taken weekly or biweekly from each site. Starting at half-inch green, flower bud samples and vegetative bud samples were taken separately. Each sample consisted of 25 buds, enough to fit into a 20 x 20 cm bag. Flowers were sampled through complete petal fall. Two of the most common plant species on the ground were selected for sampling at each site. Four samples were taken weekly and placed into the

[1] Determination of adult thrips specimens has not been completed as of November 2002.

same size bag. Plant specimens or vegetative parts with flowers were selected over those without flowers. Actively growing leaf buds were also given preference. Three to four species of plants were selected in the surrounding steppe habitat. The sampling methods were the same as for plants on the orchard ground. Sampling was not done until the species started to grow and was discontinued when the plant entered dormancy or, in the case of annuals, went to seed and died.

Plant tissue samples were washed with soapy water to dislodge the thrips. Adults and larvae were isolated by pouring the rinse through a series of sieves. The thrips were stored in 70% EtOH until sorted. After the adults and larvae were counted, representative specimens were slide-mounted for identification to species.

Results and discussion:

When sampling began in early March apple trees were still fully dormant. When the apple buds reached half-inch green, about the second week in April, adult thrips began to appear in the buds (Table 1). Thereafter, adult and immature thrips were found in flowers from pink to petal fall. After petal fall, thrips populations increased in the growing shoots. There was no apparent break between one generation and the next, but the rise in thrips numbers in the samples just after petal fall was apparently due to the flight of the second generation. By June, adult thrips were a little less than one per shoot in one of the sampled orchards. Both adult and immature thrips were found in shoots until the tips formed dormant buds, about mid-September. As expected, samples of the dormant buds in October yielded no thrips.

Thrips were present on dandelion, *Taraxacum officinale,* and grasses before apple trees broke dormancy. Some dandelion plants had over 10 flowers opened at peak flowering, just before peak flowering of apple. Thrips continued to breed in dandelion during the spring. However, most of the plants went to seed as apple reached full bloom, forcing the thrips to find new hosts. Flixweed, *Descurainia sophia*, a yellow winter annual, was common at one site. This plant flowered after apple petal fall. Thrips were found on the flowers until mowing occurred in late May. Afterward, thrips were less abundant on flixweed and declined until the plant went to seed and died back, around late June. Following apple petal fall and throughout the summer and fall, thrips were abundant on dandelion flowers. Most dandelions did not produce flowers after the spring, but about 10% produced one flower at a time until frost. Grass species on the orchard floor, such as downy brome, *Bromus tectorum*, Italian ryegrass, *Lolium multiflorum,* and orchard grass, *Dacylis glomerata,* rarely had any thrips all season. One orchard sampled had a ground cover of orchard grass with no broadleaf weeds. This orchard apparently benefited from the lack of a preferred thrips host on the ground because the number of thrips counted in apple flowers in spring tended to be low.

In adjacent land with undisturbed native vegetation, thrips were found in early March on gray rabbitbrush*, Chrysothamnus nauseosus*. As the plant grew new leaves, thrips were found occasionally on the vegetation. In early May, arrowleaf balsamroot, *Balsamorhiza sagittata,* began to bloom, and thrips were abundant in the flowers until they went to seed. Antelope bitterbrush, *Purshia tridentata,* also began to bloom in early May which attracted some thrips. By July, bitterbrush had begun to grow new shoots, and thrips were found on the succulent growth. Flower heads began to form on big sagebrush, *Artemisia tridentata,* in July; and by September and October thrips were abundant on the open flowers. Other fall blooming plants, such as rabbitbrush and snow desert buckwheat, *Eriogonum niveum,* attracted thrips to their flowers. Native grasses such as bluebunch wheatgrass, *Agropyron spicatum, s*teppe bluegrass, *Poa secunda,* and needlegrass, *Stipa spp.,* had very few thrips. Further, these grasses were dormant after producing seed in early summer. Thus, they were not capable of supporting thrips for most of the season. Both orchard and adjacent native habitats were able to sustain a breeding population of Western flower thrips from early spring until fall frost. In the orchard, the primary breeding sites found were in growing apple shoots and dandelion flowers. In adjacent wild areas, because of the diversity of plants and their different growth habits, thrips can find open flowers or growing shoots throughout the year. Orchards that are isolated from uncultivated areas and have few broadleaf plants on the ground may harbor few thrips in the fall generations and few thrips emerge in the spring. Indeed, few thrips were collected from one of the sites, which had almost no broadleaf plants in the ground cover.

Budget:

Project duration (one year):	2002
Current year:	2002
Summary of total cost of project:	$27,385

Table 1. Mean adult thrips/sample[1] on common host plants within and next to apple orchards.

Rep	Habitat/Plant Sample	March			April				May				June				July				Aug				Sept				Oct				Nov
	Apple Tree																																				
A[2]	Flowers				0	0	<1	3	2	6	0																										
C	Flowers					0	0	<1	<1	0																											
B	Flowers					<1	0	0	<1	0	2																										
D	Flowers			0	0	<1	3	8	0																												
A	Shoots				0	0	<1	<1	0	1	<1	6	9	11	10	8	3	4	4	x	x	x	x	x	x	x	x	x	x		-		
C	Shoots					0	0	0	0	0	0	0	<1	3	<1	1	1	2	4	1	2	<1	x	x	x	x	x	-	-		-		
B	Shoots					<1	0	0	<1	0	0	<1	1	1	<1	<1	7	7	7	2	1	0	x	x	x	x	x	-			-		
D	Shoots			0	0	0	<1	<1	<1	2	1	5	16	13	3	9	16	5	10	2	4	2	x	x	x	x	x	-			-		
	Ground cover																																				
A	Dandelion	<1	2	0	5	2	<1	0	3	15	<1	20	51	42	50	58	35	93	81	x	x	x	x	x	x	x	x	x	x	x	x	x	x
C	Dandelion	0	0	0	0	0	<1	<1	<1	1	4	138	24	104	22	72	80	66	21	21	3	5	5	x	x	x	x	x	x	x	x	x	x
D	Flixweed	<1	0	0	0	0	0	<1	0	37	23	4	6	3	10	x	x																
A	Downy brome	<1	0	0	0	0	0	0	0	0	0	0	0	0	<1	1	<1										-	-	-	-	-	-	-
D	Downy brome	1	4	2	1	<1	0	0	0	0	0	<1															-	-	-	-	-	-	-
C	Italian ryegrass	<1	0	0	0	0	0	0	0	0	0	0	<1	<1	<1	0	<1	0	<1	0	0	0	<1	-	-	-	-	-	-	-	-	-	-
B	Orchard grass	0	0	0	0	<1	0	0	0	0	0	0	0	<1	0	0	0	0	0	<1	0	0	-	-	-	-	-	-	-	-	-	-	-
	Wild areas																																				
C	Bitterbrush	0	0	0	0	0	0	0	1	2	0	0	0	<1	<1	7	1	1	<1	0	0	0	0	-	-	-	-	-	-	-	-	-	-
B	Bitterbrush	0	0	0	0	0	0	0	1	2	0	0	<1	0	0	0	0	1	0	0	<1	0	-	-	-	-	-	-	-	-	-	-	-
B	Balsamroot				0	<1	0	2	11	28	61	23																					
A	Big sagebrush	0	0	0	<1	0	0	0	<1	0	0	0	<1	0	0	0	6	<1	3	-	x	x	x	x	x	x	x	x	x	x	x	-	-
B	Big sagebrush	0	<1	0	0	0	0	0	0	0	<1	0	0	0	0	<1	0	0	0	0	0	<1	x	x	x	x	x	x	x	x	-	-	-
D	Big sagebrush	0	0	<1	0	<1	0	0	0	0	0	0	<1	<1	0	<1	<1	0	3	0	0	0	-	-	x	x	x	x	x	x	-	-	-
D	Rabbitbrush	1	0	0	0	0	0	0	0	0	<1	0	<1	<1	0	<1	<1	<1	<1	<1	6	8	x	x	x	x	x	-	-	-	-	-	-
A	Buckwheat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<1			x	x	x	x	x	x	-	-	-	-	-	-
C	Buckwheat		0	0	<1	0	0	0	<1	<1	0	<1	<1	0	0	0	0	0	0	<1	0	1	4	x	x	x	x	-	-	-	-	-	-
D	Bluegrass	0	0	0	0	0	0	0	0	0	0																						
A	Needlegrass	0	0	0	<1	0	0	0	0	0	<1	0	1	0	0																		
C	Needlegrass		<1	0	0	0	0	0	0	0	0	<1	0	0																			
B	Wheatgrass	0	0	0	0	0	0	0	0	0	0	0	2	<1	<1																		

[1] Numbers are mean thrips/sample. Letter x indicates presence of thrips in preliminary observation. Minus indicates no thrips seen in preliminary observation. No number indicates no sample taken because of plant dormancy.

[2] Letters indicate different sample locations: A=Brewster, B=Bridgeport, C=Orondo, D=Vantage.

FINAL REPORT

TITLE: Plum curculio biology, host range, distribution, and control in Utah

PRINCIPAL INVESTIGATOR: Diane G. Alston, Department of Biology, Utah State University (USU)

CO-INVESTIGATOR: Anchalee Stark, Technical Research Assistant, USU

FUNDING HISTORY: Funded in 2000 (Year initiated): $15,000

SIGNIFICANT FINDINGS:

- Plum curculio (PC) in northern Utah appears to be limited to an approximately 50 sq. mile narrow strip from Honeyville to Willard, centered on Brigham City, in Box Elder County.
- PC infestations are primarily in neglected or unmanaged sites: home yards, roadside wild plum, and neglected orchards. The majority of PC-infested-sites are in the residential areas of Brigham City (74%), and in the majority of these, sweet cherry is the host tree (68% of Brigham City home yards, and 51% of all infested sites).
- In laboratory host tests, PC adults fed and laid eggs in all cultivated pome and stone fruits offered, and in wild plum (*Prunus americana*) and black hawthorn (*Crataegus douglasii*). Although eggs were laid in peach and pear, no larvae developed. No oviposition scars, eggs or larvae were found in ornamental crabapple (*Malus* spp.).
- A single diazinon treatment applied to 23 home yards with host trees just after petal fall of sweet cherry and apple was fairly effective (0-3 larvae/100 fruit per site as compared to 31 larvae/100 fruit in nearby untreated wild plum). Applying the insecticide earlier, at petal fall, and a reapplication 10-14 days later may improve the efficacy of insecticide treatment.

OBJECTIVES:

1. To better define the boundaries of PC distribution in northern Utah.
2. To determine the potential host range of PC for commercial, ornamental, and wild fruits in the West.
3. To evaluate the effectiveness of PC controls applied to host trees.
4. To continue to test any new or modified lures for PC adults in traps.
5. To evaluate the effectiveness of trunk exclusion devices on the reduction of PC injury to fruit and adult populations in the tree.
6. To evaluate the effectiveness of PC controls that target adults in the spring as they congregate on the ground underneath host trees.

PROCEDURES:

PC Survey

There were 302 new sites surveyed in 2000 to extend the survey area and delimit the boundaries of PC infestation. A total of 657 fruit host sites have been surveyed for PC in Box Elder, Cache and Weber Counties in 1998-2000. Surveys were conducted from mid June to mid July when all types of host fruit were available on trees. At each survey site, 100-200 fruit was examined for feeding and oviposition injury. The survey results have been mapped.

PC Host Range

The potential host range for PC in Utah was investigated by placing field-collected adults on green fruits of commercial, ornamental, and wild fruit trees in cages in the laboratory. One each active male and female were placed together on 5-10 fruits attached to a twig kept in floral preservative inside cages kept at room temperature. Cages were replicated 10 times each. Fruits were evaluated 5-8 days later for the number of feeding and oviposition scars, and eggs and larvae inside.

PC Control

The Box Elder/Brigham City Mosquito Abatement District applied diazinon for control of PC to 23 home yards in Brigham City from May 6-19, 2000. The efficacy of diazinon treatment for control of PC was determined by sampling 100 fruit from each site on June 21.

Adult Trapping

Circle and pyramid traps with possible adult PC attractants (fruit essence, ethanol, acetic acid, and aggregation pheromone) were evaluated in 3 sweet cherry home yard, 3 wild plum, and 1 sweet cherry orchard sites in 2000. Most trapping sites had a single replication of circle traps (1 home yard) or pyramid traps (2 wild plum) or both types of traps (1 home yard and 1 wild plum). One home yard site consisted of 3 adjacent back yards with 10-15 sweet cherry trees each, and each yard contained a replication of circle traps. The orchard site had 2 replications each of circle and pyramid traps. First traps were placed in mid to late April and PC were collected at approximately weekly intervals through September.

Exclusion of PC from Trees

Wire screen collars were placed on five trees each at two sites. Five trees without screen collars at each site were used for comparison of the percentage of PC infested fruit between the two treatments.

PC Control on the Ground

This objective was not addressed in 2000.

RESULTS AND DISCUSSION:

PC Survey

During 1998-2000, 657 sites in Box Elder, Cache, and Weber Counties were surveyed for the occurrence of plum curculio (PC) and injured fruit. The surveyed area was divided into four regions and the occurrence of PC is reported in the table below and Figure 1.

PC infestations were found almost exclusively in neglected or unmanaged sites: home yards, roadside wild plum, and neglected orchards. The majority of PC-infested-sites were in the residential areas of Brigham City (74%). Sixty-nine percent of the Brigham City home yards infested with PC were sweet cherry, and 51% of all sites infested with PC were found in sweet cherry in home yards.

Region	# Sites Surveyed	# Sites Positive for PC
1 – Tremonton to northern border of Brigham City	168	14
2 – Northern border of Brigham City to Weber Co.	414	85
3 – Weber Co. line to North Ogden	60	0
4 – Cache Co.: Wellsville to Mendon and Avon to Paradise	15	0
Total	657	99 (99/657=15.1%)

PC Host Range

In the field, PC larvae have been found in apple, apricot, cherry (sweet and tart), peach, and plum (cultivated and wild). No PC injury or larvae have been found in pear fruit in the field. Results of laboratory fruit host trials are reported in the table below. PC adults fed and laid eggs in all cultivated pome and stone fruits offered, and in wild plum (*Prunus americana*) and black hawthorn (*Crataegus douglasii*). Although eggs were laid in peach and pear, no larvae developed. No oviposition scars, eggs or larvae were found in ornamental crabapple (*Malus* spp.). No adult leaf feeding was observed on black hawthorn or ornamental crabapple.

	Number per cage (5-10 fruits) after 5-8 days				
Fruit Host	# Feeding Scars	# Oviposition Scars	# Eggs	# Larvae	Leaf Feeding?
Cultivated Stone Fruits					
Apricot	40.6	13.8	--	9.2	Yes
Sweet Cherry	9.7	8.3	0.9	2.4	Yes
Tart Cherry	17.8	17.1	6.3	0.6	Yes
Peach	2.7	0.8	0.9	0	Yes

	Mean number per cage (5-10 fruits) after 5-8 days				
Fruit Host	Feeding Scars	Oviposition Scars	Eggs	Larvae	Leaf Feeding?
Cultivated Pome Fruits					
Apple	9.4	8.6	4.8	1.0	Yes
Pear	2.4	0.4	0.2	0	Yes
Wild and Ornamental Fruits					
Wild Plum	21.1	7.8	6.1	0.2	Yes
Black Hawthorn	7.0	3.0	2.2	0.6	No
Ornamental Crabapple	3.4	0	0	0	No

PC Control

The Box Elder/Brigham City Mosquito Abatement District applied a single diazinon spray for control of plum curculio to each of 23 home yard sites in Brigham City from May 6-19, 2000. Petal fall of cherry and apple, the optimal time to control PC with insecticides, occurred from the last week of April to first week of May in the Brigham City area. Petal fall for apricot and peach was earlier, approximately mid April. The efficacy of diazinon for control of PC was determined by sampling 100 fruit from each site on June 21. The number of feeding and oviposition scars, eggs, larvae, and exit holes are presented below as a mean value for each fruit type. Most of the larvae recovered from fruit were near mature size, indicating that they were close to leaving the fruit to pupate. Although feeding and oviposition scar counts were high for most fruit types sampled, most of these were probably made by PC before the diazinon treatment was applied. Survival of eggs and larvae was low (0-3 larvae/100 fruit) in comparison to a nearby untreated wild plum site (31 larvae/100 fruit) which suggests that control with diazinon was reasonably good. Applying the insecticide earlier, at petal fall, and a reapplication 10-14 days later may improve the efficacy of diazinon treatment. No oviposition scars, eggs or larvae were found in peaches sampled at four sites.

		Mean number per 100 fruit				
Fruit Host	# Sites	Feeding Scars	Oviposition Scars	Eggs	Larvae	Exit Holes
Apricot	3	116.7	50.0	0	3.0	0
Sweet Cherry	11	31.1	12.1	0	0.8	0.2
Tart Cherry	1	24.0	23.0	0	3.0	0
Peach	4	17.0	0	0	0	0
Apple	4	37.8	25.5	0	1.3	0
Comparison untreated wild plum						
Wild plum	1	68	115	0	31	0

Adult Trapping

Pyramid traps caught more adult PC than circle traps in a wild plum site, however, trap catch was lower and similar between trap types in sweet cherry yard and orchard sites (Figure 2). No difference in trap capture between circle and pyramid traps was found in 1999 studies. Circle traps are more appropriate to use in home yard and some orchard sites because they are placed on tree trunks rather than on the ground where they may interfere with landscape or orchard activities. There was no significant increase in adult PC trap catch in circle traps baited with any attractant (fruit essence, ethanol, aggregation pheromone, acetic acid or any combination) as compared to unbaited traps (Figure 3). Circle traps with fruit essence (plum or sweet cherry), ethanol, essence + ethanol, and acetic acid + essence performed as well as no lure at most sites, but the pheromone alone or in any combination generally caught fewer PC than unbaited traps. Slight, but non-statistically different increase in trap catch was also observed for fruit essence in 1999 studies, but no clear or consistent trend in increased catch for any of the attractants stands out.

Exclusion of PC from Trees

The percentage of fruit infested with PC was no different between trees with exclusion collars and without at either of the two study locations. Flight and crawling appear to both be major modes of movement for PC into host trees.

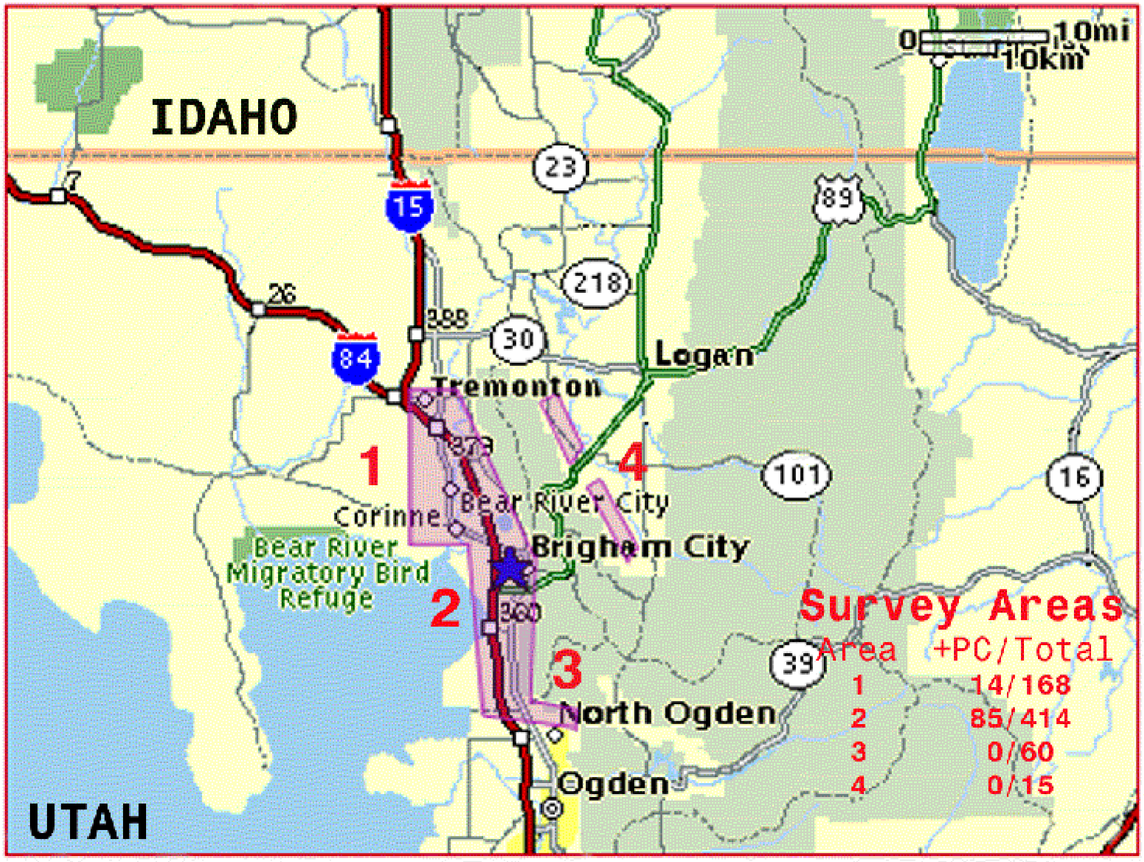


Figure 1. Map of plum curculio survey areas in northern Utah (Areas 1 & 2, Box Elder County; Area 3, Weber County; Area 4, Cache County), 1998-2000. The number of sites infested with PC out of the total number of sites surveyed in the area is indicated in the lower right-hand corner.

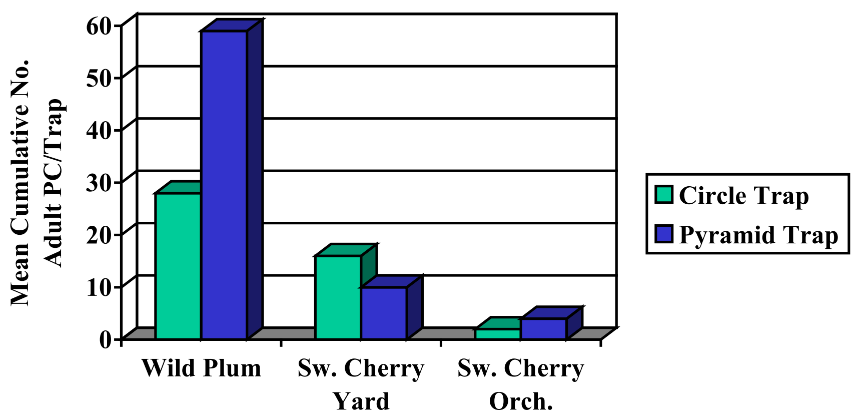


Figure 2. Comparison of adult plum curculio capture in circle and pyramid traps at three sites from May 2 – September 8, 2000.

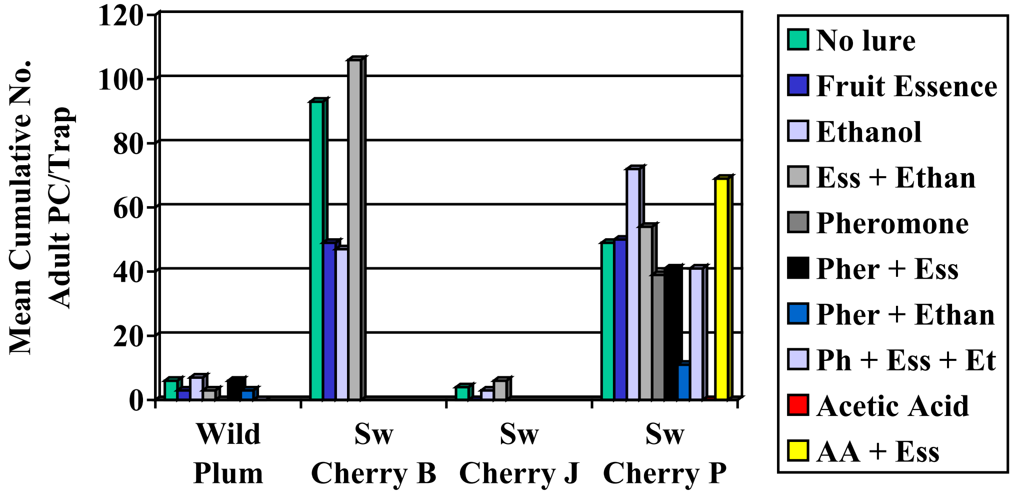


Figure 3. Comparison of adult plum curculio attractants in circle traps at one wild plum and three sweet cherry home yard sites from May 2 – September 29, 2000. The acetic acid and acetic acid + essence lures were only in place from June 9 – September 29.

CONCLUSIONS:

- **Where is PC?** The PC population in northern Utah has been delimited to an approximately 50 sq. mile narrow strip along highways 38 and 89 from Honeyville to Willard, centered on Brigham City, in Box Elder County. PC is unlikely to be found beyond this area because of large stretches of vegetation without host trees (dominated by sage brush) surrounding the infested area. Survey traps and fruit injury samples conducted in surrounding areas of Box Elder County and in other fruit producing counties of northern Utah (Cache, Davis, Weber, Utah Counties) have all been negative for PC.
- **What is PC's primary habitat and host fruit?** 74% of the sites positive for infestation with PC are in Brigham City home yards. In the majority of these (69%), sweet cherry is the host tree. Therefore, the primary target for reduction and elimination of PC should be removal and treatment of home yard trees, especially sweet cherry.
- **What is the potential for PC to attack native, naturalized or ornamental hosts?** In the laboratory, PC demonstrated the ability to attack a native western U.S. pome fruit, black hawthorn (*Crataegus douglasii).* PC did not lay eggs in ornamental crabapple (*Malus* spp.). A non-native, naturalized host, American plum (*Prunus americana*), is a common host of PC in the infested area. All cultivated stone and pome fruits are potential hosts for PC, although peach and pear do not seem to be as readily attacked as others.
- **Can PC be controlled with insecticides?** The primary control of PC in the eastern U.S. is insecticides. Control of PC with diazinon or other insecticides appropriate for home yard use shows promise. Applying the insecticide at petal fall and repeating treatment 10-14 days later may improve the efficacy of diazinon over a single spray applied 1-2 weeks after petal fall.

- **Adult attractants and trapping.** No attractants evaluated show consistent increase in capture of adults in traps. Continued development and improvement of adult attractants, along with increased knowledge of PC host-finding behavior is needed.
- **PC population reduction program.** Removal of PC infested trees is the best approach to reduce and eliminate PC in northern Utah. Application of well-timed insecticides is an additional tool that has been implemented to a degree. Beyond these approaches, development of tactics for long-term population suppression is needed, such a biological control, mass trapping, removal or disturbance of overwintering sites, and others.

FINAL REPORT
PROJECT NO.: 13C-3343-3123

Title: *Lygus* Bug and Western Flower Thrips Ecology in Washington State Apple Orchards

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

Organization: WSU Prosser

Cooperator(s): R. Wight, Field Research Dir., IR4 Project, WSU-Prosser

OBJECTIVES: *Lygus*
1. Compare *Lygus* sampling techniques for Lygus on the orchard floor.
2 Develop an early season threshold for Lygus on apples in the Columbia Basin.
3. Attempt to fine tune the UC Lygus phenology model to fit eastern Washington Lygus populations

OBJECTIVES new for 2002: Thrips
1. Evaluate edge effects of migration of thrips from adjacent riparian habits into apple orchards.

OBJECTIVES: Riparian Buffers
1. Develop riparian habitats that will not serve as a point source for *Lygus* and thrips infestations.

SIGNIFICANT FINDINGS- *LYGUS*
Orchard Sampling. In 2001 and 2002 several *Lygus* abundance sampling techniques were investigated. Techniques tested included sweep net samples of the orchard floor, beat sampling of trees, and colored sticky cards. Sweep net sampling of the orchard floor was the only technique tested that caught substantial numbers of *Lygus*. Tree beating was inefficient at capturing Lygus after a cover spray for codling moth had been applied. Additionally the use of colored sticky cards in measuring abundance of *Lygus* was not efficient at capturing Lygus.

***Lygus* Damage.** Branch cage studies in 2001 and 2002 helped quantify proportional *Lygus* abundance to fruit damage. *Lygus* feeding in April resulted in greater proportional amount of fruit injury then *Lygus* feeding in feeding in May 2002 or July 2001. Weekly assessments of Lygus infestation at high abundance of Lygus (1 bug per fruit) resulted in high levels of damage when cages were established on any given week from 17 June through19 August 2002. A cover spray of azinphos methyl applied to our research orchard on 5 July effectively killed the Lygus we caged on 8 and 15 July. Azinphos methyl's residual effectiveness for suppression of Lygus appeared to break down after 15 to 17 days after application.

Phenology Model. A phenology model currently used in California proved effective at predicting the first generation hatch of *Lygus* in late-May in Eastern Washington and the subsequent peak hatch event in mid-July. However, the model lost predictive accuracy as the season progressed and would provide little predictive value for when adult migration into orchards might occur in April or May.

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 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 apple growing areas

SIGNIFICANT FINDINGS-THRIPS

Flight monitoring. Thrips flight activities were monitored in apple orchards with yellow and blue sticky card traps in 2001. Blue cards are proving to be significantly ($P<0.01$) more effective then yellow cards at catching western flower in apple orchards. We have observed a definite orchard edge effect with the orchard floors bordering riparian buffers having significantly ($p<0.01$) greater populations of thrips then the orchard floor 92 meters inside the orchard. There were also significant ($P<0.05$) differences in abundance of thrips as measured by blue sticky card with the orchard floor having a greater abundance of thrips then cards placed in the canopy on both the orchard edge and 92 meters in.

SIGNIFICANT FINDINGS-RIPARIAN BUFFERS

We have developed considerable evidence that riparian areas are confirmed sources of hemipteran and thrips pests. These include both stinkbugs, (Jay Brunner, personal com), and *Lygus* bugs and flower thrips (Walsh unpl. data). We have also documented an increase in the populations of several beneficial arthropods in riparian buffers. We have also identified host plant on which Lygus can complete development and feral plants around which the abundance of flower thrips are greater then plants that appear to be non-hosts for flower thrips

Methods Lygus

1. We compared several sampling techniques to assess Lygus populations in the riparian sites and nearby orchard floors in spring and summer 2001 and 2002 to assess when Lygus adults become active and to determine when subsequent generation's egg hatch takes place. Sampling techniques tested included sweepnet, and colored sticky cards.
2. Lygus damage 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 trilals were repeted 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 in which 3 replicate cages were established weekly from 17 June to 19 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 Lgus feeding changed over time as the growing season progressed.
3. Phenology model. Data collected in section below "Riparian habitats" was used for this study. Collected weather information from Washington Public Access Weather Systems data and imported the data into the UCIPM degree day calculator/ phenolgy model calculator for Lygus bugs in a single-sine model with 54°F serving as a horizontal developmental threshold. An arbitrary biofix date of January 1 was used in 2001 and 2002 as the biofix for potential egg-laying.
4. **Biolgical control**. Extensive surveys in 2002 determined the presence of Lygus parasitism by *Persitenus* spp. Sites were chosen opportunistically during a 2 week period of driving-about eastern Washington State. Sweep nets were used to capture Lygus nymphs from crop plant/ weed hosts. Lygus nymphs were then aspirated into vials and placed on ice and transported to a freezer located at IAREC in Prosser. As time permitted laboratory assistants dissected the nymphs to

determine and quantify the presence of the nymphal parasitoid *Peristenus* spp. In total over 40 sites were surveyed and over 4,000 Lygus were dissected.

Methods- Thrips

We have evaluated thrips population abundance in riparian buffers and on the the perimeter of orchards and in the interior of orchards within the tree canopy and on the orchard floor with blue and yellow sicky cards that were placed every 2 weeks in May through October 2001.

Methods- Riparian habitats.

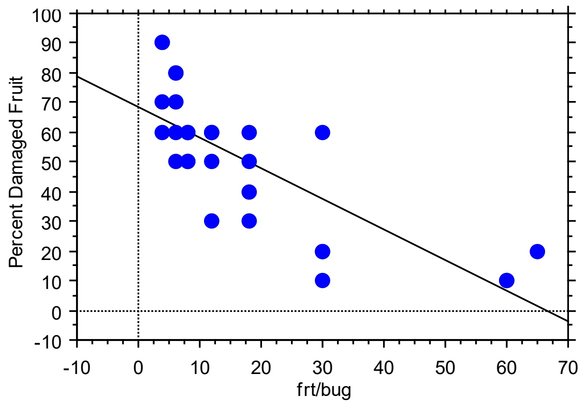
1. In 2001 we identified apple orchards in the Yakima Valley that had "protected waterways" running in, next to, or near their orchards. In early spring 2001 we established 3 field survey sites of 180' by 180' along protected waterways and monitored; a) the ambient plant species, and b) the abundance of *Lygus*, thrips and other pest and beneficial insects present at each respective riparian site and at the adjacent orchard edge and orchard interior every 2 weeks from March through November. In spring 2002 we identified or established distinct specific plant stands on which we began monitoring for the ambient insects present on these plants.

RESULTS/ DISCUSSION LYGUS-

Orchard Sampling. In 2001and 2002 several *Lygus* abundance sampling techniques were investigated during spring. Techniques tested included sweep net samples of the orchard floor, beat sampling of trees, and placement of yellow and blue colored sticky cards on the orchard edge and interior of 4 apple orchards on the Roza near Prosser, WA. Sweep net sampling of the orchard floor was the only technique tested that caught any observable numbers of *Lygus*. Tree beating at 10 beats per sample site was ineffective at quantifying Lygus and both colors sticky cards of sticky cards were ineffective. Lygus nymphs were observed on orchard floors but were never directly observed within the tree canopy. At each orchard site application of cover-insecticide sprays resulted in an observed reduction of Lygus abundance.

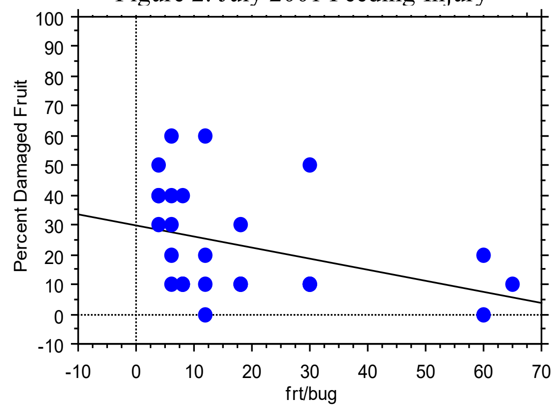
***Lygus* Damage.** *Lygus* feeding has been likened to chemical injury. *Lygus* feeding damage in apple orchards is a significant concern after fruit set, however feeding damage can result in fruit disfigurement during the fruit growing season. Branch cage studies in 2001 and 2002 have helped quantify 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 we also designed a sequential sample experiment in which we established cages weekly from June 17, 2002 through August 19, 2002. 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. On 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. Lygus feeding damage was greatest on the June 17 establishment date at about 70% of the fruit being damaged (Figure 4). Damage for every other week appeared to be fairly consistent. A application of azinphos methyl was made on July 5 and this effectively reduced Lygus feeding in tyhes cages on the July 8 & 15 cage-loading dates. This indicates that a benefit of azinphos-methyl application includes a 2 week suppression of Lygus fe

Figure 1. April 2001 Feeding Injury



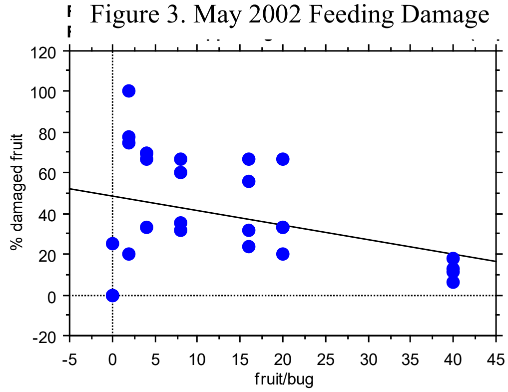
Y = 68.628 - 1.037 * X; R^2 = .599

Figure 2. July 2001 Feeding Injury



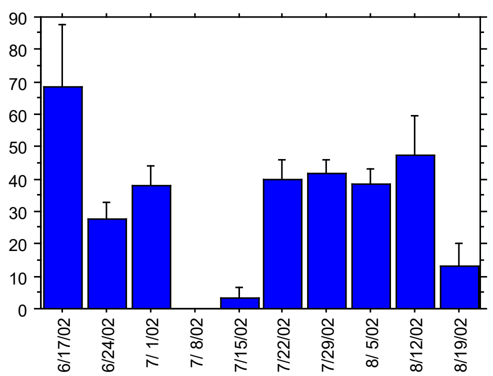
Y = 29.889 - .372 * X; R^2 = .134

Figure 3. May 2002 Feeding Damage



Y = 48.927 - .717 * X; R^2 = .115

Figure 4. Sequential feeding injury 2002



Phenology Model. *Lygus* overwinter as adults in plants and plant debris. Russian, thistle, Kochia, smotherweed, mullein, horseweed, sweetclover, wild mustards, ragweed, and sagebrush are among many plants that will serve as good overwintering hosts for *Lygus*. Overwintering *Lygus* adults became active as temperatures warmed in spring 2001 and 2002. We observed our first adult Lygus on 2 April and 9 April 2001 and 2002, respectfully. A phenology model Walsh helped develop in 1990 (www.ipm.ucdavis.edu) proved effective at predicting the first generation hatch of *Lygus* in spring in Eastern Washington (Table 1) and the subsequent peak hatch periods for the 2^{nd} and 3^{rd} generations of Lygus in 2001 and 2002. In running this model degree days are accumulated starting on January 1 with 54° Fahrenheit serving as a horizontal lower-cutoff for development. Eggs laid by adult Lygus in late-fall or winter will require approximately 252 degree days in order to hatch. This corresponded well with when we observed our 1^{st} generation of nymphs in both orchards and in our riparian survey sites (Table 1) in mid to late May. Although this model proved fairly effective at predicting hatch periods for Lygus during the summer months it has been our observation that the majority of feeding injury on apples is caused by adult Lygus and that this model does little towards predecting when adult Lygus will migrate into apple orchards. Rather rainfall patterns and the subsequent dry-down of the over-wintering hosts of Lygus is a prime cause of spring movement of adult Lygus. During the summer months harvest of field and forage crops (primarily alfalfa) also contributes to movement of adult Lygus. A highly preferred weed-host for adult Lygus during the summers of 2001 and 2002 was Kochia. Preferred hosts for nymph development included alfalfa and wild mustards.

Table 1. Cumulative degree day accumulations 54°, predicted dates of peak hatch for the 1st, 2nd, and 3rd generations of Lygus and the actual average number of Lygus caught per sweep among all of the samples taken bi-weekly at our riparian survey sites (n=72) for 2001 and 2002.

	2001			2002		
Date	Cumulative DD	Model Prediction	Nymphs /sweep	Cumulative DD	Model Prediction	Nymphs / sweep
March 1	0		n/a	9		n/a
April 1	35	(1st adult 4/2)	n/a	27	(1st adult 4/9)	n/a
April 15	39		n/a	69		n/a
May 1	109	1st hatch	0	119	1st hatch	0
May 15	198	(May 23)	0	166	(May 27)	0
June 1	384		**1.6**	307		**0.6**
June 15	484		1.2	473		1.3
July 1	675		0.8	713		1.5
July 15	961	2nd hatch	1.3	991	2nd hatch	**3.2**
August 1	1196	(July 23)	**3.0**	1351	(July 17)	0.3
August 15	1513		1.6	1573		1.2
September 1	1784	3rd hatch	0.7	1870	3rd hatch	**3.4**
September 15	1984	(Sept. 8)	**2.4**	2052	(Sept. 8)	1.3
October 1	2153		1.2	2173		1.3
October 15	2215		0	2235		0
November 1	2237		0	2272		0

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 (unpublished data/ Table 2) in 2002 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 apple growing areas (Table 2).Extensive surveys in 2002 determined the presence of Lygus parasitism by *Persitenus* spp. In total over 40 sites were surveyed and over 4,000 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.

Table 3. Presence of the parasitoid *Peristinas* spp. attacking the nymphs of Lygus bugs- July 2002.

Location	State	Vegetation Type	Number of nymphs examined	Number of nymphs parasitized	Percent parasitism
Touchet	Washington	mustard	320	101	32%
Crosby Site	Washington	yellow mustard	88	11	13%
Yakima Valley Hwy near Donald and Snokist	Washington	alfalfa	140	15	11%
Naches	Washington	alfalfa	90	8	9%
Monitor	Washington	wild alfalfa	207	14	7%
Roza	Washington	alfalfa	45	3	7%
Corner Snipes & Wilson (Sunnyside)	Washington	feral alfalfa	168	9	5%
Sommers Rd.: 4 Miles North of Hwy 26	Washington	canola	70	3	4%
Hwy 12: Burbank	Washington	alfalfa	25	1	4%
Chaffee Rd. near Kershaw Hgts.	Washington	mustard	153	5	3%
Othello Station	Washington	alfalfa	81	2	2%
Othello	Washington	alfalfa	167	4	2%
Factory Rd: Sunnyside	Washington	alfalfa	196	4	2%
Corner of Robblard and Komnawack Pass Rd	Washington	alfalfa	275	4	1%
4 Miles West of Pomeroy	Washington	alfalfa	142	1	1%
Wapato: Hwy 97 & Branch Rd.	Washington	alfalfa	145	1	1%
3735 "Bjur Ranch" near Clark Rd.	Washington	dry alfalfa	181	1	1%
10 Miles Southwest of Ephrata: Hwy 282	Washington	alfalfa	186	1	1%
Kahlotus Hwy Pasco	Washington	Russian thistle/ mustard	134	0	0%
6 Miles East of Othello	Washington	mustard on edge of alfalfa	209	0	0%
15 Miles East of Ephrata	Washington	lettuce seed	133	0	0%
Road 0: 10 Miles East of Quincy	Washington	alfalfa by apples	175	0	0%
Across from Trinidad	Washington	apple orchard floor/ferrel alfalfa	129	0	0%
Wenatchee: Wildwood/Hwy 97	Washington	alfalfa next to apple orchard	343	0	0%
Wenatchee: TFREC	Washington	Russian thistle & knapweed	69	0	0%
Sunnyside	Washington	concord grapes/mustard	129	0	0%
IAREC HQ	Washington	alfalfa	124	0	0%
Orondo Orchard: 2 Miles North of Orondo	Washington	feral alfalfa next to apples	112	0	0%
Corner Scoon & Williamson (Outlook)	Washington	alfalfa	90	0	0%
Yakima: Birchfield & SR24	Washington	alfalfa	43	0	0%
Hwy. 22 & Fisher (Mabton)	Washington	alfalfa	77	0	0%

Thrips.

Thrips Sampling. Thrips flight activities were monitored in 3 apple orchards in the Yakima Valley near Prosser. This study was run in conjunction with other projects that we had ongoing on thrips management on several other crops comparing blue or yellow sticky cards as a monitoring tool. At present thrips abundance has been counted on 756 blue cards and 886 yellow cards. Blue cards are proving to be significantly (P<0.01) more effective then yellow cards at catching western flower thrips across a range of crops.

Thrips per 3” by 5” sticky card

	Count	Mean	Std. Dev.	Std. Err.
bl stky cd	756	369.504	798.592	29.045
yl stky cd	886	226.445	447.793	15.044

In apple orchards this same pattern remained consistent as well across 3 sample dates

Thrips per 3” by 5” sticky card

	Count	Mean	Std. Dev.	Std. Err.
6/29/01, bl stky cd	31	224.839	280.040	50.297
6/29/01, yl stky cd	31	178.871	227.104	40.789
7/13/01, bl stky cd	31	440.968	320.108	57.493
7/13/01, yl stky cd	31	324.032	316.892	56.916
7/27/01, bl stky cd	13	359.615	382.777	106.163
7/27/01, yl stky cd	13	183.077	231.755	64.277

Riparian Habitats. Populations of agricultural pest insects in Pacific Northwest irrigated agro ecosystems are increased by the improper establishment and maintenance of riparian buffers adjacent to ephemeral creeks that can serve as habitat corridors. Exotic species of plants persisting in degraded riparian buffers are serving as better hosts to problematic species of arthropods including Lygus than are native plant species. In contrast, populations of some beneficial arthropods including spiders and Carabid beetles increase in the presence of several exotic plant species. Arthropod and vegetation surveys conducted in representative riparian buffers adjacent to apple orchards have determined trends in associations between pest and beneficial arthropods and the species of plants they persist on. Pending regulations that may arise from the Endangered Species and Clean Water Acts necessitate these studies. Knowledge of insect plant associations when re-habilitating degraded riparian buffers will enable land owners/ growers to establish plant species that are more likely to host beneficial predacious arthropods, and less likely to serve as hosts to pest insects. From our studies we have identified plants which appear to serve as host for the pest arthropods Lygus and Thrips. In spring 2002 we established distinct plant stands of selected plants (Table 4) on which we will conduct comprehensive study in future years.

Our research on riparian habitats clearly points out the need for weed and insect management tools during the establishment/rehabilitation phase of riparian buffers.

Table 4. Plants selected for comprehensive study in 2002/2003 field studies

Common Name	Genus species	Native	Insect Pest host
Reed canary grass	*Phalaris arundinacea*	Yes	Yes
Wild rose	*Rosa* spp.	Yes	Yes
Big sagebrush	*Artemesia tridentata*	Yes	Yes
Canada thistle	*Cirsium arvense*	No	No
Perennial pepperweed	*Lepidium latifolium*	No	No
Kochia	*Kocia scoparia*	No	No
False london rocket	*Sisymbrium loeselii*	No	No
Spike rush	*Eleocharis* spp.	Yes	No
Gray Rabbit brush	*Chrysothamnus nauseosus*	Yes	No
Common mullein	*Verbascum thapsus*	No	?
Cheat grass, downy brome	*Bromus tectorum*	No	?
white horehound	*Marrubium vulgare*	No	?
Tumble mustard	*Sisymbrium altissimum*	No	?
Yarrow	*Achillea millifolium*	Yes	?
Stinging nettles	*Urtica dioica*	Yes	?
Prickly lettuce	*Lactuca serriola*	No	?
Saltbush	*Atriplex patula*	Yes	?

FINAL REPORT
Project #: AE-01-26

PROJECT TITLE: Bisexual Monitoring of Codling Moth and Establishing a female Biofix

PI: Alan L. Knight USDA, ARS, Wapato, WA
Co-PI: Doug Light USDA, ARS, Albany, CA

SIGNIFICANT FINDINGS:

- DA lures effectively monitored both generations of codling moth in > 200 apple and pear orchards during 2000, 2001, and 2002.

- The cumulative catch of codling moth in DA-baited traps was higher versus sex pheromone-baited traps in apple orchards under mating disruption (MD) during both flights, except during the first flight in 2000.

- The DA lure was less effective than the sex pheromone lure in conventional apple orchards – catching ca.50% as many moths during the first flight and 25% during the second flight.

- Apple cultivar influences the performance of the DA lure. Compared with moth catch in a sex pheromone-baited trap the DA lure was most effective in late-season cultivars, such as 'Granny Smith' and 'Fuji', and least effective in 'Gala'.

- The DA lure catches < 20% as many moths as sex pheromone lures in MD Bartlett pear orchards. However, this lure is more effective than the sex pheromone lure in MD Anjou orchards.

- The first moth caught in a DA-baited trap is usually a male. Greater than 90% of the first females caught were mated. During the 2000-02 seasons there was no pattern in whether a pheromone-baited or DA-baited trap caught the first moth.

- The sex ratio of moths caught by the DA lure in MD orchards is nearly 50:50 male:female.

- Greater than 75% of all females caught by the DA lure during the season were mated in MD orchards. The proportion of mated females is only slightly reduced in MD vs conventional orchards.

- A comparison of the female mating status of moths caught with a passive interception trap and the DA-baited trap suggested that the proportion of virgin females is underestimated by the DA lure.

- The DA lure allowed us to see that nearly all females are mated only once during the first generation across all orchard types. In contrast, multiple matings are common in non-MD orchards during the second flight. Up to 50% fewer total matings occurred in MD orchards versus conventional orchards during the second flight though only 10% more virgin females were trapped.

- Factors such as trap height and trap position within the canopy can affect the numbers and the sex ratio of moths caught. Higher traps catch more moths and traps placed away from fruit and in open spaces within the canopy catch more females.

- ❖ The beginning of sustained female moth captures in DA-baited traps plus 155 degree-days was as effective as the beginning of sustained male captures in sex pheromone traps plus 250 degree-days in predicting codling moth egg hatch. However, timing egg hatch from the first moth caught in either type of trap was a variable predictor of egg hatch. Frequent trap checking plus consideration of dusk temperature and wind speed improves the prediction of egg hatch.

- ❖ Cumulative first generation moth catches in DA-baited traps were more effective than similar catches in sex pheromone-baited traps in eliminating unnecessary first generation cover sprays (false-positives). Cumulative moth captures in DA-baited traps were more closely correlated than catch in sex pheromone-baited traps with the levels of codling moth fruit injury prior to harvest. Establishing action thresholds based on the capture of female moths or low number of total moths in DA-baited trap during the first flight was shown to be a useful monitoring tool. However, false negatives occurred with about 10% of traps baited with either the DA or sex pheromone lure.

OBJECTIVES:

1. Evaluate the effect of crop and cultivar on the seasonal attractiveness of the DA lure.
2. Evaluate the utility of measuring the mating status of female codling moth with the DA lure.
3. Evaluate the use of the DA lure to establish an improved Biofix and prediction of egg hatch.
4. Evaluate the use of the DA lure to establish action thresholds for applying supplemental insecticide sprays to orchards under mating disruption.

PROCEDURES:

CM Flight Curve: A large number of orchards of Gala, Golden, Red, Fuji, Granny, and Anjou under mating disruption in the Brewster area were monitored with the DA (ethyl (2*E*,4*Z*)-2,4-decadienoate) and Mega sex pheromone lures (Trécé Inc., Salinas, CA) from 2000-2002. In addition, two Bartlett orchards in Brewster in 2002 and six Bartlett orchards in Yakima from 2000-2002 were monitored. Within each orchard two traps baited with each lure type were spaced 100 m apart. Fruit injury within 50 m of each trap was assessed prior to harvest by sampling thirty fruits from 20 trees. All female moths were dissected to determine their mating status.

Predicting Egg Hatch: The phenology of codling moth was monitored in three orchards in the Yakima Valley with DA and sex pheromone lure-baited traps and passive interception traps each year. All traps were checked twice per week. Passive interception traps treated with STP™ were replaced on each date. Egg density and egg hatch were monitored 2-3 times per week until mid June. Degree day totals from key trap catch events until first observed egg hatch were determined.

Development of Action Thresholds: Cumulative moth catches for each trap prior to the predicted date for the 1st cover spray were compared with the level of nearby fruit injury at harvest to assess the use of these lures to establish reliable action thresholds in apple. Orchards were typically treated with 1-3 cover sprays during the season. Thus these data do not provide an accurate correlation of moth catch and injury. The data are not analyzed but are presented to provide a comparative view of each lure's performance relative to pest pressure.

RESULTS AND DISCUSSION:
CM Flight Curve:
Captures of codling moth exhibited two well-defined flight curves with both the DA and pheromone lures in the Brewster apple and pear orchards (Figs. 1-5). During this three-year study nearly a 1:1 M:F sex ratio was found and > 85% of all females were mated in the DA-baited traps. The DA lure revealed that the flight of female codling moth in each generation lasted 40-50 d. However, in abandoned orchards with high levels of codling moth there was continuous flight of female moths all season (Fig. 6). This suggests that immigration of mated females from these unmanaged sites can occur throughout the season and may require additional control measures.

The DA lure outperformed the pheromone lure across all cultivars in this study, except in 2000 and in Gala during 2001 (Table 1). These results may suggest that the Mega pheromone lure was more attractive in 2000 then in the later two years. The male / female sex ratio in DA-baited traps varied among cultivars and years. In general, the M / F ratio appears to be influenced by the degree that the moth catch consists of moths from inside the orchard versus moths flying into the orchard. For example, the Grannies have a low M/F ratio and they also have the highest levels of fruit injury. The Goldens in 2001 included one orchard with high moth counts and levels of fruit injury. Conversely, sex ratios are higher in pear blocks where no injury was detected (due to male moths flying into orchards treated with sex pheromone).

These data show that a very high level of mating by codling moth occurs in MD orchards (Table 1). In general, >85% of females trapped were mated. The exceptions occurred in Reds in 2000 and in Anjou where a somewhat higher percentage of virgin moths were caught. The data from pear suggest that the DA lure can be used effectively in Anjou, but is much less attractive in Bartlett.

Table 1. Summary of the relative attractiveness of the Pherocon DA lure versus the Pherocon Mega lure for codling moth within various cultivars of apple and pear.

	DA / PHER Ratio			**DA - M / F Ratio**			**DA - % Virgin F**		
Cultivar	**00**	**01**	**02**	**00**	**01**	**02**	**00**	**01**	**02**
Grannies	0.71	8.79	2.57	1.00	1.20	0.95	15.5	13.8	12.4
Fuji	-	2.62	3.03	-	1.97	2.73	-	13.3	13.5
Reds	0.85	1.40		1.63	2.68	-	30.1	14.0	-
Gala	-	0.93	1.31	-	1.60	1.41	-	14.3	9.5
Goldens	-	3.21	-	-	0.69	-	-	12.7	-
Anjou	-	1.67	5.21	-	2.75	1.40	-	25.0	20.6
Bartlett	-	0.11	0.47	-	1.50	1.91	-	13.6	20.1

Data collected from the three high-pressure sites in the Yakima Valley also showed that a high level of mating occurs in orchards under MD, conventional, or abandoned conditions during both generations (Table 2). Interestingly, we found that almost all mated females in the first generation have only one spermatophore. Reductions in the mean number of spermatophores in the MD orchard during the second generation suggests that though the percentage of mated females does not differ overall among orchard types, the number of mating were reduced up to 50% (Table 2).

Examining these data over three years and across generations I found that the DA lure versus the interception trap appears to be somewhat biased for mated females, 32% lower percentage of virgin moths, 17.32 (2.50) vs 25.42 (2.93)%; $t = -2.73$, df = 16, $P = 0.01$.

Table 2. Summary of the mating status of female codling moth in high-pressure orchards in the Yakima Valley.

Orchard type	Year	Gen.	% mated females	Mean # Spermat.	Gen.	% mated females	Mean # Spermat.
Pher.	01	1	81.9	1.02	2	78.2	1.08
Conv.	01	1	86.5	1.03	2	96.6	1.62
Abd.	01	1	84.3	1.08	2	76.0	1.39
Pher.	02	1	54.6	1.01	2	88.0	1.04
Conv.	02	1	72.7	1.00	2	81.4	1.18
Abd.	02	1	95.7	1.03	2	84.7	1.44

Predicting Egg Hatch: The DA lure can be used to accurately predict the timing of egg hatch. However, interpretation of moth catches can be difficult due to the relative low moth catches, infrequent trap checks, and the use of an accepted definition of Biofix. The prediction of egg hatch using both DA and sex pheromone lures is shown in Table 3. First moth catch in traps typically generated an early prediction of the beginning of egg hatch. Using the sustained catch improved the model. First female catch was the most variable predictor. But, the beginning of sustained catch with the DA lure was an excellent tool in predicting egg hatch. Using traps to precisely predict the population emergence of codling moth remains difficult. Establishing the start of a sustained period of moth catch would be difficult without daily trap checking. Regardless, of which method a pest manager uses to time sprays, he needs to consider daily maximum and minimum temperatures, as well as wind speed at dusk, to interpret moth counts and improve their correlation with female mating and egg laying events.

Table 3. Difference (d) between predicted and actual start of egg hatch using 250DD after pheromone catch and 155 DD after DA catch.

Orchard / year	Date of 1st Egg Hatch	1st Male caught w' Pheromone	Sustained Pheromone Catch	1st Female w' DA	Sustained DA Catch
Pher / 02	2 June	-4 d	0	-8 d	0
Conv /02	29 May	-6 d	-1 d	-7 d	0
Abd / 02	23 May	-3 d	-1 d	-7 d	0
Pher / 01	25 May	-1 d	+1 d	-1 d	+1 d
Conv / 01	21 May	-1 d	0	-9 d	0
Abd / 01	20 May	-6 d	-1 d	-5 d	+1 d
MEAN	**-**	**3.5 d**	**0.7 d**	**6.2 d**	**0.3 d**

Development of Action Thresholds: During 2000 we showed that the use of DA lures would reduce the over-spraying of uninfested orchards during the first flight. We suggested that sex pheromone traps pull male immigrants into the orchard and are less effective in reflecting the local population density of females than the DA lures. Seasonal cumulative catches of moths in DA-baited were more closely correlated with fruit injury at harvest than similar traps baited with sex pheromone lures in both years (correlation coefficients for DA versus Pherocon Mega lures: 0.84 versus 0.71 in 2000 and 0.69 versus 0.36 in 2001, respectively). However, false negative catches of moths occurred with both types of lures in about 10% of orchards.

Cumulative moth catches in traps baited with the DA lure prior to the 1st cover spray timing were useful indicators of pest pressure during both 2001 and 2002 (Table 4). The lowest mean injury occurred in plots where no moths were caught by the DA lure. A threshold of 1-2 total moths or no female moths in DA-baited traps appeared to also be a good predictor of low injury levels. Capturing no moths in the pheromone-baited trap appeared to be a more variable predictor of injury between years. These data were obtained from sprayed orchards and cannot be used to establish a quantitative relationship between moth catch and injury. However, they suggest that there is a relationship between the number of moths caught by the DA lure and CM fruit injury.

Table 4. Relationship between moth captures (Action Threshold) prior to 1st Cover Spray timing in DA and pheromone-baited traps and mean % fruit injury at harvest.

	Mean % CM Fruit Injury	
Action Threshold	2001 Fuji, Reds, & Gala N = 60	2002 Fuji & Gala N = 40
0 moths in DA	0.01	0.04
1-2 moths in DA	0.04	0.08
0 Females in DA	0.09	0.16
0 moths in Pheromone	0.04	0.20
1-2 moths in pheromone	0.13	0.24
1 female in DA	0.20	0.32
3-5 moths in DA	0.17	0.60
>5 moths in DA	0.42	0.68
2 females in DA	0.28	1.24
3-5 moths in pheromone	0.42	-
>5 moths in pheromone	0.48	-
3 females in DA	-	1.32

CONCLUSION:

The availability of the DA lure for monitoring CM has been a significant development in pest management. The DA lure appears to be a good predictor of local codling moth populations. However, due to the apparent short range of activity of the DA lure we do not know, yet, how to best use them lures to monitor entire orchards. The occurrence of any moths in the DA-baited trap or of 0-1 F per trap is a good indicator of low moth pressure. Higher moth counts correlate well with the occurrence of fruit injury. The DA lure would seem to be a good alternative to the use of sex pheromone lures in MD orchards for all cultivars, except perhaps Bartlett pear. The use of the DA lure allows pest managers to track the emergence and activity of female CM. Development of a timing model based on female moth activity shows promise. The DA lure has provided a better portrait of the seasonal activity of female moths and subsequently egg laying and egg hatch. The DA lure has allowed us to see that a very high level of mating occurs under mating disruption programs for codling moth. Evaluation of the relative mating success within MD orchards does not seem to provide useful information.

Fig. 1. Seasonal mean captures of codling moths in DA- and sex pheromone- baited traps within 22 apple orchards treated with MD in Brewster during 2000.

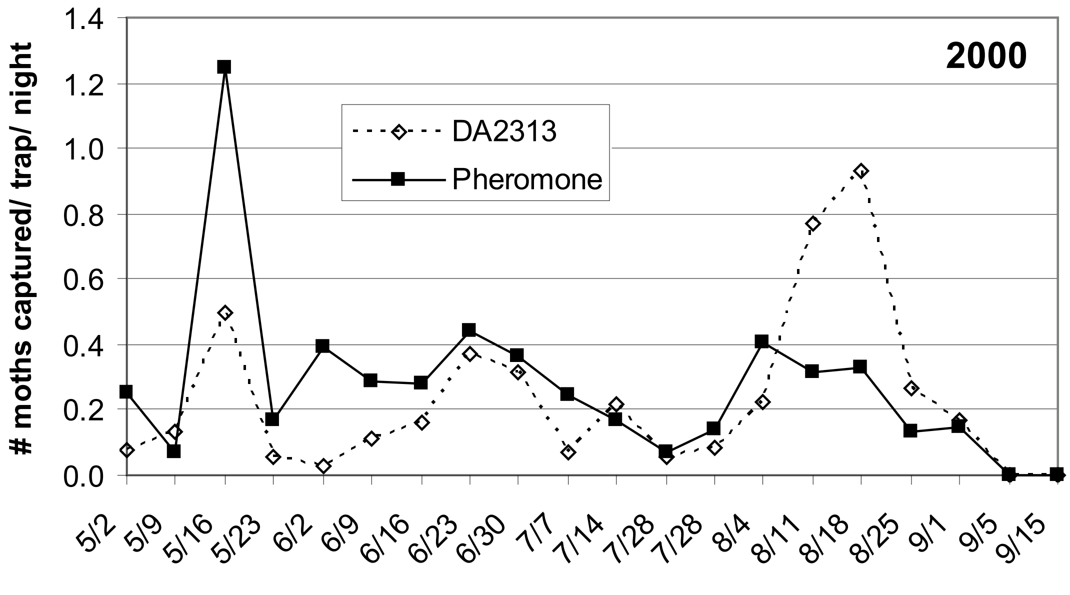


Fig. 2. Seasonal mean captures of codling moths in DA- and sex pheromone- baited traps within 57 apple orchards treated with MD in Brewster during 2001.

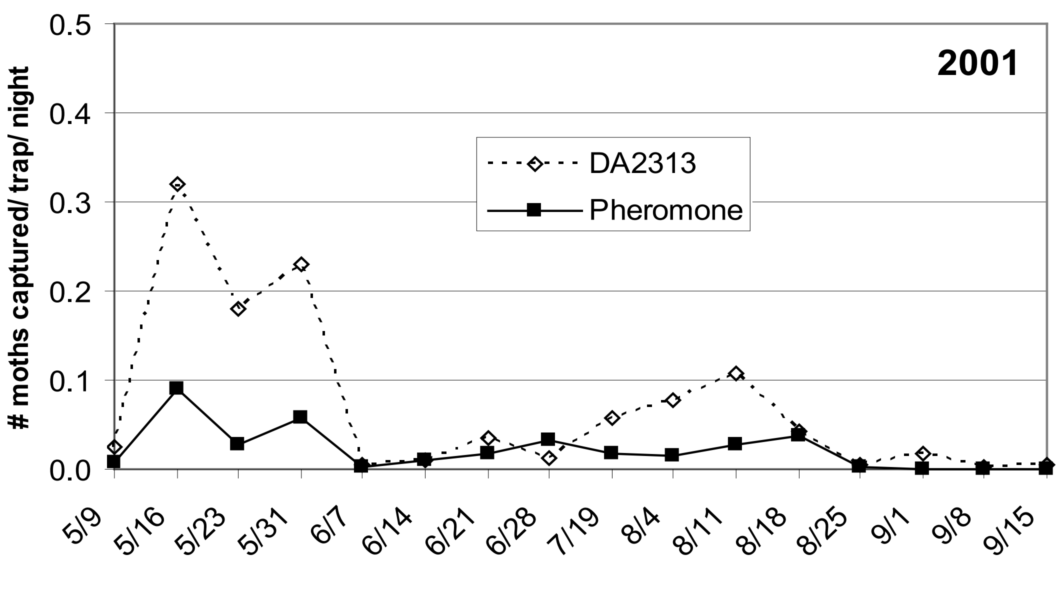


Fig. 3. Seasonal mean captures of codling moths in DA- and sex pheromone- baited traps within 30 apple orchards treated with MD in Brewster during 2002.

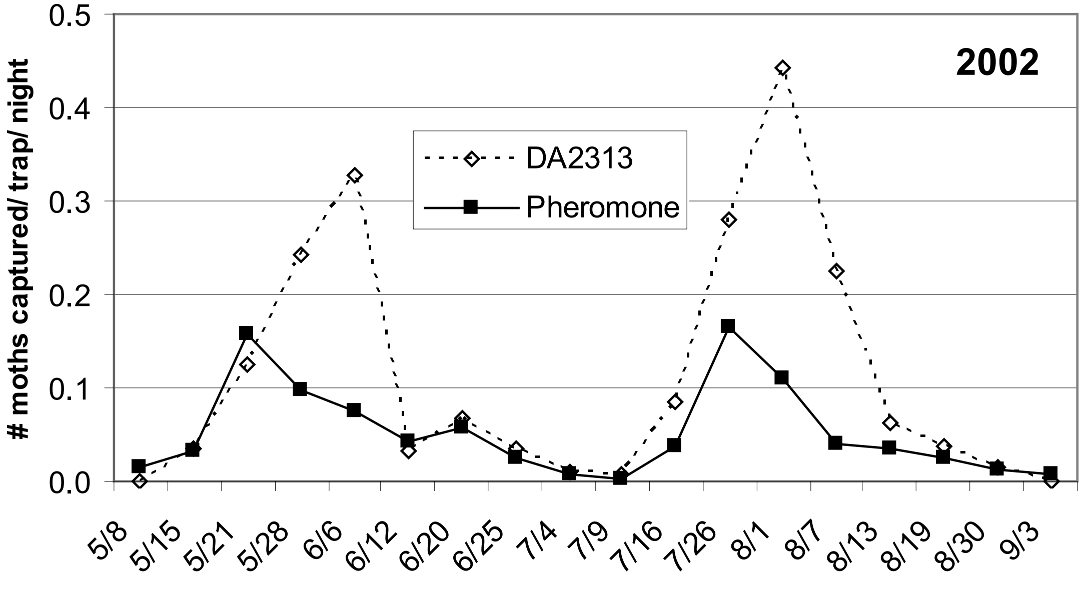


Fig. 4. Seasonal mean captures of codling moths in DA- and sex pheromone- baited traps within 10 pear orchards treated with MD in Brewster during 2001.

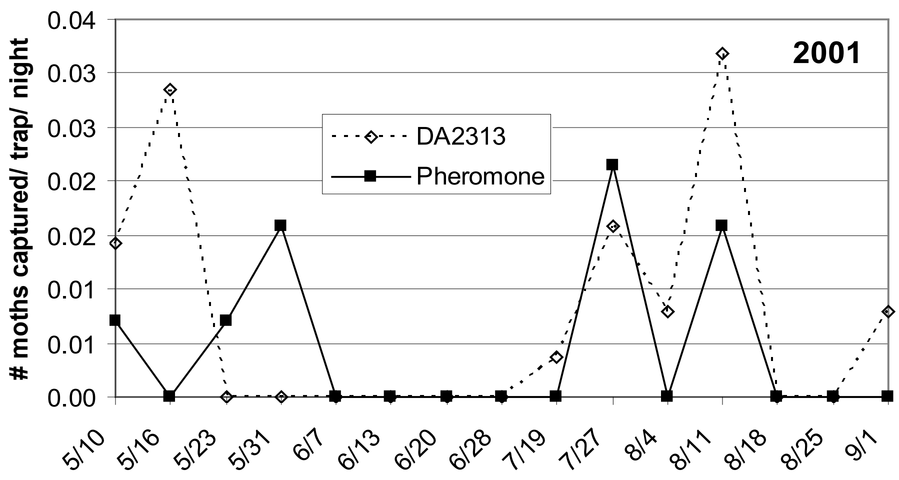


Fig. 5. Seasonal mean captures of codling moths in DA- and sex pheromone- baited traps within 10 pear orchards treated with MD in Brewster during 2002.

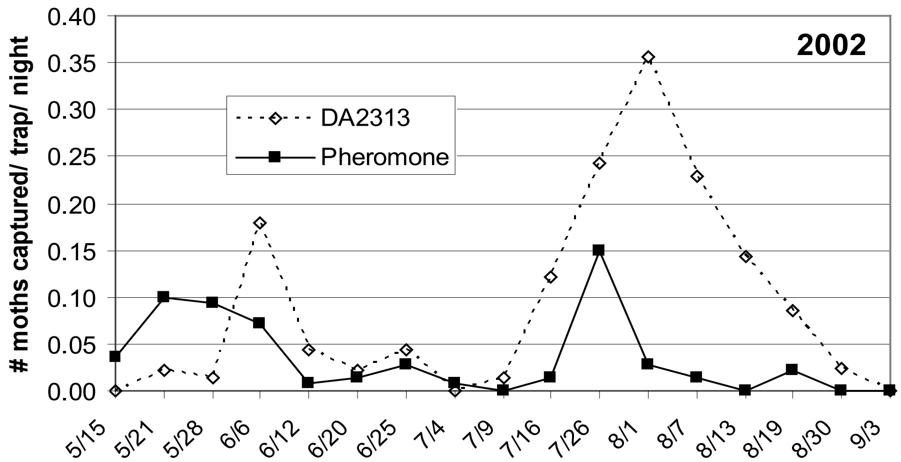
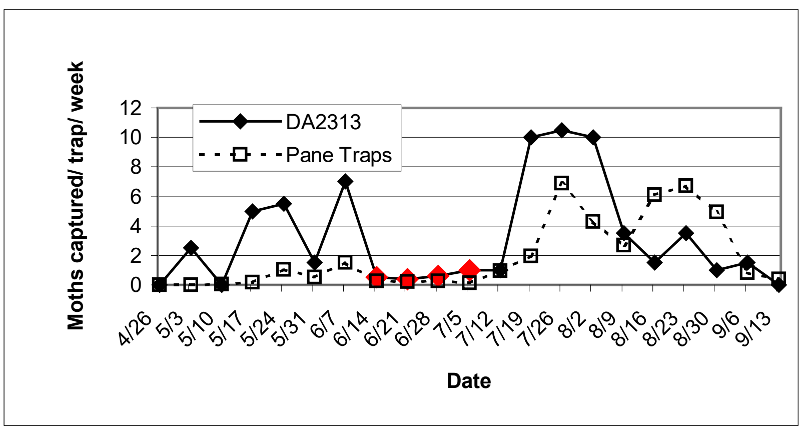


Fig. 6. Seasonal captures of codling moths in DA-baited and passive interception pane traps within an abandoned apple orchard, Wapato, WA 2002.



Budget Summary

Title: Bisexual monitoring of codling moth and establishing a female biofix
PI: Alan L. Knight
Project duration: 2000-2002
Project total: $77,000

Year	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Total	31,000	31,000	15,000

Current Year Breakdown

Item	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Wages	21,000	21,000	10,000
Benefits (10%)	2,100	2,100	1,000
Supplies	6,900	6,900	3,000
Travel (local)	1,000	1,000	1,000
Total	31,000	31,000	15,000

FINAL REPORT
Project #: AE-01-28

PROJECT TITLE:	Management of Codling Moth with a Bisexual 'Attract and Kill' Formulation
PI:	Alan L. Knight USDA, ARS, Wapato, WA
Co-PI:	Doug Light USDA, ARS, Albany, CA

SIGNIFICANT FINDINGS:

- An effective insecticide-treated bait station was developed.
- A range of studies were conducted to develop an improved trapping system for CM.
- Field trials using the technique of mass trapping males and females were only moderately effective in reducing fruit injury by CM.
- Field trials using insecticide-treated bait stations baited with the DA and pheromone lure reduced fruit injury 60-98% at mid-season and 30-60% at harvest.

OBJECTIVES:

1. Develop and test the use of mass trapping using a combination DA and sex pheromone lure for management of codling moth.
2. Develop and test the use of bait stations using a combination DA and sex pheromone lure for management of codling moth.

PROCEDURES:

Development of the Insecticide-treated Bait Station. Several synthetic pyrethroid insecticides were examined for their direct toxicity and repellency to adult CM. The sublethal effects of insecticide exposure on mating and fecundity was also examined.

Development of an Improved Trapping System. The effect of loading rate of DA on the number of female moths captured was examined in a replicated field trial. A new no-maintenance trap (C.A.P.T.) was designed and built for these studies. The efficacy of this trap was evaluated in flight tunnel tests and later in field trials. Alternative traps were examined and the use of fluon was evaluated to improve several non-saturating trap types. The effect of trap design on the number of female moths captured was examined.

Field Trials for Mass Trapping. Mass trapping studies were established in 12 orchard plots (0.7 – 1.5 ha) during 2002. Twenty-four traps per acre were deployed for the first generation. All traps were baited with a dual DA/sex pheromone lure. Fruit injury was assessed at mid-season and prior to harvest. Trap density was increased to 50-100 per acre for the second flight. Trap type was changed in most plots during the season as new information was gained on relative trapping efficiency. All plots were treated with 11 applications of 1.0% horticultural oil during the season.

Field trials with Insecticide-treated Bait Stations. Four 0.5-1.5 acre plots were established in both 2001 and 2002 using pesticide-treated delta traps at a density of 24 per acre. During 2002 trap density was increased to 50/acre in the second generation. Traps were coated with 1% esfenvalerate (0.18 :g per cm^2). Traps were retreated with insecticide every 2-3 wks all season. Fruit injury was assessed at mid-season and prior to harvest in all plots plus check plots.

RESULTS AND DISCUSSION:

Mass trapping. Table 1 shows that mass trapping of codling moth was not very effective in reducing fruit injury by codling moth. Studies were terminated in four orchards mid-season due to lack of control. Several factors likely contributed to this poor level of control. Unfortunately, once the season began I found that the C.A.P.T. trap did not catch many moths. We switched to Multipher traps in most plots in the second generation and increased trap density from 24 to 50 -100 per acre. Subsequently, we learned that Multipher traps catch a lower percentage of female codling moths than sticky traps. Replacing the 9010 lure with a combination of the Biolure 10X plus the DA lure increased the number of male moths captured by 4-fold, but this did not appear to improve the level of control. Fruit injury was reduced in only one of the thirteen plots (Table 1). This was the only plot where we used sticky traps (Pherocon IIB & IIC) for the entire season. The level of fruit injury reduction that we achieved in this plot was similar to results obtained directly by Trècè Inc. and by Doug Light whom also used similar sticky traps.

Table 1. Summary of three apple plots treated with mass trapping for control of codling moth. Data from 10 plots are not shown but were similar to Plot SW.

Plot # traps/ac	Flight	Trap / Lure	# males per tree	# females per tree	% injury	% reduc.
SE 24 - 50	1st 2nd	IIB / 9010 IIB / 9010	6.0 18.5	1.1 4.3	8.8 42.1	30 59
SW 24 - 100	1st 2nd	CAPT / 9010 Mult. / 9010	3.5 19.0	0.6 2.4	8.5 22.5	0 0
NW 24 - 100	1st 2nd	CAPT / 9010 Mult / Biolure	4.4 36.5	1.5 2.8	9.9 29.80	0 15

Insecticide-treated Bait Stations. We started the 2001 season with a 1-ft^2 flat station but switched to a delta station on 7 June. The entire trap was treated with esfenvalerate at a rate of 0.18 mg A.I./cm^2. Concurrent residual studies of trap surfaces suggested that traps would be effective killing stations for up to three weeks. Therefore the insecticide residue was renewed ca. every two weeks: 11 and 24 May, 7 and 19 June, 6 and 26 July, and 9 August. Unfortunately, only the outside of traps was retreated on 9 August and no further reapplications were made after this date. Initially we baited 25% of the traps with both DA and Pherocon L2 lures and the remainder with only DA. However, beginning in July all stations were baited with both lures. Four replicated plots were established in May with either 24 or 40 bait stations per acre in several heavily infested orchards under MD. However, in June it appeared that both treatments were effective and plots were rearranged to include only four 1-3 acre plots treated with only 24 stations per acre. Two of these plots received 11 oil applications (1% oil in 200 gallons of water) during the season (7 sprays during 1st flight and 4 sprays during 2nd flight).

Fruit injury at mid-season and pre-harvest is reported in Table 2. Fruit injury was dramatically reduced at mid-season but this success deteriorated during August. We were unclear on whether these poor late season results are solely due to our failure to maintain a toxic deposit on the traps during August or also due to a reduction in the attractiveness of the lures. A final problem with this study was that the plots were surrounded by trees with > 90% injury at mid-season and the effectiveness of the bait stations in removing this huge number of adults was likely inadequate.

Table 2. Percent codling moth fruit injury at mid-season and prior to harvest in 2001 within 1-3 acre plots treated either alone or combinations of MD, DA2313 baited, insecticide-treated stations, and 1.0% horticultural oil.

TrTreatment	**Mid-Season**		**Pre-harvest**	
	East	West	East	West
Untreated	75%	75%	90%	95%
MD only	15%	25%	60%	85%
MD + DA stations	1%	4%	30%	60%
MD + Oil	0.5%	3.0%	7.5%	17.3%
MD + DA stations + Oil	0.1%	0.3%	3.4%	7.5%

During 2002 the use of pesticide-treated traps ('lure and kill') appeared to be more successful than mass trapping, especially through mid-season (Table 3). Levels of fruit injury reductions at mid-season were somewhat lower than those achieved in 2001 except in the Pack plot. Reductions in fruit injury averaged ca. 40% at harvest which was similar to the 2001 results (Table 2).

Table 3. Summary of insecticide-impregnated bait station studies used to control codling moth in mating disrupted orchards in 2002.

Plot	1st Gen % fruit injury	% reduction from MD check	2nd gen. % fruit injury	% reduction from MD check
Ware N	3.9	50	24.4	33
Ware S	7.9	62	39.7	39
Office	5.4	81	16.3	46
Pack	0.1	98	8.3	44

The greater effectiveness of the 'lure & kill' approach versus mass trapping may be due to the inefficiencies of standard trapping. For example, I previously found that the larger delta trap used in 'lure & kill' caught 50% more moths than the Pherocon IIB. Additionally, using adhesive on both the top and inside of this trap increased moth catch by 200-300%. However, residual analysis showed that the loss of the trap's effectiveness to kill moths occurred much faster on the outside versus the inside of the trap. This was thought to be due to both degradation of the insecticide and accumulation of debris on the residue. Significant sublethal effects were found following brief exposure to aged insecticide residues such as reduced mating, longevity, and fecundity.

CONCLUSION:

Due to the direct feeding habit of codling moth growers cannot tolerate having ***any*** female moths laying eggs in their orchards. The DA-based attract and kill program appeared to be very effective during the first generation. However, several factors appear to currently limit the utility of using the DA lure to remove females and manage codling moth in commercial apple orchards. First, a very high proportion of female moths trapped in these orchards are already mated and have likely laid some eggs before capture. Mass trapping would work better in conjunction with an effective mating disruption program. Secondly, it is unfortunate that this situation is made worse by the bias for mated females seen with the DA lure. Third, the difficulty in trapping female moths with standard traps is an important factor. The greater effectiveness of the insecticide-treated traps is likely due to the trap's much larger surface area and the sublethal and lethal effects from insecticide exposure. Fourth, the expense of using a large number of traps and lures may be prohibitive. An alternative design has been hypothesized by Trécé Inc. that should make this approach cost effective. Finally, the effectiveness of the DA lure to remove a high proportion of female moths in the population appears to be limited, especially during the second generation. For example, we found that the capture of female moths in a

DA lure-baited trap during the first flight is equivalent to the captures on three passive interception traps. However, during the second flight, this drops to parity between traps. In comparison, a light trap is equivalent to only a single interception trap in the first generation (cooler temperatures limit the period of nocturnal moth flight), but its effectiveness increases to three traps in the second flight.

These data are promising and suggest several alternatives. First, the use of light traps could be an effective alternative to manage codling moth during the second generation. Currently, Ron Britt uses light traps in several hundred acres to supplement mating disruption. His program uses up to 4 traps and costs $100 – 200 per acre. Certification of this approach for organic growers can be achieved by using the kaolin powder instead of an insecticide strip in the traps to kill the moths. Perhaps the most effective program would be the use of DA-baited insecticide-treated traps during the first flight and light traps during the second flight. Second, efforts to reduce mating of females by either the use of higher dispenser densities or use of the pheromone-based attract & kill droplets should be evaluated. Third, to date all mass trapping studies have been conducted in Red, Goldens, and Fuji. Additional studies are needed in blocks of Granny Smith because the DA lure appears to be most attractive in this apple cultivar and in Anjou pear. Finally, efforts to improve the attractiveness of the bait would likely improve the performance of this approach. Recent reports and continued work by Dorn's and Witzgall's research groups plus the work by Landolt, have already suggested a few and may lead to additional kairomones that are more attractive alone or in combination with DA.

Budget Summary:
Title: Management of Codling Moth with a Bisexual 'Attract and Kill' Formulation
PI: Alan L. Knight
Project duration: 2000-2002.
Total project cost: $94,000

Year	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Total	24,000	34,000	36,000

Current Year Breakdown

Item	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Wages	16,000	21,000	22,000
Benefits (10%)	1,600	2,100	2,200
Supplies	5,400	8,900	10,800
Travel (local)	1,000	2,000	1,000
Total	24,000	34,000	36,000

FINAL REPORT
Project #: AE-01-27

PROJECT TITLE: Pheromones and Attraction Inhibitors for Codling Moth Mating Disruption

PI: Alan L. Knight USDA, ARS, Wapato

SIGNIFICANT FINDINGS:

- Polyethylene dispensers emitting an equilibrium blend of codlemone plus isomers were shown to be very effective in disrupting male CM orientation to virgin female-baited traps. The level of disruption achieved with these dispensers was significantly better than with Isomate C+ dispensers. These results were in contrast to our 1998 study where the disruption of 1.0 and 10.0 mg lure-baited traps was more effective in codlemone-treated than codlemone+isomer-treated plots. The isomeric blend was shown to be as effective as codlemone in reducing mating by CM in small plastic containers in laboratory bioassays.

- I conceived the idea of using aerosol puffers in a widely-spaced internal grid (I.-H.E.L.P.) (0.25 puffers per acre) to manage codling moth at a lower cost than the current dispenser technology. This idea was first tested in 1999 in a single orchard and these studies were subsequently expanded each year from 2000 to 2002.

- From 1999 to 2001 the I.-H.E.L.P. array of puffers were shown to be as effective as the use of hand-applied dispensers in 1200 acres of commercial orchards.

- During 2001 and 2002 I had formulated and successfully tested the use of puffers emitting 33% less pheromone than the standard machine in nearly 500 acres of commercial orchards.

During 2001, I conceived and successfully tested the idea of using a grid of screened cages of 100 Isomate C+ dispensers (MBA) (1.6 cages per acre) in 200 acres.

- During 2002, I developed the idea of the Pheromone Mop that was tested at a density of 1.6 to 4.0 mops per acre in 24 apple and three walnut orchards. Mops used either 100 Isomate C+ or 50 Isomate C-tt dispensers. Mops using the C-tt dispenser were as successful as the hand-applied dispensers in controlling CM. However, the C+ mops did not perform as well as the hand-applied dispensers.

Growers preferred the use of these I.-H.E.L.P. approaches to the standard hand-applied system due to greater ease and lower cost. Growers reported that the use of Mops in 2002 reduced their application cost by 35%.

OBJECTIVES:

1. Investigate whether any alternative chemical blend can be used to improve the performance of sex pheromone for mating disruption of CM.

2. Develop and test alternative strategies using sex pheromones to disrupt codling moth that might be less expensive and/or more effective.

PROCEDURES:

Testing of An Alternative Pheromone Blend: Replicated (n = 4) field studies were conducted within an apple orchard with dispensers emitting codlemone, codlemone + isomers, codlemone acetate, and codlemone + codlemone acetate (50:50) in 1998 and 1999. Plots were 0.25 ha and were separated by > 50 m. During 1998 plots were monitored with traps baited with 1.0 and 10.0 mg codlemone loaded lures. Two thousand sterilized moths were released into each plot weekly. During 1999 plots were monitored with 5 virgin female-baited traps plus three lure-baited traps (Isomate C dispenser) were spaced ca. 10-20 m apart. Sterilized CM moths were released across the entire 30 ha orchard all season at a rate of 1,000 moths per acre weekly. The experiment was repeated for four weeks from 15 July to 18 August 1999.

Laboratory studies were conducted with CM in plastic arenas to examine mating disruption. One hundred-microliter hexane-pheromone solutions were tested on filter paper in 1.6-liter containers using two loads of codlemone, isomerized codlemone, codlemone acetate, and a mixture of codlemone and codlemone acetate. Three virgin females plus five males were added to each plastic container just prior to the initiation of scotophase. Females were dissected the following day to determine their mating status.

Development of An Alternative Pheromone System: The use of aerosol emitters (puffers) was evaluated for codling moth in commercial orchards from 1999-2002 in the Brewster, WA area. Our initial studies evaluated the use of a grid of puffers spaced 50 m from the borders of the orchard and 100 m apart plus treating a 10 m perimeter with hand-applied dispensers. This grid approach was named I.-H.E.L.P. for the integration of several technologies and the use of high emission low point density pheromone sources. Puffers were provided by both Paramount Farms and Consep Inc and released 7.5 mg codlemone every 15-30 minutes for 12-24 hours per day.
The M.B.A. (Multi-unit Box Approach) and Pheromone Mop arrangements used 1.6 units per acre. These units were arranged in a grid starting 25 m from the border and spaced 50 m apart.

In all studies, comparison orchards treated with 200 Isomate C+ dispensers (Pacific Biocontrol, Vancouver, WA) per acre were established and similarly monitored. Orchards were paired based on their similar size, location, pest pressures, cultivar, ownership, and expected spray practices. From 1999 to 2001 all orchards were 40 acre in size. During 2002, orchards varied from 10 to 40 acres.

All orchards were monitored with Pherocon VI delta traps (1 trap per 5 acres) baited with a sex pheromone lure (Mega lure from 1999-2001 and Biolure 10X in 2002). Traps were placed near (<10 m) the perimeter of orchards. Traps were checked weekly, and liners were replaced as needed. Lures were replaced after 8 weeks. Spray records were obtained from the growers and field managers at the end of the season. Fruit injury was assessed just prior to harvest by sampling thirty fruit from twenty trees within each quadrant of the orchard (2,400 fruit sampled per orchard). An equal number of fruit were sampled from the interior and from the edge of each quadrant.

Changes in the weight of Isomate C+ and Isomate C-tt dispensers hung individually or situated within the center or on the outer rim of Pheromone Mops were evaluated during the 2002 season. Dispensers were first weighed on 29 April and again 149 days later on 25 September. Data are expressed as mg loss per d.

RESULTS AND DISCUSSION:
Testing of An Alternative Pheromone Blend: In 1998 the codlemone alone treatment provided the best disruption of lure-baited traps, but differences among treatments were not significant ($P = 0.15$). In contrast, during 1999 male moth capture was significantly higher in the codlemone acetate and the codlemone acetate:codlemone blend plots than with either codlemone or codlemone + isomers in the pheromone dispenser-baited traps (Table 1). Male capture by virgin female-baited traps was significantly lower in the codlemone + isomer plots than in all other treatments except the codlemone acetate plots. Mating was significantly reduced by both rates of codlemone and codlemone + isomers

compared with the check in the plastic arena bioassays (Table 2). Mating was significantly lower at the higher dose rate for both of these treatments and these two treatments did not differ.

These data are consistent with my work from 1994 and 1996 that showed that the codlemone + isomers can enhance mating disruption. The Z,E isomer has been shown to be an attraction inhibitor and the E,Z isomer has been shown to enhance attraction. Further studies are needed to evaluate the potential of this blend for disruption.

Table 1. Field tests comparing polyethylene dispensers releasing different blends of pheromone. Each treatment replicated in four 0.25 acre blocks.

Treatment	Proportion traps baited with a dispenser catching moths	Proportion of virgin female-baited traps catching moths
Check	0.78 (0.06)ab	0.18 (0.04)b
Codlemone	0.47 (0.15)a	0.10 (0.05)b
Codlemone + Isomers (63:37)	0.42 (0.21)a	0.01 (0.01)a
Codlemone acetate	0.89 (0.06)b	0.08 (0.04)ab
50:50 blend of codlemone + codlemone acetate	0.89 (0.07)b	0.15 (0.05)b

Table 2. Proportion of female codling moths mating in plastic crispers with various pheromone treatments. Three virgin females plus five males were placed in each container for 24 h.

Treatment		**1 µg dose**	**10 µg dose**
Check	0.88a		
Codlemone		0.60b	0.23c
Codlemone + Isomers (63:37)		0.67b	0.30c
Codlemone acetate		0.75ab	0.85a
50:50 blend of codlemone + codlemone acetate		0.70ab	0.55b

Development of An Alternative Pheromone System: I began the I.-H.E.L.P. project in 1998 to develop the use of aerosol puffers using a much lower density of emitters than originally recommended by Dr. Harry Shorey in California. My goal was to develop a program that would be cost effective when compared with the reduced rates of hand-applied dispensers commonly used in Washington. In 1998 we only treated a single 50-acre orchard with a 10 m-band of Isomate-C+ dispensers at the full rate around its perimeter and one puffer per hectare (2.5 acres) deployed internally. Puffers were placed in a grid beginning 50 m from the orchard's edge and spaced 100 m apart. All puffers released 7.5 mg of sex pheromone per puff. The cost of this program was $42 per acre. Control of codling moth in the first year was acceptable so we continued and expanded the project. During 1999 we compared the degree of trap shut-down achieved with the I.-H.E.L.P. approach versus both full and half rates of Isomate C+ using releases of sterile Canadian moths in replicated 40-acre orchards. During the season we evaluated several cycles of puffing (12 h vs. 24 h per day and every 15 or 30 min). The puffers performed similarly to both rates of Isomate C+ dispensers in these tests. We accepted the California protocol of releasing pheromone every 15 minutes for 12 h. Mark-recapture studies demonstrated that a puffer placed on the downwind edge of an apple orchard had an effective range of < 10 m for disruption, while a puffer placed on the upwind edge was effective for ca. 50 m. These data supported our deployment of an internal grid of puffers spaced 100 m apart.

Studies conducted during 1999 and 2000 suggested that the I.-H.E.L.P. use of puffers could be successful. During 2001 no significant difference was found between the standard hand-applied programs and the I.-H.E.L.P. programs ($P = 0.19$) (Table 3). In addition, we showed that the pheromone emission rate from puffers could be reduced to 5.0 mg per puff and still be effective. This was again demonstrated in 2002 with a new puffer machine in seven orchards. Phytotoxic problems associated with puffer use were prevalent but considered to be minor problems. Mechanical problems with puffers occurred every year and have slowed the adoption of this technology. The manufacturer (Suterra) is currently redesigning their units for the 2003 season.

We expanded our study in 2001 to include a passive system of dispensing high rates of pheromone from a low number of point sources due both to continuing mechanical problems with the aerosol puffers and concern that a closer array of high emission point sources would be more effective. The concept of a passive high-emission point source such as the M.B.A. was found to be effective in five orchards during 2001 (Table 3).

Table 3. Comparison of five I.-H.E.L.P. strategies (5 orchards and 200 acres per treatment) paired against similar orchards treated with Isomate C+ dispensers (200 dispensers per acre) in the Brewster area during 2001.

Treatments	**Monitoring** (moths/trap/season)			**Sprays**		**%** **fruit injury**	
	Mega	***DA***	***OBLR***	***CM***	***OBLR***	***CM***	***OBLR***
M.B.A.	**0.53**	**2.60**	**-**	**0.8 border**	**-**	**0.00**	**-**
Isomate C+	**0.55**	**2.50**	**-**	**1 border**	**-**	**0.14**	**-**
Paramount CM puffers	**0.80**	**-**	**-**	**½ border**	**-**	**0.02**	**-**
Isomate C+	**-**	**-**	**-**	**1 border**	**-**	**0.12**	**-**
Consep CM puffers	**1.5**	**1.5**	**-**	**½ cover**	**-**	**0.01**	**-**
Isomate C+	**-**	**-**	**-**	**½ cover + 1 border**	**-**	**0.16**	**-**
Paramount Low-CM/LR	**4.25**	**-**	**30.1**	**1 cover + ½ border**	**1 cover**	**0.20**	**0.18**
Isomate C+	**-**	**-**	**-**	**2 covers + 1 border**	**1 cover**	**0.54**	**0.43**
Paramount CM/LR	**2.2**	**2.8**	**37.3**	**½ cover + 1.5 borders**	**1 cover**	**0.12**	**0.09**
Isomate C+	**-**	**-**	**-**	**½ cover + 1.5 borders**	**1 cover**	**0.29**	**0.11**

The subsequent development of the Pheromone Mop further reduced the cost of this passive approach and they are disposable. During 2002 the C-tt Mops performed similarly to the use of hand-applied dispensers (Table 4). However, fruit injury was significantly higher in the C+ Mop-treated orchards than in the hand-applied orchards. We also found that the C+ dispensers released significantly less pheromone when placed in the Mop versus dispensers clipped individually on branches (Table 5). No similar effect was seen with the C-tt dispensers in mops.

A major difference in our approach with I.-H.E.L.P. during 2002 versus the previous three seasons was in the selection of orchards. Previously, all studies were conducted in replicated 40+ acre blocks, generally within large contiguous areas under mating disruption. In comparison, this year Pheromone Mops and puffers were tested in primarily 10-20 acre orchards that were distant from larger blocks under mating disruption. The relative success of the I.-H.E.L.P. approach in these smaller, more isolated blocks is encouraging.

Several other groups also tested the Pheromone Mop during 2002. Pacific Biocontrol evaluated this approach in several orchards and reportedly had no problems. Joe Grant (UC Farm Advisor) also tested the Pheromone Mop at a higher density (4 mops per acre) in two walnut sites in California. Grant reported that the Mop performed as well as the standard pheromone treatment of 400 dispensers per acre. Currently, the C-tt dispenser is being tested in Australia in Pheromone Shields™ using the I.-H.E.L.P. approach.

Table 4. Summary of I.-H.E.L.P. studies conducted during 2002.

Treatment (# orchards)	Mean Moth Catch (SEM)	Mean % Injury (SEM)	Mean # Cover Sprays (SEM)
5.0 mg Puffers, n=7	6.89 (1.94)	0.11 (0.03)	1.75 (0.31)
C-Plus	7.82 (3.63)	0.08 (0.07)	1.36 (0.39)
t-tests, p-value	*0.81*	*0.58*	*0.64*
P-Mops-tt, n = 11	8.42 (2.71)	0.15 (0.08)	1.49 (0.51)
C-Plus	10.13 (3.62)	0.14 (0.06)	2.03 (0.52)
t-tests, p-value	*0.49*	*0.87*	*0.11*
P-Mops-C+, n=13	10.72 (2.89)	0.14 (0.06)	1.61 (0.42)
C-Plus	6.46 (2.07)	0.05 (0.03)	1.83 (0.44)
t-tests, p-value	*0.14*	*0.02 ***	*0.80*

Table 5. Summary of weight loss of dispensers (mg per d) placed in the field on 29 April and re-weighed on 25 September.

Dispenser	Indiv. dispenser	Center of Mop	Outer Rim of Mop	P-value
Isomate C-tt	1.75	1.85	1.89	*0.51*
Isomate C+	0.68a	0.56b	0.53b	*0.003***

CONCLUSION:

Studies have consistently shown that the use of dispensers emitting an isomerized blend of codlemone and its geometrical isomers can improve the disruption of male attraction by virgin females. The mechanism(s) for this approach are not well understood and this approach will require additional testing to develop commercial products.

The major objective for implementing I.-H.E.L.P. strategies with codling moth sex pheromone in tree fruits is to develop less expensive, and equally or more effective management programs. Results from 1999-2002 suggest that the use of widely separated pheromone sources can be used to effectively manage codling moth. Both the mechanical puffers and the passive grouping of dispensers to create a low-density grid of point sources releasing higher levels of pheromone appear to be valid approaches. The I.-H.E.L.P. strategy utilizes a full rate of hand-applied pheromone dispensers on the perimeter of orchards, where codling moth is typically more of a threat. The success in using puffers or Mops in an internal grid may benefit from the typically lower codling moth populations in the interior of most orchards.

Budget:

Title: Pheromones and Attraction Inhibitors for Codling Moth Mating Disruption
PI: Alan Knight
Project duration: 1998-2002
Project Total: $178,600

Year	Year 1 (1998)	Year 2 (1999)	Year 3 (2000)	Year 4 (2001)	Year 5 (2002)
Total	38,000	38,000	36,600	35,000	31,000

FINAL REPORT
WTFRC Project # AE-01-33

Project Title: Development of feeding attractants for control of moths pests of apple.
PI: Peter J. Landolt
Organization: USDA, ARS, Yakima Agricultural Research Laboratory, Yakima, WA
Cooperator(s): Jay Brunner and Mike Doerr, WSU, TFREC, Wenatchee, Washington, Everett Mitchell (deceased 2002), USDA, ARS, Gainesville, Florida

Objectives:
1. Develop formulation for a bait station, to incorporate an attractant, a feeding stimulant, and toxicant.
2. Verify attractant release rates and moth attraction, contact, and mortality in response to bait station.
3. Develop bait station visual characteristics.
4. Conduct flight tunnel, field cage, and field tests with a bait station model.

Significant Findings:
1. A bait station design was used that includes a controlled release dispenser for the feeding attractant (acetic acid and 3-methyl-1-butanol), a permethrin formulation (Teflon grease), and a badminton birdie or shuttlecock as the target (artificial flower visual design).
2. Numbers of male and female Lacanobia fruitworm moths were significantly reduced in apple orchards with bait stations with a feeding attractant as the lure.
3. Reductions in moth numbers can be accomplished with relatively low rates of bait stations per acre (50).
4. Most female moths responding to the lure have not yet laid most of their eggs, supporting the idea that bait stations to kill females will reduce oviposition.

METHODS:
Bait Stations: Bait stations were made of a combination of a physical target, a chemical attractant in a controlled release dispenser, and a toxicant in a suitable formulation. The target in this case was a white plastic badminton birdie or shuttlecock. Controlled release dispensers were 8 ml polypropylene vials, each loaded with 5 ml of a 50:50 mixture of acetic acid and 3-methyl-1-butanol (isoamyl alcohol), onto cotton balls within the vial. Three mm diameter holes were drilled into the vial lids for release of the attractant. The vial was glued into the base of the shuttlecock, replacing the rubber cap which was removed from the shuttlecock. The inside of the shuttlecock was coated with a Teflon grease which contained 6% by weight technical grade permethrin. Bait stations (shuttlecock with lure and coated with permethrin in Teflon) were suspended by wire hangers from branches on apple trees, in the upper half of the canopy.

Bait station efficacy was evaluated with a flight tunnel assay and a contact assay. In the flight tunnel, Lacanobia fruitworm moths were tested for attraction to, contact with, and mortality following contact, after exposure for 2 minutes to a bait station. Moths were captured after 2 minutes in the flight tunnel and were held in vials to determine mortality rates. In the contact assay, used on shuttlecocks exposed in the field, moths held with forceps were lightly touched to the shuttlecock and then held in vials to determine mortality rates in comparison to untreated moths.

Plot monitoring.: Orchards were selected to provide space for four 5 acre plots, with all plots of the same variety and similar canopy structure. Each plot was monitored with one blacklight trap, two pheromone traps, and two feeding attractant traps. Blacklight traps possessed 8 watt blacklight bulbs run off of 12 volt batteries. Pheromone traps were Agrisense Universal Moth Traps (green lid, yellow cone, white bucket), baited with one mg of a three component sex pheromone applied to a rubber septum. Pheromone lures were placed in a small plastic basket at the center of the inside of the trap top. A one inch square piece of Vaportape was placed within each bucket to kill trapped moths. These same traps were used as feed attractant traps, but were baited with 8 ml vials of acetic acid and 3-methyl-1-butanol with 3 mm diameter holes in the lids. Vials were suspended within the bucket of each trap with a thin wire. When traps were checked, all contents were placed in pre-labled ziplock plastic bags for transport to the lab, and were stored in a freezer until moths were sorted and identified under a dissecting microscope. All female Lacanobia fruitworm and spotted cutworm moths were dissected to determine their reproductive status (mating and egg development).

<u>Experiment 1: Bait Station Density</u>. Five-acre plots in apple orchards were monitored starting at the beginning of the *Lacanobia* flight in mid-May 2002. Where and when trap captures were high enough (several moths per trap per day), experiments were begun. The four treatments were 0, 2, 10, and 50 bait stations per acre. Plots were monitored daily for 6 days prior to bait station deployment and daily for 6 days after bait station deployment. Three replicates of this experiment were conducted from late May into early June, while an additional three replicates of the same experimental design were conducted from mid to late June. Orchards used in the 4th to 6th replicates were not the same as orchards used in the 1st to 3rd replicates. Statistical comparisons were made of numbers of Lacanobia moths captured in traps before and after bait station deployment, to determine if numbers of moths in those plots were reduced with bait stations. Additionally, numbers of moths captured after bait station deployment were compared between treatments to determine if there were significantly fewer Lacanobia moths captured in plots treated with bait stations, compared to untreated controls.

<u>Experiment 2: Efficacy of Bait Station Deployment</u>. Five-acre plots in apple orchards were monitored as described above, beginning the first week of August 2002. When trap catches were high enough experiments were begun. This experiment provided comparisons of unbaited plots with plots treated with 50 bait stations per acre. Plots were paired to provide the same apple variety and canopy structure for both treatment and control. Plots were monitored daily for 6 days before bait station deployment, daily for 6 days after bait station deployment, and then intermittently until mid. In early to mid September, depending on variety and other activities in orchards, trees were sampled for larvae, using a beating technique. A white bedsheet was placed on one side of the tree, starting at the trunk, and all limbs above the sheet were knocked with a pole to attempt to dislodge caterpillars on limbs and foliage. Larvae on the sheet were collected into a paper cup with apple leave and were transported to the laboratory for identification. Early instar larvae were reared to third instar for final identification as to species.

Experiment 3. Evaluation of pheromone baited and feeding attractant bait stations. This experiment was not conducted due to a lack of time. Because of difficulties with early season weather contributing to variance in the data, it was decided to repeat the first experiment during the latter half of the first Lacanobia flight rather than conduct this experiment, which was scheduled for that time period.

Results and Discussion:

A total of 48 moths were tested to the final bait station design in the flight tunnel. 22 were attracted, 20 contacted the bait station, and 18 died in the assay. The other two were paralyzed During field tests, bait stations were brought in from the field and tested with the contact assay after 6 days exposure and after 3 weeks exposure. Mortality of Lacanobia moths that were touched to bait stations was 80% and 68% for bait stations that were 6 days and 3 weeks old respectively, with the majority of the remainder paralyzed.

Bait Station Density.

Data from monitoring traps are summarized below in Table 1.

Numbers of male and numbers of female Lacanobia fruitworm moths that were captured in traps baited with the feeding attractant were reduced in plots for the 6 day period following treatment (bait station deployment) compared to the 6 day period before treatment, for the 0, 2, 10 and 50 bait stations per acre treatments. For the 6 day period of monitoring following bait station deployment (post-bait), numbers of male and numbers of female Lacanobia fruitworm moths in traps baited with the feeding attractant were significantly reduced in plots with 50 bait stations per acre, compared to untreated plots.

The reductions in captures of moths from pre to post-baiting appear to be due to changes in weather, and are not attributable to the deployment of bait stations. Experiments were begun when a threshold of several moths per trap per day was reached, which probably was when night time temperatures were relatively warm. The warm weather in May and June did not last longer than the five day pre-baiting period, and cooler weather prevailed for the post-baiting period. However, the post-baiting reductions in moths in traps in treated plots (50 bait stations per acre) compared to control plots can be attributed to the deployment of bait stations and supports the hypothesis that numbers of Lacanobia fruitworm moths in plots were reduced by the bait stations.

Numbers of male and numbers of female Lacanobia fruitworm moths that were captured in blacklight traps were also reduced in plots for the 6 day period following treatment (bait station deployment) compared to the 6 day period before treatment, for most treatments as well as the controls. For the 6 day period of monitoring following bait station deployment (post-bait), numbers of male and numbers of female Lacanobia fruitworm moths in blacklight traps were significantly reduced in plots with 50 bait stations per acre, compared to untreated plots.

These data support the discussion above, that bait station deployment reduced the presence of Lacanobia in plots with 50 bait stations per acre, but that there was a strong reduction in moth activity following the dates of baiting in all plots, probably in response to changes in weather. It is encouraging that similar results were obtained with blacklight and feeding attractant traps, because a reduction in captures of moths in feeding attractant traps might be interpreted as evidence of disruption of moth ability to find lures in baited plots, rather than evidence of mortality of moths at bait stations. It is less likely that odors from lures of bait stations would effect moth response to blacklight.

Numbers of male Lacanobia moths captured in pheromone traps were reduced during the post baiting period compared to the pre-baiting period, in control and treated plots. During the 6 day post-baiting period, numbers of males in baited plots (2, 10, or 50 bait stations per acre) were not significantly different than in unbaited plots.

It is generally assumed that male response to female sex pheromone involves much greater distances than moth responses to either blacklight or feeding attractant. Also, males are more likely to be wide-ranging in their search for mates, compared to moths searching for food. Thus, it is not surprising that the any mortality of males from bait stations in 5 acre plots did not significantly impact numbers of males in pheromone traps.

Table 1. Numbers of Lacanobia fruitworm moths captured in monitoring traps in 5 acre apple plots treated with different numbers of bait stations. May-June 2002. Monitoring traps were baited with acetic acid and isoamyl alcohol (AAIAA), blacklight, or sex pheromone lure (Pheromone).

	Bait Stations per Acre			
	0	2	10	50
AAIAA				
female prebait	0.63 ± 0.15	0.79 ± 0.17	0.64 ± 0.15	2.15 ± 0.56
female postbait	0.22 ± 0.06a	0.25 ± 0.07a	0.21 ± 0.07a	0.04 ± 0.02b
male prebait	0.34 ± 0.09	0.49 ± 0.12	0.45 ± 0.13	1.21 ± 0.26
male postbait	0.11 ± 0.04a	0.15 ± 0.05a	0.13 ± 0.05a	0.06 ± 0.03b
Blacklight				
female prebait	0.24 ± 0.11	0.47 ± 0.29	0.35 ± 0.28	0.63 ± 0.24
female postbait	0.14 ± 0.07a	0.13 ± 0.07a	0.09 ± 0.07a	0.03 ± 0.03b
male prebait	0.13 ± 0.06	0.45 ± 0.32	0.18 ± 0.17	0.63 ± 0.21
male postbait	0.14 ± 0.07a	0.21 ± 0.13a	0.06 ± 0.04b	0.06 ± 0.04b
Pheromone				
male prebait	10.23 ± 1.97	6.71 ± 1.15	8.59 ± 1.47	10.21 ± 1.49
male postbait	5.08 ± 0.96a	2.79 ± 0.44a	3.96 ± 0.66a	4.38 ± 0.71a

Means within a row followed by the same letter are not significantly different at $p < 0.05$ by an lsd test.

Efficacy of Bait Station Deployment

Data from monitoring traps are summarized below in Table 2.

In plots treated with bait stations, numbers of female Lacanobia fruitworm moths that were captured in traps baited with the feeding attractant were significantly reduced during the 6 day period following baiting compared to the 6 day period before baiting. Numbers of males in those plots were numerically but not significantly lower following baiting. For the 6 day monitoring period following baiting, numbers of male and numbers of female Lacanobia in feeding attractant traps were significantly lower in treated plots compared to control plots.

In plots treated with bait stations, numbers of female Lacanobia captured in blacklight traps were significantly reduced during the 6 days after baiting compared to the 6 days before baiting. Numbers of males in those plots were numerically but not significantly lower following baiting. For the 6 day monitoring period following baiting, numbers of male and numbers of female Lacanobia in blacklight traps were significantly lower in treated plots compared to control plots.

Numbers of male Lacanobia in traps baited with pheromone were significantly reduced in baited plots compared to control plots during the 6 day post baiting period. Male numbers were not significantly reduced in baited plots during the post baiting period compared to the pre-baiting period.

This trap catch data supports the hypothesis that the bait stations deployed at 50 per acre reduced populations of Lacanobia fruitworm moths in plots, particularly numbers of females. Numbers of female Lacanobia moths captured in feeding attractant traps were reduced by about 3/4 while numbers in blacklight traps were reduced about ½.

Numbers of Lacanobia larvae collected were not sufficient for statistical analysis. However, all larvae collected were in unbaited plots.

Table 2. Numbers of Lacanobia fruitworm moths captured in monitoring traps in 5 acre apple plots that were treated with 50 bait stations per acre or were not treated (control). August-September 2002. Monitoring traps were baited with acetic acid and isoamyl alcohol (AAIAA), blacklight, or sex pheromone lure (Pheromone).

	Bait Stations per Acre 0	50
AAIAA		
female prebait	0.74 $\pm$ 0.19ax	0.76 $\pm$ 0.14bx
female postbait	1.35 $\pm$ 0.23ax	0.32 $\pm$ 0.07by
male prebait	0.65 $\pm$ 0.17ax	0.69 $\pm$ 0.16ax
male postbait	1.10 $\pm$ 0.17ax	0.44 $\pm$ 0.12bx
Blacklight		
female prebait	3.36 $\pm$ 0.45ax	2.79 $\pm$ 0.78ax
female postbait	2.08 $\pm$ 0.32ax	1.14 $\pm$ 0.21by
male prebait	16.91 $\pm$ 3.53ax	6.00 $\pm$ 2.06ax
male postbait	11.94 $\pm$ 2.68ax	3.31 $\pm$ 0.78bx
Pheromone		
male prebait	72.79 $\pm$ 6.77ax	49.78 $\pm$ 6.37ax
male postbait	59.34 $\pm$ 5.38ax	36.10 $\pm$ 2.66by

Means within a row followed by the same letter (a or b only) are not significantly different at $p< 0.05$ by an lsd test. Means within a column pair followed by the same letter (or or y only) are not significantly different at $p < 0.05$ by an lsd test.

These results provide a possible means of reducing populations of Lacanobia fruitworm below damaging levels without using cover sprays of insecticides. Advantages of such a bait station method are 1) minimal or no contact between pesticide and the commodity, 2) minimal contact between the pesticide and beneficial arthropods, 3) reduced contact between pesticide and workers. Costs of such a system are as yet unknown. Further work needs to be done to determine if reductions in numbers of female moths in orchards is directly related to reductions in reproduction and then in larvae on apple trees in the next generation. Costs of materials for the bait station are low (birdie, grease, pesticide, attractant, vial, wire) but can certainly be reduced.

Project Title: Development of bait stations for control of Lacanobia fruitworm.
Project Total (3 years): $84,313

Year	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Total	$32,213	$25,000	$27,000
Current year breakdown			
Item	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Salaries	$20,800	$22,000	$19,500
Benefits	6,930		2,500
Equipment			
Supplies	4,000	3,000	4,000
Travel	500		1,000
Total	$32,213	$25,000	$27,000

Additional support was provided in 2001 and 2002 by IFAFS/RAMP ($30,165 per annum).

FINAL REPORT

Project Title: Optimization of chemical attractants for the spotted cutworm
PI: Peter J. Landolt
Organization: USDA, ARS, Yakima Agricultural Research Laboratory, Wapato, WA

Objectives:
1. Determine the best blend and dose of sex pheromone components for the spotted cutworm.
2. Document non-target moths captured in traps baited with spotted cutworm pheromone.
3. Determine the best blend of floral compounds for trapping spotted cutworm moths.

Significant findings:
1. Chemical analyses of female pheromone glands showed the consistent presence of three compounds: (Z)-7-tetradecenyl acetate, (Z)-5-tetradecenyl acetae, and (Z)-7-tetradecenol, with (Z)-7-tetradecenyl acetate the major component.
2. Field tests of pheromone compounds indicated co-attractiveness of (Z)-5-tetradecenyl acetate and (Z)-7-tetradecenyl acetate, and inhibitory effects of (Z)-7-tetradecenol.
3.Field tests of single, two and multi-component blends of floral chemicals did not indicate attractiveness of any material comparable to that already attained with acetic acid and 3-methyl-1-butanol. Weak attractiveness to phenylacetaldehyde was demonstrated.

Methods:
Eggs were obtained from female spotted cutworms captured in a light trap at the USDA. Larvae were reared on a standard pinto-bean based diet for noctuid moths to obtain unmated females in the laboratory. Abdominal tips including the pheromone gland were cut and extracted in organic solvent, then analyzed by GC-MS to obtain preliminary identifications of compounds. Structures were confirmed by comparison to synthetic standards, both using spectroscopic data and GC retention times on polar and non-polar columns.

Pheromone chemicals tested were formulated in pre-extracted red rubber septa at one milligram loads, and placed in Universal moth traps in apple orchards for evaluation of attractiveness. Three experiments were conducted with pheromone chemicals. The first compared single chemicals found in gland extract to two and the three component blend to determine the importance of each chemical found to attractiveness. The second and third tests were evaluations of ratios of the two active components, to determine the significance of the relative amounts of chemicals in the lures.

Floral chemicals evaluated were based on two USDA patented floral lures and included phenylacetaldehyde, methyl jasmonate, linalool, limonene, methyl-2-methoxy benzoate, 2-phenylethanol, and methyl salicylate. These chemicals were loaded in 8 ml vials with 6 mm diameter holes in the lids. Vials were placed in the bottom of Universal moth traps for field testing in apple orchards. Three tests were conducted; 1) each chemical tested singly in comparison to an unbaited trap, 2) two component blends made up of phenylacetaldehyde and each of the other chemicals, and 3) the three and 5 component blends of the two floral blends (from honeysuckle and Gaura flowers).

Results and Discussion:

Analyses of female abdominal glands indicated the presence of three compounds that are similar to moth sex pheromone chemicals. These are (Z)-7-tetradecenyl acetate, (Z)-5-tetradecenyl acetate, and (Z)-7-tetradecenol. Ratios of these three chemicals in gland extracts were about 100 to 1 to 1 for the three chemicals respectively.

Field tests indicated some attractiveness of (Z)-7-tetradecenyl acetate alone and much stronger attractiveness of the two component combination of (Z)-7-tetradecenyl acetate and (Z)-5-

tetradecenyl acetate. The addition of (Z)-7-tetradecenol appeared to reduce attractiveness of those two compounds.

Table 1. Numbers of male spotted cutworm moths captured in Universal moth traps baited with different pheromone lures.

Chemicals tested	Mean $\pm$ SE males captured
No chemicals	0.00 $\pm$ 0.00
Z-7-14:AC	0.55 $\pm$ 0.14
Z-7-14:AC/Z-5-14AC	9.80 $\pm$ 2.62
Z-7-14:AC/Z-7-14:OH	0.20 $\pm$ 0.12
Z-7-14AC/Z-5-14:AC/Z-7-14:OH	2.10 $\pm$ 0.51

Further evaluation of the two component pheromone, (Z)-7-tetradecenyl acetate and (Z)-5-tetradecenyl acetate, at different ratios, indicated an optimum response at a ratio of 9 to 1, although there was not much variation in the responses over the range of ratios tested (Table 2).

Table 2. Numbers of male spotted cutworm moths captured in Universal moth traps baited with different ratios of Z-7-14AC/Z-5-14:AC lures, at a 1 mg load per septum.

Ratio of Z-7-14AC/Z-5-14:AC	Mean $\pm$ SE males captured
100 to 0	0.25 $\pm$ 0.18
100 to 0.3	2.65 $\pm$ 1.19
100 to 1	3.75 $\pm$ 0.80
100 to 3	3.20 $\pm$ 0.79
100 to 10	4.55 $\pm$ 1.18

Testing of single component floral lures indicated only very weak attraction to phenylacetaldehyde, and no significant response to other chemicals tested. Addition of each of the other floral compounds to phenylacetaldehyde did not significantly increase numbers of spotted cutworm moths trapped over what was obtained with phenylacetaldehyde alone.

Results of this work provide recommendations for the chemicals to be used for formulating pheromone lures for the spotted cutworm, as well as lure component loads. Such lures might be used as a means of monitoring seasonal phenology or emergence of spotted cutworm in orchards.

Budget:
Project Title: Optimization of chemical attractants for the spotted cutworm.
PI: Peter J. Landolt
Project duration: 2002
Project total: $6,000

FINAL REPORT

Project Title: Leafroller Biological Control

PI: Tom Unruh
Organization: USDA-ARS Yakima

CO-PIs Jay Brunner, WSU-TFREC, Wenatchee
Robert Pfannenstiel, USDA-ARS, Yakima

Cooperator(s): Fred Plath, Jim Doornink, Dave Alan

OBJECTIVES:
1) Complete demonstration that habitats can be modified on a functional scale to produce an orchard-wide and significant increase in parasitism of leafrollers
2) Determine the importance of parasitism versus predation in organic versus conventionally managed (=mating disrupted) orchards.
3) Complete evaluation of horticultural methods to deploy strawberries and roses in or near orchards.

Significant findings in 1999-2002

< Leafroller parasitism is typically very low in the spring generation, increasing to as much as 50% in the summer generation in typical orchards (1999-2000).

< Parasitism in a ca. 1000 hectare area near Wapato WA was dominated by Tachinidae, followed assorted parasitoids including *Oncaphanes americanum, Apantales polychrosidus*, and the exotic wasp, *Colpoclypeus florus*. Of these, *Colpoclypeus* and the other wasps showed higher parasitism near riparian habitats (1999-2000)

< Surveys of leafrollers and their parasitoids in natural habitats showed that *Colpoclypeus florus* used our pest leafrollers on various wild plants in the summer generations and used *Ancylis comptana* on rose, *Syndemis spp*. on Red Ozier Dogwood, and *Filatema spp*. on cottonwood as overwintering hosts (1999-2000).

< Use of organophosphate and other strident insecticides reduced parasitism dramatically (1999-2000).

< Parasitism by *C. florus* was high in the summer generation in orchards near a naturally occurring rose patch in Wenatchee and was high in both spring and summer generations in orchards near a rose patch in Yakima (1999-2002).

< Survival and exclusion cage studies show that predators and unknown mortality suppress leafrollers by about 80 to 90 percent and parasitoids, especially *C. florus*, eliminated 80-90% of the survivors after predation (2001).

< *C. florus* shows high activity and broad-scale dispersal in fall seeking overwintering hosts as assessed from high parasitism of sentinel hosts placed throughout orchards and in grassland habitats surrounding orchards (2002).

< 2001 and 2002 assessment of parasitism by C. florus in orchards showed high parasitism near gardens of strawberry and rose planted in 2000 demonstrating that this alternate host/overwintering habitat could be manipulated. (2001-2002)

Results and Discussion
In 1992 Jay Brunner at the Tree Fruit Research and Extension Center in Wenatchee found the parasite *Colpoclypeus florus* parasitizing leafrollers in an orchard near the station. This tiny wasp was

collected in Italy and introduced by Agriculture Canada into the Ontario area to control Red-banded leafroller in 1968. We believe it spread on its own to Washington. *C. florus* is found throughout Europe and is often the most important parasite of as many as 9 leafroller pests there. By 1996 Jay's lab including his post doctoral associate, Bob Pfannenstiel, were mass rearing and releasing *C. florus* in an effort to increase its distribution in Washington and to determine the impact it could have on *Pandemis* and OBLR. They observed that it caused high parasitism of leafrollers in the summer generation in some orchards. They also observed that the wasp parasitized very few leafrollers in the spring generation of larvae, similar to its biology in Europe. Both of our pest leafrollers and most pest leafrollers in Europe spend the winter as tiny larvae hidden in bark crevices and bud scales or as egg masses. *C. florus* over winters on its host and requires a large larvae on which to do this. The mystery in Europe and now in Washington was what were the over wintering hosts that allowed this species to persist.

In the winter of 1998-9, Bob Pfannenstiel discovered 2 leafrollers in our native habitats that pass the winter as mature larvae and act as hosts for *C. florus* to do the same. These include: *Syndemis* spp., an uncommon native on dogwood, and the exotic species, *Ancylis comptana*, or Strawberry leafroller, which is often abundant on wild rose species (*Rosa woodsii, R. canina, and R. nutkana*). Studies based on this background and designed to demonstrate the importance of riparian habitat elements in orchard parasitism are presented here.

Materials and Methods

Parasitism was assessed by exposing laboratory-reared, 4th instar, *Pandemis* leafrollers that we placed on potted apple trees in and near orchards, typically 20 leafrollers/tree and 5 trees per site. After 2-3 weeks of exposure, leafrollers were retrieved and individually reared to adulthood on apple foliage in the laboratory. Parasitism was estimated as the number of a given parasitoid emerging divided by the total of all parasitoids and moths emerging. Parasitism exposures occurred in the spring and summer generations of our pest leafrollers and also in mid-late September in some years. In 1999-2000 at one site and in 2001-2002 at a second, transects of leafrollers were deployed in orchards near naturally occurring patches of *Rosa woodsii*. In 1999-2000 over 100 deployment sites were used to characterize parasitism patterns across two nearby areas comprising over 1000 hectares near the Yakima River. In 2001 and 2002 fewer sites were used to estimate the effect of roses planted at 4 sites in this 1000-hectare area. Roses were planted in July and August of 2000 and consisted of 5 by 15 m plots containing 20-30 seedling rose plants (Rosa woodsii) collected from wild rose patches and 30-40 strawberry plants (var. Quinalt). Gardens were 10-70 m from orchard edges and were irrigated regularly by drip or impact sprinklers. Gardens were situated at sites distant from the Yakima River near sage-grassland habitat where previous 1999 and spring 2000 studies of parasitism showed no parasitism by *C. florus*. In late August 2000 gardens were infested with mid-stage *Ancylis* larvae by transferring infested strawberry leaves from our laboratory colony.

Results and discussion

In two locations where fairly large and heavily infested naturally occurring rose patches were adjacent to orchards, we found parasitism by *C. florus* was consistently higher than 50% in the summer generation of *Pandemis*, and, at one of the sites, parasitism in the spring generation also exceeded 50%. Parasitism declined with distance from the rose patches but this effect was highly variable by date and year.

Figure 1, directly below, shows parasitism increasing with distance from roses at Columbia View, near Wenatchee. Parasitism really doesn't build and move into orchards until the summer generation.

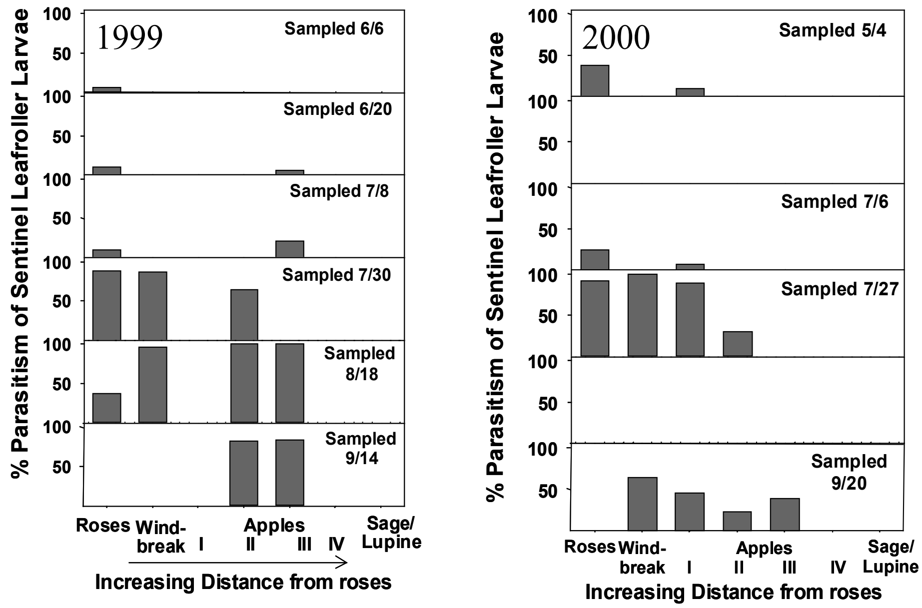
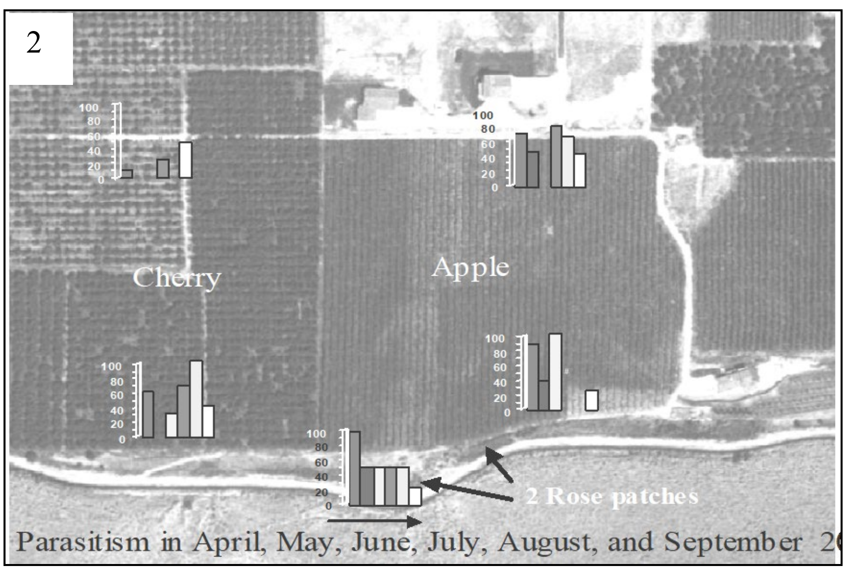


Figure 2, below, shows season long parasitism in an orchard area next to roses in Yakima. Parasitism begins in early spring (April) and persists through the season.

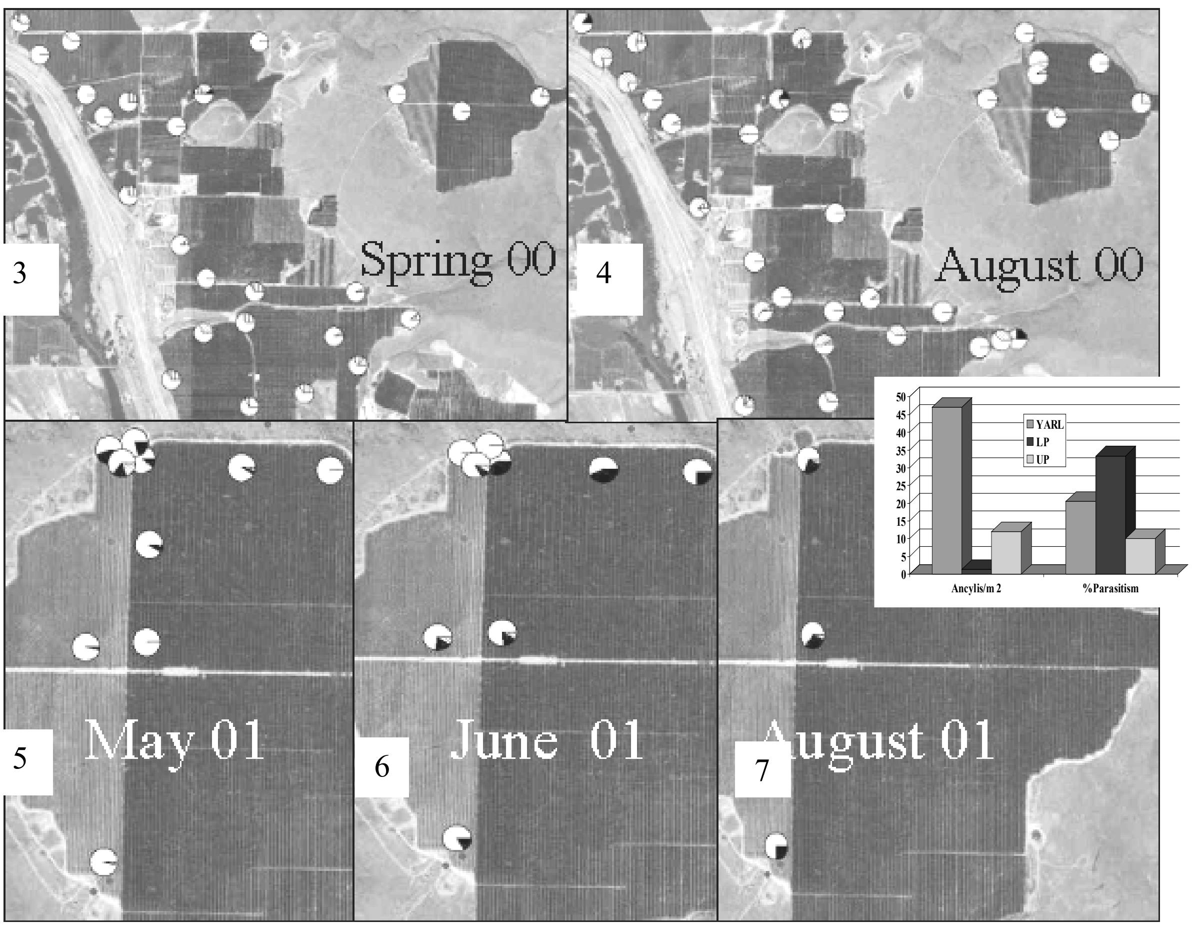


Also in 1999 and 2000, in landscape studies in over 1000 hectares of mixed apple, pear, cherry and stone fruit orchards along hillsides adjacent to the Yakima River near Parker, we found parasitism by 3 wasps, *C. florus*, *Oncophanes americana*, and *Apanteles spp*., was higher in those orchards closest to the river while parasitism by wasps was very low or absent in orchards bordering the grass-sage habitats away from the river. These observations lead us to the following fairly natural set of conclusions: 1) Parasitism is enhanced by nearby riparian habitats; 2) rose infested with the strawberry leafroller seems to be the critical element of this "riparian habitat" effect. We tested these conclusions as hypotheses by planting the 4 gardens described above.

Collections in December 2000 and February 2001 showed that 3 of the 4 gardens were successfully infested with the Strawberry leafroller and these leafrollers were also parasitized by *C.*

florus. In the spring of 2001 we observed parasitism of *Pandemis* by *C. florus* in orchards near each of these 3 garden sites. This significantly contrasts with our observations that 1) no parasitism by *C. florus* occurred in the same orchard sites the previous 2 seasons, 2) no parasitism by *C. florus* occurred near the garden that failed to become infested with strawberry leafroller, and 3) no parasitism by *C. florus* occurred in other orchard sites along the sage grassland habitat. Parasitism at these gardens has persisted and increased through the summer generation of 2001 and the spring of 2002.

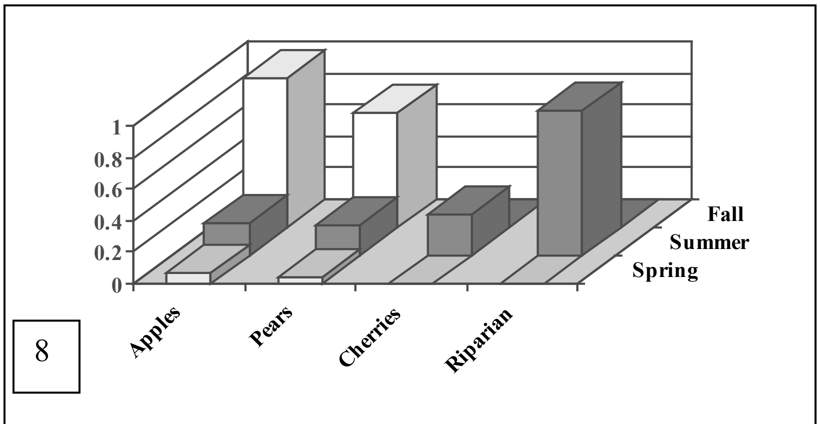
Figures 3-7, below show pattern of parasitism in the landscape in Parker Washington site in 2000 and 2001. Specifically, the top 2 panels show how parasitism increases from spring to summer generation and also show that parasitism by C> florus (black pie slices) is very low and only evident close to the riparian habitats along the river. The one exception, the high parasitism below the "g" in panel on right stems from escapes during our planting a garden of infested roses at this site. Panels on bottom show the progression of parasitism extending from garden (at top left of each panel) into the orchards through the 2001 season. The insert in the last panel shows both the abundance of the strawberry leafroller and the over wintering parasitism rate of these leafrollers in three gardens in 2001-2. The lightest bars in the insert depict the garden in figures below.

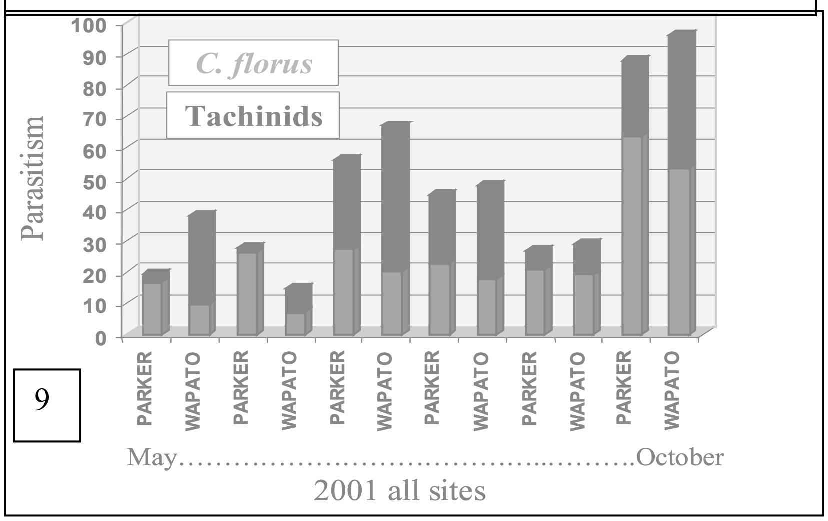


From these results it is clear that parasitism is greatly enhance by proximity to riparian habitats and especially the rose/Ancylis elements of those habitats. It is also evident that the effect can be manipulated and, from the overwintering data (insert, & unpubl. data), that the gardens form stable habitats, mimicking what we see in natural rose patches.

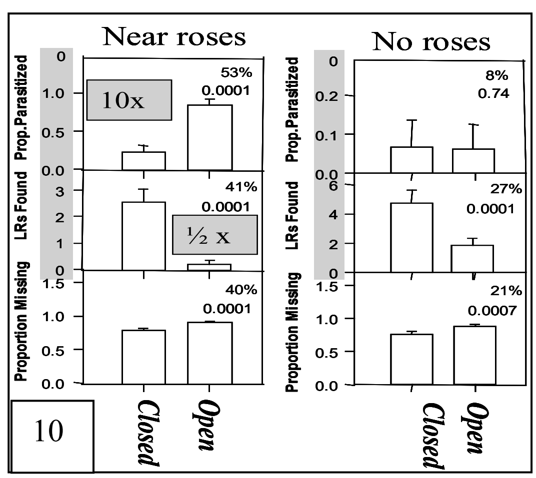
Our work also examined the effects of cultural practices and pesticide use on parasitism in orchards.

The pesticide versus parasitism data is not completely analyzed but the cultural effect information is available. In Figure 8 below we see that parasitism is most likely to occur in fall (when no hosts occur naturally in our orchards). In spring parasitism is greater in apples than in pears than in cherries but in the summer generation parasitism is equally distributed among crop types. In figure 9, second below, we see the pattern of parasitism in 2001. Parasitism by mid summer exceeds 50% but the potential for parasitism is under estimated because ,many of the sites used were distant from gardens and the orchards were sprayed.





More beneficial insects in orchards near riparian habitats seem to be the rule, but studies of the details suggest that having more beneficial species may not be as important as having some of the right beneficial species. In other words, all elements of riparian habitats may not be important and the significant beneficial effects may only be associated with a few key plants and the insects they harbor. The figure on the right (10) demonstrated the importance of a rose patch (and *Colpoclypeus florus*) in the final number of leafrollers surviving in open/close cage experiments. Near roses parasitism is high and few leafrollers survive (<0.2), without roses parasitism is low and 2 leafrollers survive.



We are now taking these "proof of concept" experiments to a larger scale with 6 more gardens in Yakima, 3 in Wenatchee, and 2 each in Milton

Freewater, the Dalles and Brewster. Several of the new gardens will be larger (50 m by 50 m). It is our hope that these simple habitat modifications will prove to be an enduring low input method to provide biological control of leafrollers and eliminate this need for insecticides for this pest complex.

Additional reports of these efforts including the analysis of pesticide effects on parasitism rates, will appear in both peer-reviewed journals and in the grower oriented press. Support from the Tree Fruit research Commission and the Commission on Pesticide Registration is gratefully acknowledged.

Budget/ Funding History
Project Title: Leafroller Biological Control
PI: Tom Unruh
Project total (4 years): $166,000 WTFRC

Year	Yr 1 (1999)	Yr 2 (2000)	Yr 3 (2001)	Yr 4 (2002)
Matching	42,000[1]	48,000[1]	28,400[2]	28,400[2]
Total	83,000	100,000	66,400	63,400
WTFRC	41,000	52,000	38,000	35,000

[1] WCPR; [2] IFAFS

FINAL REPORT
WTFRC Project #AE-01-48 **WSU Project # 13C-3643-4095**

Project title: Development of bait-based monitoring systems for codling moth, leafrollers and lacanobia fruitworm

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

Co-PIs and affiliations: Vincent P. Jones, WSU-TFREC, Wenatchee, WA
Peter Landolt, USDA-ARS, Wapato, WA

Cooperator: Mike Doerr, Senior Scientific Assistant, WSU-TFREC, Wenatchee, WA

Objectives:
1. Assess the attractancy of non-pheromone baits for leafrollers, lacanobia fruitworm and codling moth compared to pheromone traps and determine the reproductive status of female moths attracted to the non-pheromone baited traps.
2. Determine the area of attractancy for non-pheromone baits using release-recovery methods.
3. Develop action thresholds for leafrollers, lacanobia fruitworm and codling moth using non-pheromone baits.
4. Evaluate pheromone based monitoring systems for both mating disruption and non-pheromone treated orchards.

Significant findings - leafrollers:
- Peter Landolt developed and optimized an acetic acid (AA) based monitoring system for pandemis leafroller (PLR) at USDA-ARS, Yakima, WA.
- WSU-TFREC focused on AA monitoring of obliquebanded leafroller (OBLR) by optimizing release rates and trap design. No consistent correlation was noted between release rate (as determined by hole size in lure) and attractancy. Previous studies indicated a hole size of 3.3 mm was optimal for PLR and thus for practical purposes a 3.3 mm hole size should be sufficient for monitoring OBLR as well.
- Various traps baited with AA lures attracted from 5-35% as many moths as the standard-load pheromone lure in a large delta-style trap. The wing-style trap captured the highest number of moths on average, but there were no significant differences among trap styles.
- The AA based technology was implemented on a larger scale to monitor both leafroller species at 21 orchards throughout Washington State as part of the Areawide II (AWII) program managed at WSU TFREC, Wenatchee. Moth captures in AA baited traps were only moderately correlated with pheromone lure captures and did not represent a significant improvement in predicting larval populations.

Significant findings - lacanobia:
- Peter Landolt developed an acetic acid+alcohol (AA+Al) based food-bait type lure for monitoring noctuid pests in orchards.
- The AA+Al lure was deployed in bucket-type traps and placed in side-by-side comparisons with lacanobia fruitworm pheromone baited traps at 12 orchards from the Royal Slope area of Washington to Brewster, as well as at 15 orchards involved in AWII.
  - Each of the bucket-style traps at all sites monitored caught lacanobia fruitworm moths during both generations.

- o Both lure technologies adequately monitored adult lacanobia fruitworm phenology.
- o The food-bait lure generally attracted fewer lacanobia than the pheromone lure, on average about 15% of the pheromone lure.
  - o The food-bait lures attracted lacanobia males and females in a 50:50 ratio over the entire season.
  - o The food-bait trap was not specific to the lacanobia fruitworm and attracted many noctuid species; those of particular importance were Bertha armyworm and spotted cutworm and therefore required some skill to accurately record the noctuid pest data because many other moths were attracted to these lures.
  - o The AA+Al lure will not prove practical for a grower or consultant to use because of the issues associated with mixed moth captures and trap maintenance.
  - o A possible use of this technology may be to develop an “Attract and Kill” product or to implement larger scale kill stations. Dr. Landolt has an active project in this area.

Significant findings - codling moth:
A novel pear-kairomone lure (“DA lure,” Trécé, Inc.) and an acetic acid (AA) based attractant developed by Peter Landolt were evaluated for their ability to monitor codling moth (CM) in non-pheromone and pheromone treated orchards. Several studies involved these two technologies in comparison with standard pheromone monitoring technology.

Non-pheromone treated orchards

- In replicated comparisons a standard-load pheromone lure consistently attracted more moths than either the DA lure or the acetic acid bait. The DA lure attracted about 10-15% of the pheromone lure, and the AA lure attracted about 1% of the pheromone lure during both the first and second generations.
- Aging studies suggest that the DA lure should last through an entire CM generation.
- The DA lure attracted both males and females during each generation. Evaluation of each sex’s flight patterns suggests that males are captured about 80 degree-days before the first female during the first generation. Thus, samples are biased strongly to males early in the flight.
- The DL lure was also biased towards older mated females and probably does not detect earliest female emergence.
- Eighty percent of the females were mated on the first day of capture, and mating levels remained high during the entire flight.
- During the first generation, capture from DA lure traps was not a good predictor of fruit injury, especially in high pressure situations, while the standard-load pheromone lure (1 mg) was a relatively good predictor of damage in orchards that were not receiving any codling moth controls.
- A possible synergistic effect of mixing pheromone and DA lures together was evaluated in three non-pheromone treated orchards. Results from this experiment show that DA lures and pheromone lures were working independently under these conditions, and no synergistic affects were apparent with the commercially available DA lure formulation.

Model validation

Additional research was conducted using pheromone and DA lure technology to evaluate the utility and accuracy of the widely used temperature-based predictive model for CM.

- Male flight as monitored by the DA lure was closely correlated to pheromone captures as well as model predictions during both generations. However, second generation observation and prediction correlations may be confounded by the size of the partial third generation.
- Detailed observations of oviposition and egg hatch reflected the same pattern as moth flight in regards to model predictions for both generations.

Pheromone treated orchards
The utility of the DA lure technology was evaluated on a large scale as a monitoring tool in the AWII project. DA lure and high-load pheromone lure captures were evaluated in replicated side-by-side comparisons.

- The DA lure and high-load pheromone lures (10X) were about equal in performance.
- The pheromone lure tended to capture moths in the first generation and the DA lure captured moths in the second generation, but the differences in overall capture of moths per season were minor.
- The frequency of when the DA or pheromone lure captured or did not capture moths was almost the same in both years of the comparison.

Methods:
Leafroller:
Peter Landolt developed a food-bait lure for monitoring tortricid pests in orchards. The food-bait was glacial acetic acid (AA), and the attractancy was relatively specific to tortricids. Optimizing the attractancy of AA baits for OBLR was conducted in several orchards using trap rotation experiments. During the first generation, the release rate of AA from polypropylene vials (5 ml vial, VWR Scientific) was controlled by varying the hole size in the vial lid. Hole sizes tested were 1.0, 1.6, 3.3 and 6.4 mm. During the second generation a vial with an optimal release rate was used to test various trap designs. AA lures with a hole-size of 3.3 mm were placed in "wet" traps (Agrisense Dome Trap, Pherotech, Inc.), wing-style traps (Pherocon II, Trécé, Inc.) and large delta-style traps (Pherotech, Inc). Further, AA captures were compared to a standard-load red rubber septum pheromone lure (OBLR W, Trécé, Inc.) placed in a large delta-style trap. The experimental design was a randomized complete block. Tests were conducted in six non-pheromone treated blocks in Milton-Freewater, OR. Captures of moths were recorded two or three times per week, and in AA lure baited traps the sex of moths was determined. To minimize position effects, traps were rotated each time they were inspected. Lures were placed during peak flight activity, and the test was conducted for four weeks.

The utility of AA monitoring systems for PLR was evaluated in a replicated test at the Wenatchee Valley College orchard in East Wenatchee, WA. The orchard was divided into three large plots (3 acres per plot) and monitored with two acetic acid baited traps and two standard load pheromone baited traps. The traps were checked twice weekly and the total number of moths recorded. Moths were removed from the acetic acid baited traps and returned to the laboratory where sex ratio and mating status were evaluated. PLR and OBLR were also monitored using acetic acid lures and pheromone lures in the AWII research plots. Monitoring was conducted at 15 locations in Washington in an effort to assess the ability of AA baited traps to accurately predict in-orchard larval populations. Each site had two treatment protocols, and each treatment was monitored with 4-6 AA and 4-6 pheromone baited traps in a side-by-side design (150 total lure comparisons). The traps were checked weekly, and sex ratio was determined in the laboratory. Leafroller larval densities were monitored during each generation.

Lacanobia:
Peter Landolt developed a food-bait lure for monitoring noctuid pests in orchards. The food-bait lure was a blend of AA and isoamyl alcohol (AA-OH) with attractancy relative specifically to noctuids. In previous tests the attractant was placed in "wet" traps where the moths were collected in a drowning solution. This was the first test of the noctuid attractant in a "dry" lure. Because of this development, the food-bait lure could be used in standard monitoring traps. This allowed for a direct comparison of the food-bait to a pheromone lure with all other conditions being equal. This attractant was deployed in general purpose bucket-style traps (Unitrap, Pherotech, Inc.) and placed in a side-by-side comparisons with bucket-style traps baited with a lacanobia fruitworm pheromone lure (Scenturion, Inc.) Twelve orchards ranging from the Royal Slope area to Brewster were monitored. Each orchard was chosen because of its history of reduced insecticide inputs. This was important in order to limit

disruption to normal phenological development. Further side-by-side evaluations were made at each of the 21 AWII research plots. This allowed for approximately 100 more side-by-side comparisons. Each of the bucket-style traps had a small piece (2" x 1") of "kill-strip" (Hercon Vaportape, DDVP toxicant strip, Great Lakes IPM) placed in the collection area. A fresh kill strip was placed at the start of each of the two generations. The pheromone lures were replaced every six weeks and the food-bait lures every four weeks. The traps were monitored every week, and the total number and sex ratio of lacanobia fruitworm, Bertha armyworm and spotted cutworm males and females were recorded.

Codling moth:

Non-pheromone treated orchards: The relative attractancy of non-pheromone baits ("DA Lure," Trécé, Inc.; Acetic Acid Lure, Peter Landolt) was compared with a standard-load pheromone lure (CM red rubber septum, Trécé, Inc.) in several orchards during both CM generations. Moth captures were recorded three times per week. The sex ratio, as well as mating status and relative age of females captured in non-pheromone lure baited traps, was assessed through laboratory dissection. The experimental design was a randomized complete block. Tests were conducted in six non-pheromone treated blocks at the Tree Fruit Research and Extension Center. To minimize position effects, traps were rotated each time they were inspected. Red septum lures were replaced after each complete rotation (9-10 days) during both generations. There were two DA lure treatments. The first was an "aged" DA lure that was placed at the start of flight and not changed, and the second was a fresh DA lure placed after each complete rotation (9-10 days). The acetic acid lure was replaced every four weeks. The traps used for this test were large delta-style sticky traps (Scenturion, Inc.). Trap bottoms were replaced after a cumulative catch of 30 moths, more often if dirty.

Several orchards that were receiving no codling moth control treatments (e.g., research blocks or abandoned orchards) were monitored with both DA lures and 1X pheromone lures in paired comparisons to model fruit injury predictions based on trap captures. Ten orchards with an expected range of codling moth pressure were selected for these comparisons. Each orchard was monitored with two DA and two pheromone baited traps. Moth captures were recorded weekly. The sex ratio and mating status were assessed through laboratory dissection.

A possible synergistic effect of mixing pheromone and DA lures together was evaluated in three non-pheromone treated orchards. Moth captures in traps baited with DA, 1X pheromone lures, or the combination in a single trap were recorded twice each week. The experimental design was a 3 x 3 Latin square.

Model validation: Male and female CM flight patterns were monitored with DA lure and pheromone lure baited traps in three orchard blocks with extremely high CM pressure. Two delta-style traps of each lure treatment were monitored three times per week. Sex ratio and mating status were determined by laboratory dissections. Detailed observations of oviposition behavior, egg hatch and fruit injury were made during each generation. During the first generation, 10 branches, each containing 5-10 fruiting clusters, were flagged. This design was replicated three times. After adult flight was detected, the flagged branches were intensively sampled every one to two days for oviposition activity. The fruit clusters and surrounding leaves were examined under magnification for egg deposition. If an egg was found it was marked and observed for hatch. Fruit injury was also recorded. During the second generation the same principle was followed although the design differed slightly. One hundred uninjured fruit clusters were selected for monitoring from each of three blocks. The clusters were examined, and eggs deposited during the first generation were either removed or marked. Weather data were collected at each site, and male flight and egg hatch were compared to model predictions. Model validation experiments were conducted in 2001 and repeated in 2002.

Pheromone treated orchards: DA lure and high-load pheromone lure (CM SuperLure, PheroTech, Inc.) captures from all 15 AWII apple sites were compared. The data set was composed of 6,122 traps that caught 797 moths at the 15 sites. The trap catch was analyzed in several ways: (1) which trap type caught moths first during the season and by how many days, (2) which trap caught moths a higher proportion of the time, and (3) which traps caught the most moths.

Results and discussion:
Leafroller:
OBLR adult captures in AA baited wet traps were low and variable during the first generation (Fig. 1A). No significant difference in moth captures or sex ratio was noted by manipulating release rates. Moth captures in the non-pheromone treated environment were biased towards females by approximately a 60:40 ratio. During the second-generation test, the 3.3-mm hole

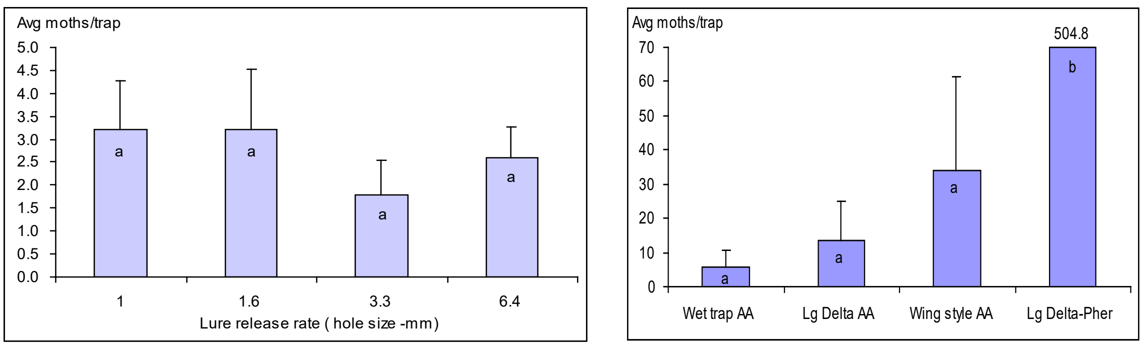


Fig. 1: Development of an acetic acid based monitoring system for obliquebanded leafroller by manipulating release rate (A) and testing trap designs (B), 2001. Statistics run on transformed data (log Y+1).

size was chosen, primarily based on previous tests that suggested it was the ideal hole size to monitor PLR. The pheromone lure/delta-style trap configuration attracted significantly more moths than any AA baited trap (Fig. 1B). Various traps baited with AA lures attracted from 5-35% as many moths as the standard-load pheromone lure in a large delta-style trap. The wing style trap captured the highest number of moths on average, but there were no significant differences among trap styles. Under the conditions of these tests (i.e., non-pheromone treated orchards), the AA lure attracted far fewer moths than the pheromone-baited traps. This may not necessarily be considered a negative. Previous studies have suggested that the OBLR pheromone lure is actually too attractive to OBLR males and may not accurately represent in-orchard population levels.

Adult PLR phenology in the high-pressure Wenatchee Valley College orchard provided evidence that the AA lure technology was biased towards older moths (Fig. 2A). The flight curve as determined by AA lures was shifted approximately two weeks later than captures from the pheromone baited traps. Further evidence of the bias towards older adults was the very high mating level in females captured in AA baited traps (Fig 2B). Approximately 90% of all captured females were already mated at least once. Under these high-pressure conditions the AA lure attracted about 10% as many moths as the pheromone lure.

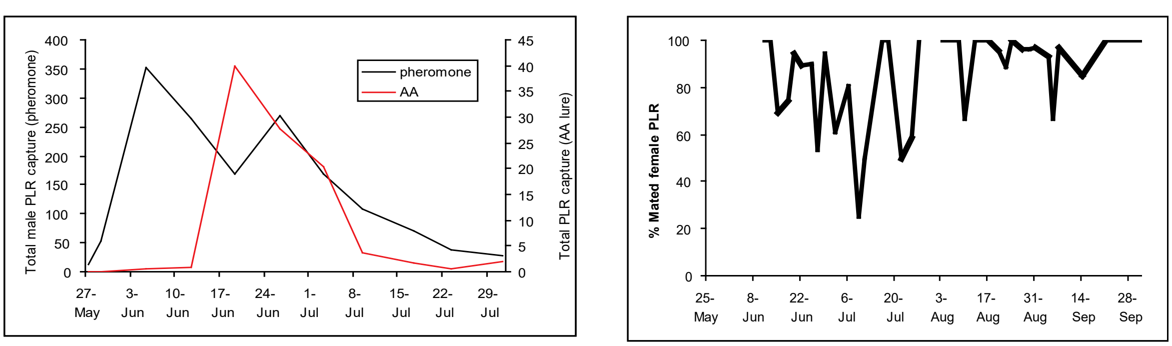


Fig. 2: Monitoring adult PLR flight (A) and mating status (B) in a high pressure orchard with pheromone and acetic acid lures, 2001.

Captures from pheromone lure as well as AA lure baited traps were poor predictors of larval populations in the AWII project. Moth captures in AA baited traps did not represent a significant improvement in predicting larval populations. Most AWII orchards had no recorded larval populations (15 of the 21 orchards). Leafroller adults were captured in pheromone baited traps at every orchard where no larvae were detected, but only 50% of the AA baited traps recorded moth captures in those same orchards. This provides more evidence that a monitoring system with reduced leafroller captures may provide a more accurate measure of in-orchard larval densities. It should be noted that orchards in the AWII program received pesticides targeted at leafrollers, and this would confound any models correlating adult captures and subsequent larval densities.

Lacanobia:
Lacanobia fruitworm was collected at each of the sites in both the pheromone and food-bait (AA-OH) traps. Adult lacanobia phenology was closely correlated between the two lure technologies (Fig. 3). The AA-OH lures attracted lacanobia males and females in a 50:50 ratio average over the entire season (Fig. 4). There did not appear to be a significant sex ratio difference either at the beginning or end of the flight periods.

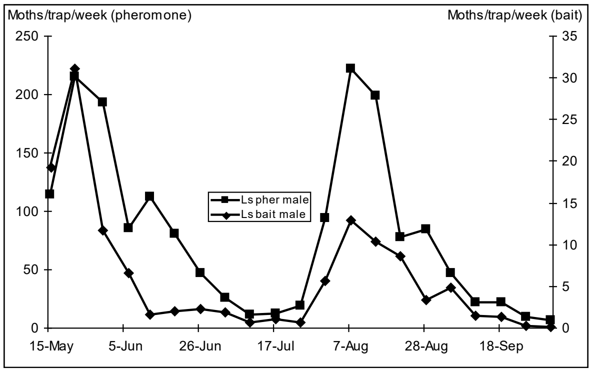


Fig. 3: Capture of lacanobia fruitworm males in bucket-style traps baited with pheromone and food-bait lures, 2000.

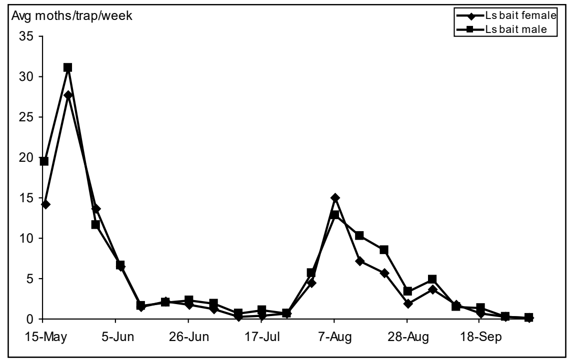


Fig. 4: Capture of lacanobia fruitworm males and females in bucket-style traps baited with food-bait lures, 2000.

The AA-OH trap was not specific to the lacanobia fruitworm and attracted many noctuid species. Included in the samples were Bertha armyworm and spotted cutworm (Fig. 5). This monitoring system may be ideal for monitoring all three of these important orchard pests, although a certain skill level is required to differentiate the important pests from various other noctuids present. The development of a "dry" AA-OH attractant that can be placed into the same monitoring device as a pheromone lure is an important innovation. These attractants appear to be capable lures for monitoring a variety of noctuid pests under all management practices.

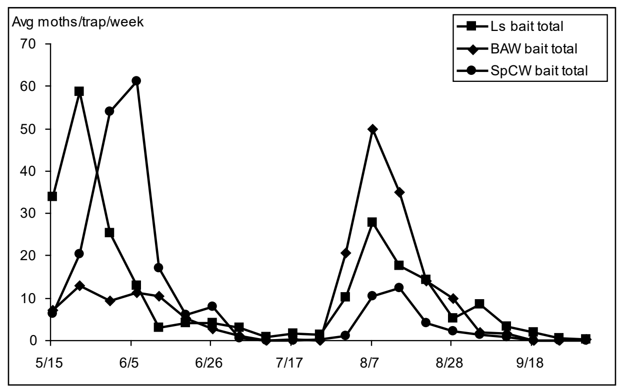


Fig. 5: Capture of lacanobia fruitworm, Bertha armyworm and spotted cutworm males and females in bucket-style traps baited with food-bait lures, 2001.

Codling moth:

Non-pheromone treated orchards: The DA lure attracted about 10-15% of the pheromone lure, and the AA lure attracted about 1% of the pheromone lure during both the first and second generations (Fig. 6A). At each evaluation period through both generations the pheromone lure attracted significantly more moths than the DA lure. The difference between the DA lure and the AA lure was statistically significant during peak flight periods of both generations and across the whole test for each flight. Aging studies suggest that the DA lure should last through an entire codling moth generation (Fig. 6B).

The relative attractancy of aged and fresh DA lures did not change over the entire 66 days of the first generation test. Evaluations of the flight patterns for each sex show that male flight was detected first and female flight was delayed by about 80 degree-days (Fig. 7A). Male flight as monitored by the DA lure was closely correlated to pheromone capture, but the peak flight may have been delayed during the second generation as measured by DA lures (Fig. 7B). As noted above, detection of male flight prior to female flight results in captures that are strongly biased toward males early in the flight. During peak flight activity about 60-70% of the moth captures are male. Interestingly, the male bias is stronger again at the end of the flight. A majority of the first females captured in the DA lure baited traps were already mated. At 100 degree-days from biofix 80% of the females were mated, and the percentage of mated females remained very high through the entire flight. This suggests that the DA lure may not detect the earliest female emergence and may be biased towards older females.

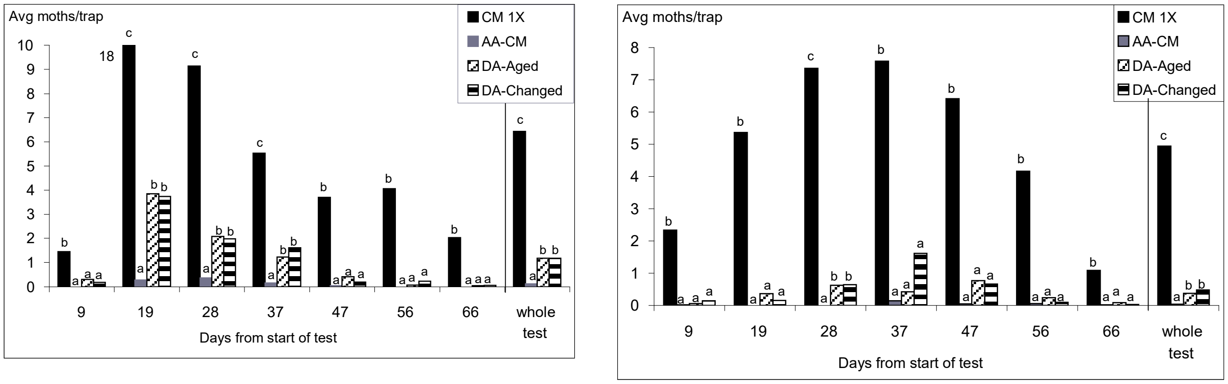


Fig. 6: First generation (A) and second-generation (B) capture of CM in traps baited with standard-load pheromone lures or non-pheromone attractants, 2000. Statistics run on transformed data (log Y+1). Columns in the same grouping with the same letter are not significantly different (p=0.05, Student's paired t-test).

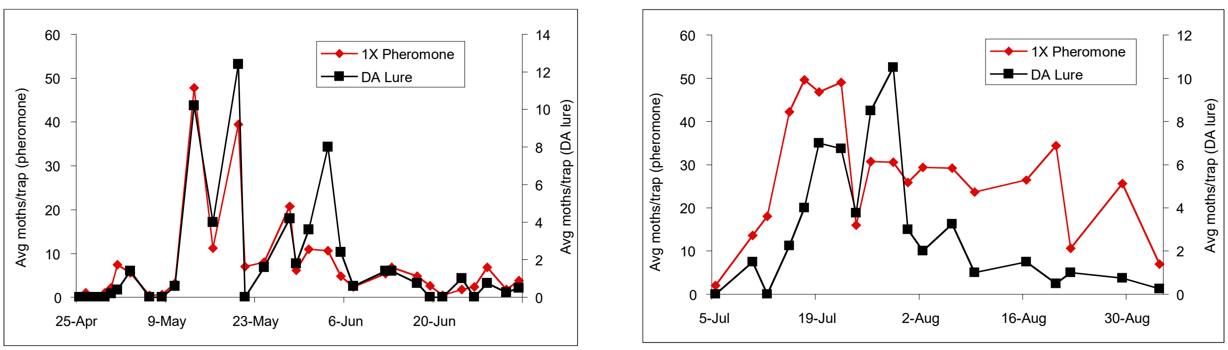


Fig. 7: Monitoring codling moth with pheromone and DA lures during the first (A) and second (B) generations, 2002.

During the first generation, capture from DA lure traps was not a good predictor of fruit injury, especially in high-pressure situations. The standard-load pheromone lure was a relatively good predictor of damage in orchards that were not receiving any codling moth controls. It is possible that plant volatiles released from apples injured by codling moth competed with the DA lure and thus suppressed capture of moths.

The experiment evaluating the interaction of the DA and pheromone lures showed that the DA lure rarely caught moths in two of the three orchards and caught approximately 20 times fewer moths than in the other two treatments in the high population level orchard in Royal Slope (Fig. 8). We found that the DA lure always attracted fewer moths than the pheromone or the combination trap, and at one site the DA + pheromone trap caught significantly fewer moths than the pheromone trap alone. In all locations, the DA lure caught more males than females, and female catches in the pheromone + DA lure treatment were between 4.4 and 1.4% of male catch.

If the data are examined using the percentage of all the trap catch that occurred at that site, the pheromone captured >62% of the moths at two of the sites and about 49% at the RS site which had the highest population of codling moth present. In a non-MD orchard, the DA lure was obviously at a major disadvantage in terms of numbers caught. Addition of the DA lure seemed to cause a reduction in trap catch unless population pressures were extremely high.

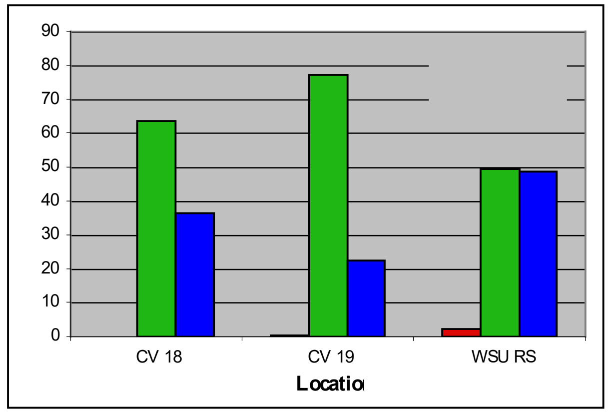


Fig. 8: The percentage of total moths caught in each orchard by the different trap types.

Model validation: Pheromone trap catch was closely correlated with model predictions during the early portion of both generations (Figs. 9-10). During the first generation (Fig. 9), captures peaked early and decreased quickly, with relatively limited flight during the later portion of the generation. This resulted in accumulated captures ahead of model predictions for most of the flight period (Fig 9B). During the second generation, observed flight was more closely correlated with predicted flight (Fig. 10A). However, a comparison of cumulative flight (Fig. 10B) showed observed flight lagging behind predictions during the later part of the generation. This analysis may be confounded by the presence of third generation adults after 2000 degree-days, which would influence observed captures not accounted for in the model.

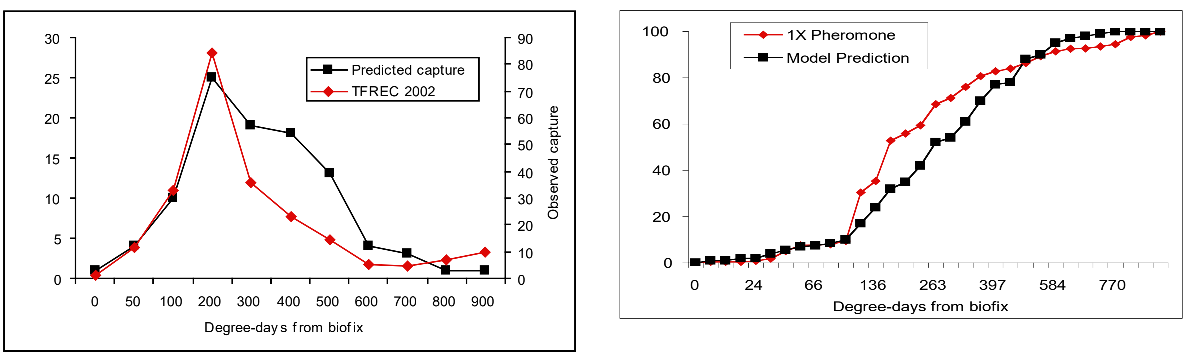


Fig. 9: Observed vs. predicted codling moth captures during the first generation in traps baited with pheromone lures, 2002.

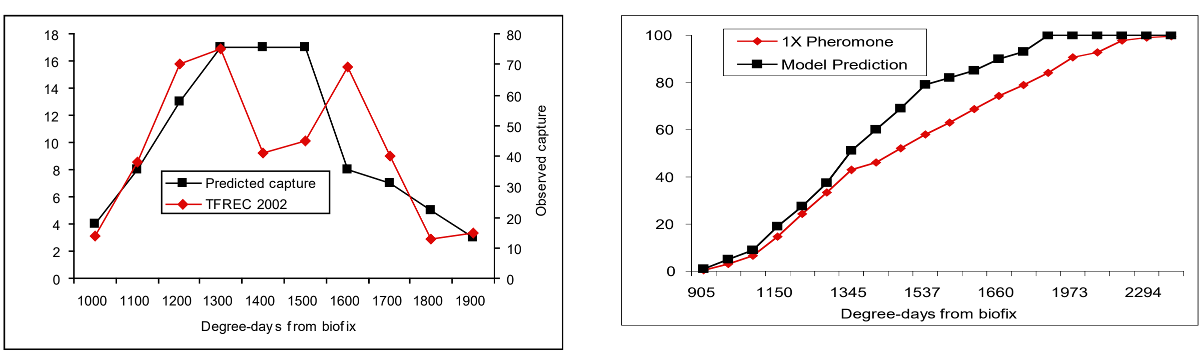


Fig. 10: Cumulative codling moth captures in traps baited with pheromone lures versus degree-day model predictions during the first (A) and second (B) generations, 2002.

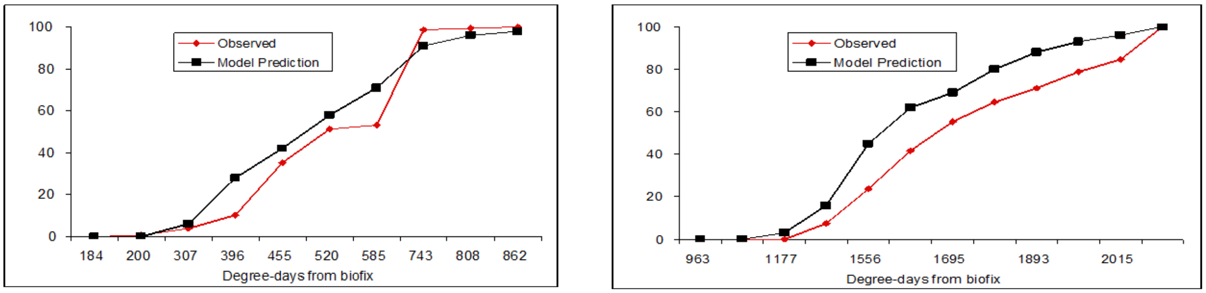
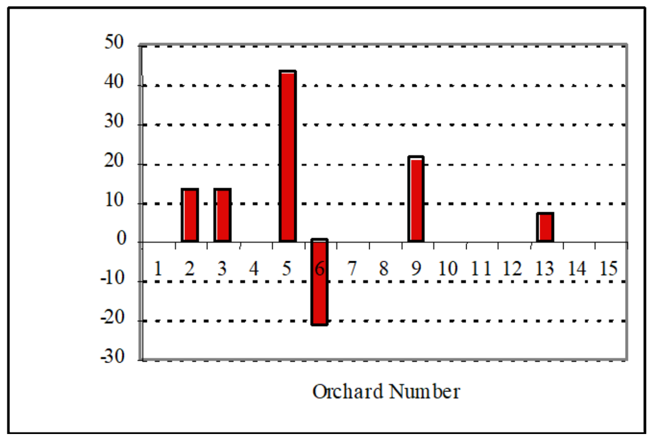


Fig. 11: Cumulative codling moth injury versus degree-day model predictions during the first (A) and second (B) generations, 2002.

Observed egg hatch and subsequent fruit injury accumulations were closely correlated with model predictions during the first generation (Fig. 11A). Second generation hatch and injury observations resembled the accumulated flight patterns seen in Fig.10B. Again, injury attributed to the partial third generation that was not accounted for in the model may be responsible for this lag in injury accumulations. It is apparent that the warmer the year, the more important it becomes to account for the late surge in injury from this partial generation.

Fig. 12: Variation in time of moth capture during the first generation at the AWII sites.



Pheromone treated orchards: The high-load pheromone lure ("bubble" or "BB") caught moths first during the season by an average of 8.4 days in the AWII apple sites. However, there was a high degree of variation by location (Fig. 12), with some sites showing no captures during the majority (or all) of the first generation. The DA lure caught moths first on only one occasion (shown as the negative bar) while the bubble lure caught moths first five times.

The proportion of times a particular lure type caught moths was analyzed in two ways: (1) assuming that the OP and NON-OP blocks were replicates, and (2) by pooling the data across that treatment. Overall, the differences between the proportion of times a trap caught moths in the OP and Non-OP blocks were minor (Fig. 13), as were the differences between the bubble and DA lures. In the OP areas, the bubble trap caught a moth fewer times than the DA, but in the N0n-OP areas the trend was reversed. As would be expected, the differences between sites in the proportion of times a moth was caught were related to the total numbers caught at each site.

These data can also be analyzed as the percentage of times that both types of lures agree, disagree (this is 100% agree), percentage when DA was negative and BB was positive and the converse (DA+, BB-). The box plot analysis shows the variability in the percentage of these traits between the different cultivars and first vs. second generation over both 2001 and 2002 (Fig. 14). There was little difference in the percentage of times that DA recorded a hit and the BB did not and the converse, with both having a median of about 14%. Agreement between the two trap types happened about 72% of the time (median). There was no indication that cultivar was the primary factor in variability; instead variability was related to population level. In terms of total trap catch, the DA traps caught more individuals than the bubble traps overall (Table 1). The majority of the moths were caught from three sites, Chinook, Hattrup and Lateral B. The overall catch of DA traps was highest because of the trap catch at the Chinook site; in all other cases, the differences in trap catch were minor and tended to favor the bubble lure.

In an overall evaluation, it appears that the DA lure is equivalent to the 10X bubble lure if used in a mating disruption orchard, except for the time of first catch. The variability in time of first catch appears to be the key difference between the bubble and the DA lure.

Fig. 13: Summary of the proportion of time caught moths in the AWII sites in 2002.

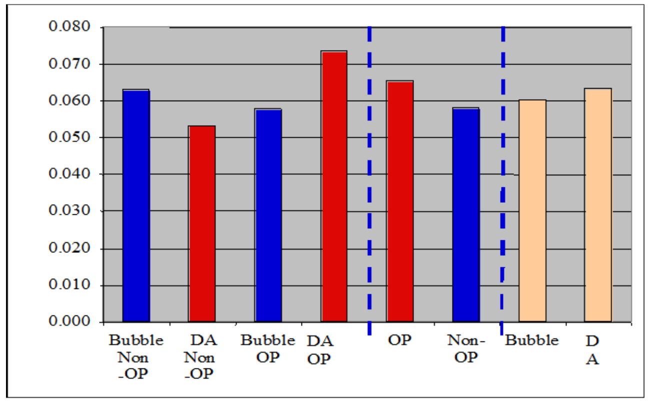


Fig. 14. Box plots showing the variability in performance of the DA and 10X pheromone lures in AWII sites.

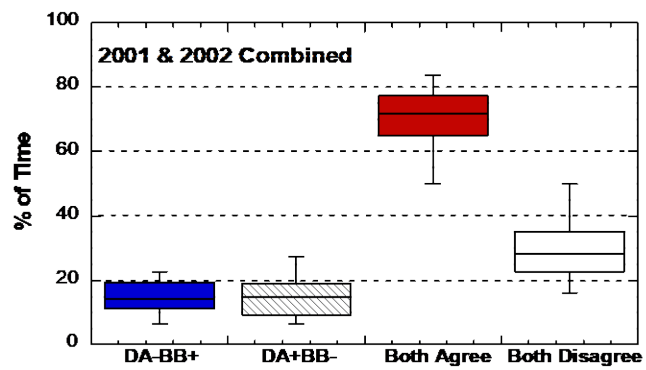


Table 1. 2002 AW trap catch summary. Numbers are the total caught per all traps season-long.

	Bubble Non-OP	Bubble OP	DA Non-OP	DA OP	Total Bubble	Total DA	Total OP	Total Non-OP	Overall Total
Totals	189	162	188	258	351	446	420	377	797

Budget: Development of bait-based monitoring systems for codling moth, leafrollers and lacanobia fruitworm, Jay Brunner, PI

Project duration: 3 years

Current year: Year 3 - 2002

Total budget (3 years): $73,208

FINAL REPORT
WTFRC Project **# AE-0143 (WSU #6408)**

Project title: Feeding enhancements for insecticides targeting neonate lepidopteran larvae.

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Co-PIs and affiliations: John Brown
Holly Boop, Sandye Bushman, Kathryn Johnson, Luis Matos, and Adam Zahand (Students)
Department of Entomology
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Cooperators: Jay Brunner and Michael Doerr, WSU-TFREC, Wenatchee
Peter Landolt, USDA, Wapato, WA
Robert Van Steenwyk, UC-Berkeley, Berkeley, CA

Preface by John J. Brown: "Dr. Pszczolkowski has taken this research project from an initial finding that Sweet'n low® stimulates neonate codling moth larvae to consume apple leaves to preliminary field studies using monosodium glutamate in Washington apples and California walnuts. His research has identified a rainfast natural occurring product that stimulates monosodium glutamate receptors in codling moth larvae. In three years he has published five refereed scientific journal articles and has three more in review on TFRC work alone. Dr. Pszczolkowski has four other publications on glutamate receptors published in: Molecular Cell Endocrinology (1999), Insect Biochemistry and Molecular Biology (2002), Journal of Insect Physiology, and the prestigious 'Proceeding of the National Academy of Sciences (2002). There is no other scientist available to continue this research at this time."

Objectives: 2000 (Objectives are numbered, followed by Significant Findings as bullets).

1. Identify a single compound or mixture of materials that will command the attention (Arrest) of wandering lepidopteran larvae and increase their feeding on leaf tissue without a toxicant.

- Established and optimized a rapid bioassay for evaluation of feeding intensity in neonates of codling moth (CM) and oblique band leaf roller (OBLR).
- Identified three commercially available substances that stimulate feeding in CM and OBLR (Sweet'n low®, monosodium glutamate, inositol) (Figure #1).

2. Use carbon 14 isotopes to demonstrate that the selected arrestment and feeding enhancement formulation will increase the efficacy of insecticides that need to be ingested for maximum toxicity to the insect pests.

- We were able to demonstrate the increased efficacy of Success® without using carbon 14 isotopes.
- Established the doses of Confirm®, Success® and Guthion® for purpose of laboratory and field studies in CM and OBLR. On the basis of this experiments we chose concentrations of 10 ppm Confirm®, 12.5 ppm Success® and 7.5 ppm Guthion® for further experiments.
- Tested the effects of feeding stimulators/pesticides combinations on mortality and feeding in CM and OBLR in laboratory experiments. We found Success® to be only slightly less toxic than Guthion® in our strain of CM, but much more toxic than Guthion® against OBLR.

Without a phagostimulant, 12.5 ppm of Success® is less toxic than 7.5 ppm of Guthion®, but addition of Sweet'n Low® to both active ingredients clearly improved the toxicity of Success® by a factor of 6x, easily doubling its toxicity compared to Guthion®.

3. Develop a micro-encapsulated or micelle formulation that will concentrate the insecticide in limited area; so that the arresting material serves to attract the lepidopteran to the 'bait.' These formulations are designed to reduce the amount of material needed and protect the insecticide from UV or other abiotic degradation.

- Compared performance of identified feeding stimulators in CM and OBLR with a feeding stimulator available presently on the market (Konsume®, Troy Biosciences). We did not observe significantly increased mortality in larvae exposed to Konsume®/Success® or Konsume®/Guthion® combinations. However, the sticky consistency of Konsume® alone caused about 10% mortality in CM and OBLR neonate populations.
- Tested persistency of feeding stimulators/pesticides combinations in the field. The results indicated that SNL and MSG are easily washed off the leaves by rain, thus simple combinations of hydrophobic pesticides, and hydrophilic feeding stimulants may maintain their enhanced efficacy only in favorable weather conditions (Figure #3).
- Tested feeding stimulators/pesticides combinations for causing "russeting" of fruit. No russeting was observed in Red Delicious apples treated with all field-tested solutions.

Objectives: 2001 (Objectives are numbered, followed by Significant Findings as bullets).

1. Extend the time spent by larvae on leaves, between emerging from the egg and reaching the fruit, thereby exposing the larvae all to pesticides for longer time.

- We demonstrated that codling moth larvae can develop through their fourth-instar feeding on apple leaves alone (Pszczolkowski, et al. 2002a).

2. Increase the ingestion of leaf tissue and thus the intake of more pesticide.

- Sweet'n Low® (1%), Inositol (1%), and Monosodium Glutamate (MSG at 25 ppm) all increased codling moth and leafroller ingestion of apple leaf tissue by 27 to 64%. We identified the active stimulant from Sweet'n Low® to be saccharine (Figure #2), which caused larvae to increased feeding even at concentrations 100x less than Sweet'n Low®.
- Mortality of codling moth larvae exposed to Success® mixed with 25 ppm MSG was 30% higher than found in populations exposed to Success® alone. This increased mortality due to adding MSG was accompanied by a 25% increase in leaf tissue ingestion.
- Both saccharine and MSG increased Oblique Banded Leafroller susceptibility to Success® by >25%; therefore both additives have become our focal point for further research.

3. Develop an encapsulated or rain protected formulation.

- We acquired 7 different particle layers, adjuvants, and 'stickers' and initially tested their acceptance or repellency alone. When tested alone all seven materials decreased codling moth larval feeding on leaves by 1 to 94%, depending on the concentration tested.
- Unfortunately batch mixing of either saccharine or MSG with particle layer did not improve neonate acceptance of the inert materials to increase rain fastness.
- We attempted 'loading' the particle layers by degassing mixtures under high vacuum and by freeze-drying. Re-formulation of particle layer material resulted in a 'solid' mass that then needed to be physically pulverized to a unit size that could be applied through a sprayer. The only additive that re-formulated well was soluble starch.
- A second problem with re-formulated matrixes was their 'stickiness' to the leaf surface, only xantan a product from 3-M Canada stuck tightly to the leaf surface. Further efforts to re-formulate xantan with saccharine or MSG were not successful and we have concluded that manufacturers of these particle layer matrixes need to 'load' them during their manufacturing process.
- However, pre-formulation by the manufacturer will add an expense to the user.

- We are still pursuing a feeding stimulant additive that can be mixed in the field. Our approaches to research for 2002 will concentrate on hydrophobic pharmacological alternatives to MSG that will stimulate MSG-receptors in neonate larvae and be rain-fast themselves.

4. By combination of these three features, our research should provide efficient pest control at remarkably lower concentrations of active ingredient.

- We can demonstrate success toward this goal under laboratory condition for spinosad.
- We have shown tebufenozide (Confirm®) does not benefit from these additives.

Objectives: 2002 (Objectives are numbered, followed by Significant Findings as bullets).

1. Finalize our characterization of Sweet'n Low®'s active ingredients.

- Saccharine hemicalcium salt is the active ingredient of Sweet'n Low®. We increased numbers of repetitions, and the final results of overall study on sugar and non-nutritive sweeteners have been summarized and submitted in a form of a manuscript to a plant protection journal, Phytoparasitica [6]. This manuscript has been revised according to the suggestions of the reviewers, and its Revised Version is attached to the report.

2. Acquire pre-formulated particle layer matrixes from commercial sources and test them under our sensitive bioassay system.
3. Explore spray technology to assure coverage of the underside of leaf to take advantage of neonate behavior and minimize lost of feeding stimulants to rain.

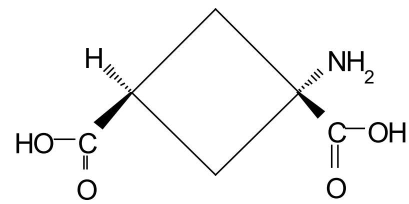
- Our success with characterization of glutamate receptor- related pharmacology of feeding (Objective #4), and the very promising properties of *trans*-ACBD made us re-think the strategy of overall research, and postpone the realization of objectives 3 and 4.

4. Investigate hydrophobic glutamate receptor agonists that could be tank mixed by growers without commercial pre-formulation of additives.

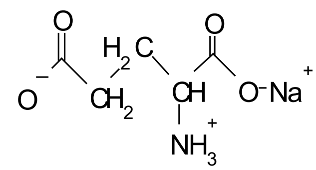
- We characterized glutamate receptor- related pharmacology of feeding by codling moth neonates. The results suggest that two levels of glutamate receptors are acting in codling moth feeding behavior:
- The metabotropic receptor level on initial steps of feeding, and NMDA receptor level during the period of sustained feeding activity (Figure #4).
- On the basis of this study, we proposed *trans*-1-Aminocyclobutane-1,3-dicarboxylic acid (*trans*-ACBD) as a hydrophobic alternative for monosodium glutamate (Figure #5), and tested its potential for spinosad enhancement in the laboratory and in the field.
- *trans*-ACBD is rain-fast beyond 20 mm, an amount of rain that completely eliminated MSG (Figure #6).
- It also significantly enhanced the consumption of leaf tissue at 75 to 100 ppm. MSG is very water-soluble (739 g/L^{-1}), whereas *trans*-ACBD is much less water-soluble ($<2g/L^{-1}$). The studies on hydrophobic alternative for monosodium glutamate resulted in two publications, one in press, and one in preparation.

- The figure below shows the structure of both monosodium glutamate (MSG) and *trans*-ACBD.

trans-ACBD



MSG



Methods: *Laboratory Studies:* Circular sections of apple leaves collected daily were treated with various concentrations of each insecticide and/or feeding stimulant and allowed to air dry. Treated sections were then mounted on a glass microscope slide and secured there by combination of adhesive tape and self adhesive reinforcement rings. Each station was then infested with one neonate larva, trapped by a cover slip and observed to record feeding activity, the amount of leave surface consumed, and mortality. All stations were examined daily for mortality, and amount of foliage consumed. Surface of the eaten leaf disc part was determined in ocular units using a stereo microscope equipped with an ocular square mesh reference scale. Next, surface area from 33 randomly chosen leaves were determined by the same method, the leave fragments dried and weighted. Based on this comparative determination, ocular units, were converted to an estimated dry weight of leaf consumed.
Field Studies: Field studies were conducted in Pullman, Wenatchee, and Hollister, CA.

- Pullman research consisted of applying insecticides with or without feeding stimulants on previously untreated trees. Sample leaves were removed immediately, one, three, or seven days later. Extirpated leaves were returned to the laboratory and challenged with neonate codling moth larvae. Comparisons were made using Guthion®, Success®, and Confirm®, plus Sweet'n low, MSG, or *trans*-ACBD.
- Wenatchee studies were conducted in collaboration with Dr. Jay Brunner (WSU-Wenatchee) MSG was added to *Bacillus thuringiensis* against an apple defoliator, obliqueband leafroller, *Choristoneura rosacea.* Leaves were removed from the field periodically taken to the laboratory and challenged with neonate obliqueband leafrollers.
- Dr. Robert Van Stennwyk conducted California field research studies in walnuts against codling moth and in orange against navel orangeworm. His results were mean number of infested nuts that dropped from the trees after the first generation, and mean percent-infested walnuts by both codling moth and navel orangeworm at time of harvest.

Results and discussion:

1. **Pszczolkowski, Maciej A**., Luis F.Matos, Sandye M.Bushman and **John J. Brown**. (2001). Feeding enhancements for insecticide targeting neonate lepidopteran larvae. 6TH International Symposium on Adjuvants for Agrochemicals. Editor: Hans de Ruiter. Pp. 420-425.
   We show that neonates of the codling moth *Cydia pomonella* (L.) are capable of feeding and development on apple leaves, and identify granulated sugar substitute, Sweet'n Low® (1%, w/v),

and monosodium glutamate (0.0025%, w/v) as substances that increase leaf feeding in codling moth neonates. In laboratory trials, addition of Sweet'n Low® or monosodium glutamate to Success®, a pesticide formulation containing Spinosad, increased its efficacy (by factor of 5.97x, and 2.94x, respectively) without increasing the amounts of toxic component. However, our semi-field experiments indicate that a more stable formulation of feeding stimulant/pesticide combination is needed to protect both from being washed from leaves by a rain. We also have preliminary data that characterize glutamate-dependent pharmacology of feeding in codling moth neonates, and improve field persistence of identified feeding stimulators. Our data suggest new strategy for rational pesticide reduction in control of lepidopteran pests. Our formulation increased the amounts of pesticide ingested by stimulation of feeding, thereby showing prospects of decreasing the amounts of toxic ingredients needed in pesticide formulation without affecting its efficacy.

2. **Pszczolkowski M.A**., Matos, L., Brown R., and **Brown J.J.** (2002). Feeding and development of codling moth, *Cydia pomonella*, (L.) (Lepidoptera: Tortricidae) larvae on apple leaves. *Annals of Entomological Society of the USA*. **95**:603-607.

We presented leaf consumption, maximum body weight, head capsule width, and testicular ultrastructure of four sequential instars in codling moth reared on solely on apple leaves of Honeycrisp® variety. The developmental parameters for Cydia pomonella (L.) larvae fed on leaves are compared with those reared on artificial diet. Head capsules in the second, third, and fourth instars reared on leaves were significantly lower in all instars fed leaves compared with larvae fed artificial diet. Maximum body weights were significantly lower in all instars fed leaves compared with larvae fed artificial diet. More than 60% of larvae fed leaves molted to the third instar, and 3% entered the 5th instar, but none pupated, demonstrating an inability to fully compensate for the reduced nutritive value of a leave-based diet. Nevertheless, this was the first research to describe the flexibility of codling moth larvae to survive several instars without burrowing into the apple itself. Leaf tissue consumption is not evident in the field, because codling moth damage would be undistinguishable from early leafroller damage.

3. **Pszczolkowski M.A**., Matos, L., Zahand, A, and **Brown J.J**. (2002). Effect of monosodium glutamate on codling moth larvae fed apple leaves. *Entomologia Experimentalis and Applicata.* **103**:91-98.

We demonstrated that monosodium glutamate (MSG) increased feeding by codling moth larvae on apple leaves. Depending on the duration of the bioassay, and the larval age at time of initial exposure, 0.05 mg/ml and 0.1 mg/ml MSG increased apple leaf consumption by 25-60% over leaves alone. The effect of monosodium glutamate was best demonstrated during the first day following hatch. Exposure to MSG also accelerated molting to the second instar. Larvae exposed to MSG initiated consumption of leaf tissue significantly earlier than control neonates. The feeding stimulatory effect of MSG was not observed if exposure to this chemical was delayed, therefore it would be advisable to have MSG present at the time of egg hatch.

4. **Pszczolkowski M.A**. and **Brown J.J**. (2002). Prospects of monosodium glutamate use for enhancement of pesticides toxicity against the codling moth. *Phytoparasitca* **30:**243-252.

Mortality of codling moth larvae exposed to Success® mixed with 25 ppm MSG was 30% higher than found in populations exposed to Success® alone. This increased mortality due to adding MSG was accompanied by a 25% increase in leaf tissue ingestion. Both saccharine and MSG increased Oblique Banded Leafroller susceptibility to Success® by >25%; therefore both additives have become our focal point for further research. However, both are very water-soluble and subject to removal form leaves by rain or overhead sprinklers used for evaporative cooling.

5. **Pszczolkowski M.A.,** Zahand. A, Bushman S.M, and **Brown J.J** (2002). Effects of calcium and glutamate receptor agonists on leaf consumption by lepidopteran neonates. *Pharmacology, Biochemistry, and Behavior.* DOI:10.1016/S0091-3057(02)01005-5.

   Calcium and glutamate receptor (GluR) agonists effect apple leaf consumption by neonates of the apple pest, the codling moth, *Cydia pomonella* (L.). Initial apple leaf consumption was advanced by the presence of *trans*-1-amino-(1*S*,3*R*)-cyclopentanedicarboxylic acid (*trans*-ACPD), but not by calcium chloride, or *N*-methyl-*D*-aspartate, (NMDA). However, during the 3h following hatch, $CaCl_2$ and NMDA increased the quantity of apple leaf tissue consumed, but *trans*-ACPD had no such effects. Stimulatory effects of $CaCl_2$ and NMDA on leaf consumption were abolished if codling moth larvae were concurrently exposed to the calcium chelator EDTA. (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) and kainic acid had no effects either on commencement or intensity of leaf consumption. We hypothesize that in codling moth larvae, apple leaf consumption is induced via metabotropic GluR, and sustained feeding is regulated via NMDA glutamate receptors. Practical aspects of these findings are discussed.

6. **Pszczolkowski M.A**. and **Brown J.J.** (200?). *trans*-1-Aminocyclobutane-1,3-dicarboxylic acid is a potential enhancer of spinosad toxicity against neonates of the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). (manuscript in preparation).

   We initiated studies on hydrophobic alternative for monosodium glutamate that could be tank mixed by growers without commercial pre-formulation of additives. We characterized glutamate receptor- related pharmacology of feeding by codling moth neonates. The results here suggest that two levels of glutamate receptors are acting in codling moth feeding behavior: the metabotropic receptor level on initial steps of feeding, and NMDA receptor level during the period of sustained feeding activity. On the basis of this study, we proposed *trans*-1-Aminocyclobutane-1,3-dicarboxylic acid (*trans*-ACBD) as a hydrophobic alternative for monosodium glutamate, and tested its potential for spinosad enhancement in the laboratory and in the field. *trans*-ACBD is rain-fast beyond 20 mm, an amount of rain that completely eliminated MSG. It also significantly enhanced the consumption of leaf tissue at 75 to 100 ppm. MSG is very water-soluble (739 g/L^{-1}), whereas *trans*-ACBD is much less water-soluble (<2g/L^{-1}). The studies on hydrophobic alternative for monosodium glutamate resulted in two publications, one in press [4], and one in preparation [5].
   We assume *trans*-ACBD could be isolated from *Atelia Herbert-smithii* (Fabaceae/Papilionoideae) a native tree of Costa Rica. This tree is known to produce the *cis*-ACBD in its seeds and most biological systems produce racemic mixtures of synthesized defensive compounds and then localize one isomer. Having a natural source of *trans*-ACBD would increase its utilization in organic agricultural systems.

7. **Pszczolkowski M.A.** and **Brown J.J**. (200?). Effects of sugars and non-nutritive sugar substitutes on consumption of apple leaves by neonates of codling moth. Phytoparasitica. (manuscript in revision).

Field Studies:
Pullman:
Wenatchee: The addition of 1.5lb/400 gal MSG to commercial *Bacillus thuringiensis* formulation, 0.3 lb/400gal DiPel® 2X DF increased mortality by a factor of 2.8x, and (3) reduced LD_{50} from DiPel® 2X DF from 1lb/400 gal to 0.3 lb/400 gal.
California: Dr. Van Steenwyk's results are unclear. After the first generation of codling moth, the nuts infested with worms following the application of MSG alone was significantly less than the controls. Likewise, after harvest the number nuts infested with codling moth in trees treated with MSG alone was significantly less than found in the control trees. Furthermore, the number of nuts infested at harvest time from trees sprayed with MSG plus Success® was less than that found in trees sprayed with Success® alone, but the difference was not shown to be significant. Dr. Van Steenwyk suggested that "MSG may increase the feeding on the husk and prevent the codling moth from infesting the nut meat."
More field research is needed and planned for 2003.

Budget:
Project total (3 years): $55,006
Current year request: (see new proposal $22,284)

Year	Year #1 (2000)	Year #2 (2001)	Year #3 (2002)
Total	$20,000	$20,860	$14,146

Current year breakdown:

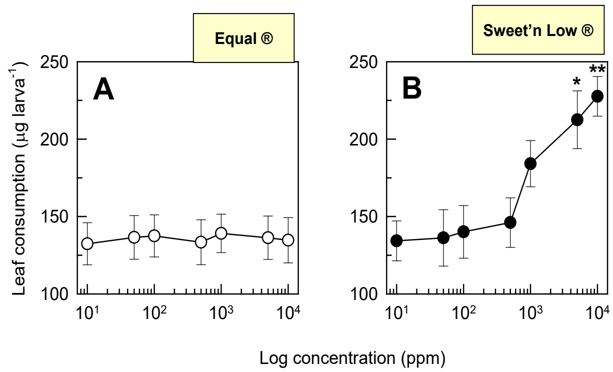
Item	Year #1 (2000)	Year #2 (2001)	Year #3 (2002)
Salaries	$13,130	$8,000	$6,675
Benefits (%*)	$4,052	$916	$2,336
Wages	$1,668	$3,160	$1,500
Benefits (0.9 %)	$150	$284	$135
Equipment		$8,200	$500
Supplies		$300	$3,000
Travel	$1,000		
Miscellaneous			
Total	$20,000	$20,860	$14,146

*Benefits for salaried post-doctorate associates varies, Dr. Pszczolkowski's was approximately 32%, whereas Dr. Yooichi Kainoh's was approximately 11%. Dr. Kainoh's visit was for 4 months and his visa was different than that of Dr. Pszczolkowski.

Dr. Maciej Pszczolkowski is supported on grant funds only! Grant funding from the 1) Washington State Commission on Pesticide Registration, federal 2) IFAFS and 3) RAMP grants, and a grant from the 4) Environmental Protection Agency have supported Dr. Maciej Pszczolkowski's research.

Figure #1. Effects of sugar substitutes on feeding of codling moth neonates

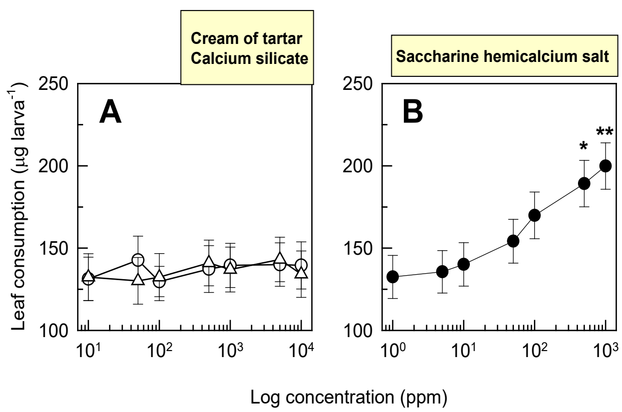
Leaf tissue consumed µg/larva



Published in 6th International Symposium on Adjuvants for Agrochemicals

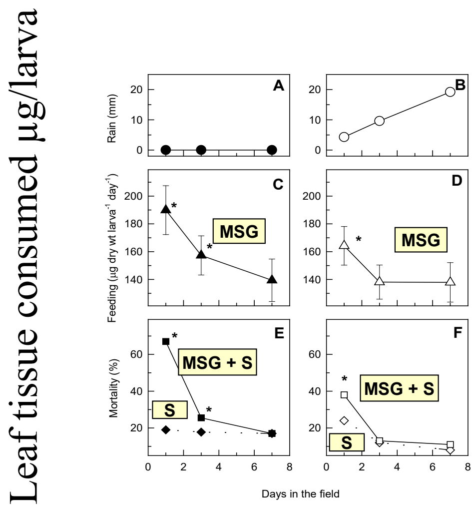
Figure #2. Identification of active ingredient of Sweet'n low ®

Leaf tissue consumed µg/larva



Accepted for publication in *Phytoparasitica*

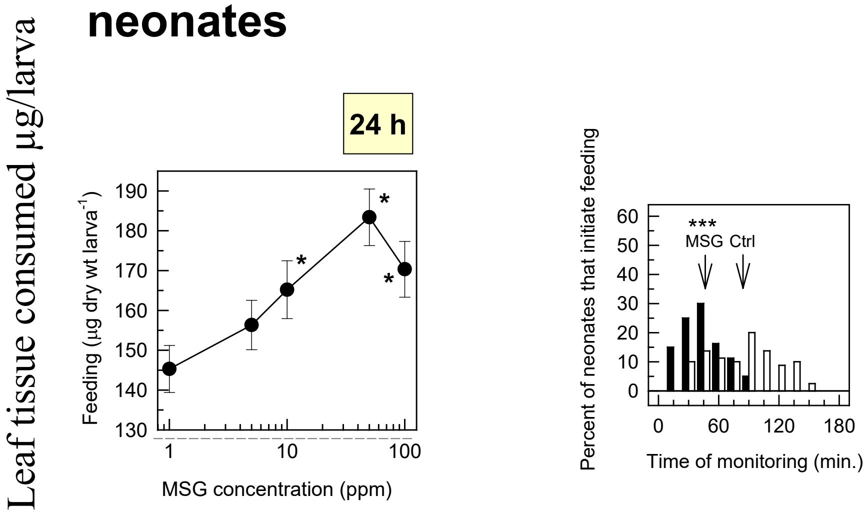
Figure #3. Field persistence of monosodium glutamate (MSG) alone or in combination with spinosad (S)



Phytoparasitca 30: 243-252.

S = Success®
MSG = monosodium glutamate

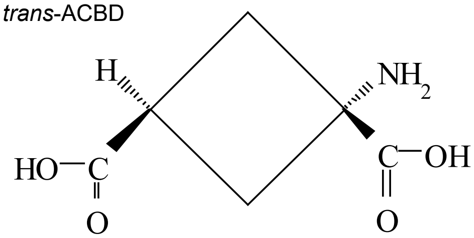
Figure #4. Effects of monosodium glutamate on feeding of codling moth neonates



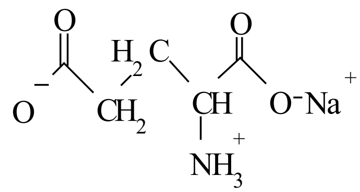
Published in Entomologia Experimentalis and Applicata. 103:91-98.

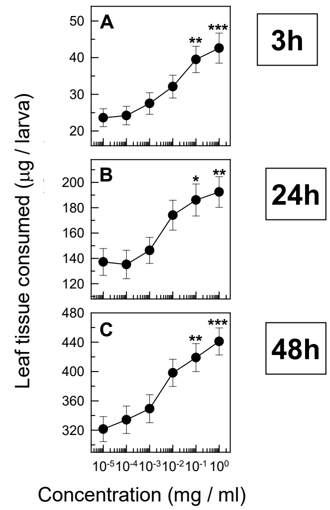
Figure #5. Effects of NMDA receptor agonist, *trans*-ACBD, on feeding of codling moth neonates

trans-ACBD



Monosodium glutamate





Pest Management Science (Submitted).

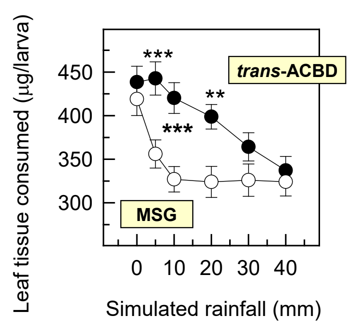
Figure #6. Field persistence of *trans*-ACBD in comparison to that of monosodium glutamate (MSG)

Water solubility

***trans*-ACBD < 2 g l^{-1}**

MSG 739 g l^{-1}

Leaf tissue consumed µg/larva



FINAL REPORT
WTFRC Project # AE-01-37 **Organization Project #** 5352-22000-013-22T

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's: David Horton, USDA-ARS, Wapato, WA
Tom Unruh, USDA-ARS, Wapato, WA

Objectives:
1. Identify plant species in extra-orchard habitats that may be important to natural enemies of pear and apple pests as alternative feeding, mating, oviposition, or overwintering sites.
2. Determine if natural enemy densities within an orchard decrease as distance from extra-orchard habitat increases.
3. Survey plants in extra-orchard habitat for the presence of leafrolling caterpillars. Rear the caterpillars and associated parasitoids.
4. Determine the occurrence and phenology of the western flower thrips and its predators, particularly the minute pirate bug, Orius, on native and introduced plant species in extra-orchard habitat.

Significant Findings:
- Many species of plants found in extra-orchard habitat host beneficial arthropods (insects and spiders) including species found in adjacent orchards which are known or presumed to contribute to biocontrol. Beneficial arthropods were found on some plants for much of the season although their number and kind varied. Sagebrush, bitterbrush, oak, and ponderosa pine are examples. Beneficial arthropods in considerable number and variety were found on other species for a more limited time, particularly around flowering. Examples included gray and green rabbitbrush, tall buckwheat, and yarrow.

- Which beneficial arthropods present in an orchard also occur in adjacent extra-orchard habitat may depend on the type of habitat present. Some orchard beneficials also occurred on a variety of plants typical of central Washington's sagebrush steppe habitats. Others were rarely found in sagebrush habitats although they were found in other types of extra-orchard habitat such as woodland or riparian. The reverse situation also occurred as some species that are common in sagebrush habitats were rare in adjacent orchards.

- Natural enemy densities in some (although not all) orchards decreased as distance from extra-orchard habitat increased, consistent with the idea that extra-orchard habitat may serve as a source of colonists for the orchard.

- The western flower thrips occurred on many of the 126 species of plants that were sampled and was sometimes present in large numbers. Extra-orchard plants supporting thrips may act as a source of pest thrips moving into orchards. Thrips predators, especially minute pirate bugs and the young of several spider species, were found on many of the same plants and could be quite abundant.
- Caterpillars that exhibited leafrolling or leaf-tying behavior were found on 33 species of plants in extra-orchard habitat. Some of the moths reared from these caterpillars appear to be

tortricids (ie., related to pest leafrollers) whereas others obviously represent different families. Many parasitoids were reared from the caterpillars although none appear to be the important leafroller parasitoid, Colpoclypeus florus. Lupines and alders were particularly heavily infested with leafroller type caterpillars.

Methods:

Orchard Sampling: Ten pear and 8 apple orchards were sampled in 2001 to determine if beneficial arthropod density decreased with distance from extra-orchard habitat. All were mating disruption or organic and each was divided into 2 or 3 sections at different distances from extra-orchard habitat depending on size: 0' - 200', 200' – 400', and > 400'. Twenty-six beat tray samples were taken in each section of an orchard once a month from May to October. All beneficial arthropods, except predatory mites, were collected and identified. Pest insects were noted on a presence/absence basis.

Extra-orchard habitat sampling: Fifty-one species of native and introduced plants in extra-orchard habitats near the 18 orchards were sampled in 2001. Each site was visited once a month from May to October. A species was sampled 1 to 6 times at a site. Ten to 20 beat trays were taken per sample. All beneficial arthropods were collected as was a sample of potential prey and host insects. Seven plant species were selected for more intensive sampling during 2002 based on their importance to beneficial arthropods as indicated by 2001 collection data. These plants were sampled at 1 – 2 week intervals, particularly during the pre-bloom, bloom, and post-bloom periods. Ten beat trays were taken per sample. Most species were sampled at more than 1 site.

Phenology and host plant utilization of western flower thrips and its predators: Samples of flowers and foliage were collected from 126 species of native and introduced plants in extra-orchard habitats to document the occurrence of flower thrips and its predators. For most species, samples were taken during the pre-bloom, bloom, and post-bloom periods. Thrips and other arthropods were extracted with Berlese funnels which employ heat from a light bulb to drive the organisms into a jar filled with 70% alcohol which in turn kills and preserves the specimens. Numbers of thrips, predators, and other organisms will be counted or estimated. Dry weights of the flower and foliage samples were obtained.

Survey of extra-orchard host plants for leafrolling caterpillars and their parasitoids: Plant species were visually inspected for the presence of leafrolling type caterpillars. Most plants were native species found in extra-orchard habitat near study orchards or at more distant locations. Most larvae were reared on foliage of the plants on which they were collected. Adult moths and any parasitoids obtained were frozen and mounted on pins or points. They will be sent to experts for identification.

Results and Discussion:

Orchard sampling: Raw collection data for some orchards indicated that natural enemy density decreased as distance from extra-orchard habitat increased. Such a trend was not apparent for all orchards, however. In some instances density of beneficial arthropods as a group decreased as distance from extra-orchard habitat increased while in other cases density of some subgroup of beneficials, or even a single species, showed the trend. We plan to analyze the distance data in greater detail this winter. Some data will be presented at the research review.

Extra-orchard habitat sampling: Beat tray samples were taken from 51 species of extra-orchard host plants during 2001. A complete list of the plants is given in Appendix 1. Occurrence of beneficial arthropods on several species of extra-orchard plants is summarized in the following table.

	Green Lacewing	Brown Lacewing	Lady Beetle	Stethorus	Deraeocoris	Campylomma	Orius	Anthocoris	Damsel Bug	Big-eyed Bug	Aphid Parasitoid	Jumping Spider	Lynx Spider	Crab Spider	Dwarf Spider	Sac Spider	Anyphaena	Orb Weaver	*
Big Sage	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	
Gray Rabbitbrush	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Green Rabbitbrush		X	X	X	X		X		X	X	X	X	X	X	X		X	X	X
Yarrow			X			X	X		X	X	X	X		X	X		X		X
Balsamroot		X	X				X					X	X	X					X
Cusick's Sunflower												X	X	X			X		
Bitterbrush	X		X	X	X		X	X	X	X	X	X	X	X	X		X	X	X
Tall Buckwheat	X						X			X	X	X	X	X	X		X		X
Clematis		X	X		X	X	X			X	X	X	X	X	X	X	X	X	
Cottonwood	X		X	X			X	X				X		X	X	X	X	X	X
Milkweed			X			X	X					X		X	X				
Oak	X	X	X	X	X							X	X	X	X	X	X		X
Ponderosa Pine	X		X		X		X					X	X	X	X				X
Deerbrush	X				X		X		X			X	X	X	X	X	X		X
Snowberry	X			X	X		X		X			X	X	X	X	X	X	X	X
Douglas Fir	X	X			X		X					X		X	X				X
Blackberry	X				X	X	X		X			X	X	X	X			X	X
Goatsbeard	X				X				X			X	X	X	X				X
Diffuse Knapweed	X						X		X	X		X							
Willow	X			X	X	X	X	X	X			X	X	X		X	X	X	X
Tumbleweed							X		X	X		X	X	X				X	
Yellow Star thistle							X		X	X		X	X	X					
Hoary Aster							X			X		X		X					
Oregon Grape		X										X	X	X		X			X
Big-leaf Maple	X	X										X		X	X		X		X
Wild Rose	X				X							X	X	X	X				X
Ocean Spray	X											X		X	X		X		X

*Cobweb spiders

Beneficial arthropods listed in the table as occurring on an extra-orchard host plant are species (sometimes more than 1) that also occur in apple and pear orchards. Several species of lady beetles, for example, feed on pest aphids in orchards. Lady beetles are listed in the table as occurring on a plant only if 1 or more of the species that occur in orchards was also taken on the plant. Several other species of lady beetles were collected on various extra-orchard host plants but were not collected in orchards during the study. These species are not included in the table and if 1 or more of them were the only lady beetles taken on a plant, then an X does not appear in the table under lady beetles for that plant.

Some beneficials were represented by 2 or more species. Jumping spiders, for example, include several species in 3 common genera (*Pelegrina*, *Phidippus*, and *Sassacus*) and 2 less common genera (*Phanias* and *Salticus*) that have all been collected from orchards. One or more of these were taken on each of the host plants in the table. Other beneficials were represented by only a single species, the minute pirate bug *Orius* and the lynx spider *Oxyopes scalaris*, for example. Both species were taken on most plants listed in the table.

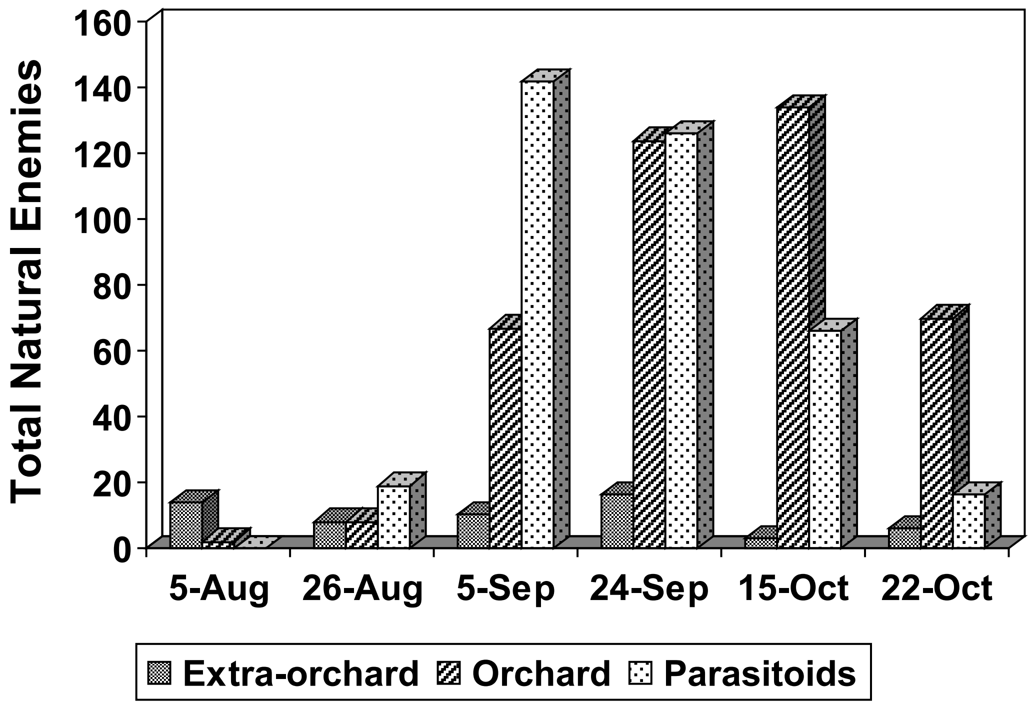
Sampling effort on the various plants was not equal. Some species, big sage and bitterbrush for example, were sampled far more frequently, and often at more than 1 location, than others such as hoary aster and ocean spray. Additional sampling on the less frequently sampled species would undoubtedly fill in some of the gaps in the table.

The 7 species of extra-orchard host plants intensively sampled during 2002 are listed in the table below, along with some sampling parameters. All 7 are native and, with the exception of western clematis, are dry adapted and typical of the sagebrush steppe habitats most commonly found adjacent to central Washington orchards. Gray and green rabbitbrush are considered to be invasive increasers and are often more abundant in overgrazed or disturbed areas than in more pristine sagebrush steppe. Such situations often occur near orchards, as was the case with a number of our study sites. Western clematis generally seems to occur at sites where water is more abundant. Our samples were taken from a dense stand that grew along an irrigation canal that paralleled the eastern edge of an organic pear orchard. It also received water during orchard irrigations and the abundance of water may have been partly responsible for its lengthy flowering period at this site.

Plant Species	**# of Sites**	**# of Samples/site**	**Sampling Period**
Gray Rabbitbrush	3	12 – 15	7/10 – 10/24
Green Rabbitbrush	3	6 – 11	7/17 – 10/24
Big Sagebrush (early)	1	10	5/17 – 8/8
Big Sagebrush (late)	2	10	8/5 - 10/22
Yarrow	2	5	6/3 – 7/1
Bitterbrush	2	7, 8	5/17 – 7/30
Tall Buckwheat	2	13, 14	7/11 – 10/16
Western Clematis	1	12	6/17 – 9/26

The natural enemies found on extra-orchard host plants can be divided into 3 groups. One group consists of predatory insects and spiders that are rarely found in orchards. They are apparently adapted to the sagebrush steppe environment and rarely move out of it. Insects include certain lady beetles as well as snakeflies, assassin bugs, and ambush bugs (the latter 3 are occasionally taken in orchards but are far more common on extra-orchard plants). Spiders include jumpers like Pelegrina helenae and P. clemata. (Interestingly, Pelegrina aeneola was not found on sagebrush associated plants but it is often very abundant in organic orchards.) A second group of beneficial arthropods consists of predatory insects and spiders also commonly found in nearby orchards where they are known or presumed to contribute to orchard biocontrol. Insects in this group include Deraeocoris, minute pirate bugs, damsel bugs, green and brown lacewings, and certain lady beetles. Spiders include jumpers like Sassacus and several species of Phidippus, the lynx spider Oxyopes, some crab spiders in the genera Misumenops and Xysticus, and several others. The third group is the parasitoids, primarily small wasps. These can be very abundant on some extra-orchard host plants at certain times but few representatives of species important in orchard biocontrol were found in the beat tray samples. Figure 1 presents data for the 3 groups of natural enemies taken on gray rabbitbrush at 1 site during 2002.

Figure 1. Numbers of natural enemies in 3 groups taken in beat tray samples from gray rabbitbrush in 2002. Flowering began between 26 Aug and 5 Sept and ended by 22 Oct. Data for only 6 of 15 collections are shown for simplicity.



During the pre-bloom period of gray rabbitbrush (sampling actually began on 10 July) samples contained low numbers of parasitoids and orchard-associated predators. Moderate numbers of extra-orchard associated natural enemies were present during this period and numbers of natural enemies in this group remained quite stable throughout the sampling period. As gray rabbitbrush came into flower, however, parasitoids and orchard-associated predators increased markedly. Minute pirate bugs increased from 4 to 48 (all adults) between 26 Aug and 6 Sept and the crab spider, Misumenops lepidus, increased from 0 to 12 (small and medium sized immatures). These 2 species were the most abundant predators on gray rabbitbrush during bloom. Both fed on flower thrips which were very abundant during bloom as well. Pirate bugs reproduced on the plant as indicated by the presence of numerous nymphs during the middle bloom period: 19 immatures (57 adults) on 24 Sept and 44 immatures (48 adults) on 1 Oct. Immature numbers are actually too low because first and second stage nymphs were all but impossible to see on the beat tray. The parasitoid fauna during the flowering period was dominated by several species of tiny wasps in the family Platygastridae whose numbers increased from 18 to 126 between 26 Aug and 5 Sept. The host for these wasps is probably a species of small fly associated with the rabbitbrush.

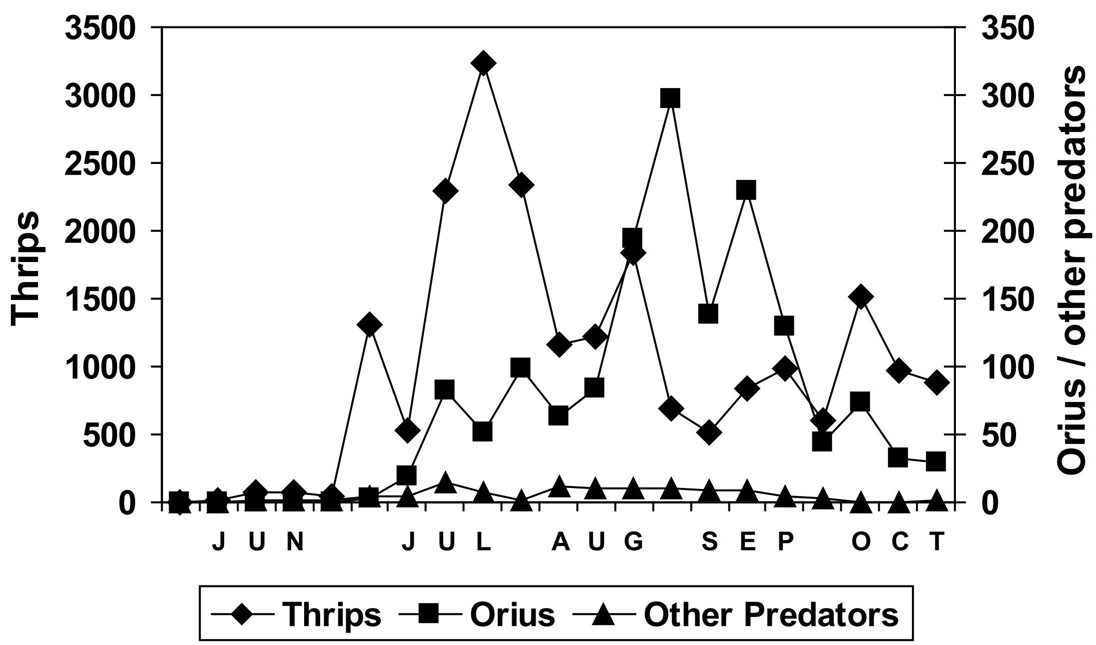
This pattern of orchard-associated natural enemy abundance was also observed on gray rabbitbrush at the other 2 sample sites and, indeed, a generally similar pattern was seen on the other intensively sampled plants as well – that is a marked increase in the number of orchard-associated natural enemies during the bloom period.

Phenology and hosts of western flower thrips and its predators: Flowers and foliage were collected from 126 species of native and introduced plants in extra-orchard habitats near our study orchards (see Appendix 2 for a complete list). Collections from many species were obtained during the pre-bloom, bloom, and post-bloom periods for a total of more than 800 samples. Most of the samples will be processed this winter but data for tall buckwheat (Eriogonum elatum) will serve as an example.

Tall buckwheat was abundant at 2 study sites in the Cowiche-Tieton area where the extra-orchard habitat was little disturbed and the native flora very diverse. Population dynamics of thrips and their predators on tall buckwheat at 1 of the sites are shown in Figure 2.

Tall buckwheat proved to be a very interesting plant with a long flowering period. First bloom was noted on 10 June and the last flowers had not dried up until mid-October. Samples were collected at approximately weekly intervals throughout this period. Most thrips were Frankliniella, the western flower thrips, although some Thrips sp. were also present. Thrips numbers built up rapidly during late June and early July by which time the buckwheat was in good bloom. Immature thrips began to dominate the collections by early July and heavily outnumbered adults until early October, when their numbers fell below those of the adults. Minute pirate bug adults showed up in low numbers on 1 July and nymphs were present in the 11 July collection. Pirate bug numbers built to a peak on 27 August then gradually declined, but adults and a few last stage nymphs were still present on 15 October when the plant was all but out of bloom. Another important group of thrips predators were small immatures of several genera of spiders that were present in low numbers (at least relative to pirate bugs) throughout the flowering period of the plant. In beat tray samples from tall buckwheat and other plants these spiders were frequently observed with thrips as prey. In many cases there appeared to be few suitable alternative prey for these small, immature predators. Important species included the jumping spiders, Sassacus papenhoei and Phidippus spp., and the crab spiders, Misumenops lepidus and Xysticus cunctator, all of which are commonly found in apple and pear orchards. Population dynamics of thrips and their predators were similar on tall buckwheat at the second study site.

Figure 2. Numbers of thrips and thrips predators (the minute pirate bug, Orius, and others, primarily small, immature spiders) extracted via Berlese funnel from samples of tall buckwheat flowers at a site near Cowiche, WA in 2002.



Berlese funnel collections from the other plants that were sampled are currently being processed. For most species the number of samples was fewer than that collected from tall buckwheat as no other species flowered for such an extended period of time. A general impression, based on superficial examination of "alcoholized" samples that had been processed through the Berlese funnels, was that thrips occurred on most of the plant species that we sampled, sometimes in high numbers. Pirate bugs and other predators occurred on many species as well.

Survey of extra-orchard host plants for leafrolling caterpillars and their parasitoids: Approximately 400 larvae that exhibited some type of leafrolling or leaf-tying behavior were obtained from 33 species of plants in extra-orchard habitat either adjacent to or at some distance from orchards. To date more than 150 adult moths and about 100 parasitoids have been obtained with about 80 specimens still in rearing. Some of the moths appear to be in the family Tortricidae (as are the pandemis and oblique-banded leafrollers) whereas others represent other families. The vast majority of the parasitoids are small wasps (Hymenoptera) representing several families including Braconidae, Ichneumonidae, and Chalcidoidea. A few flies in the family Tachinidae have also been obtained. Several hyperparasitoids were apparently reared as well. It does not appear that Colpoclypeus florus, the most important leafroller parasitoid, was obtained. Moths and parasitoids will be sent to experts for identification.

Plants that were more heavily infested with leafrolling type caterpillars included various species of lupine (Lupinus), alder (Alnus), chokecherry (Prunus virginiana), tall buckwheat, Thompson's paintbrush (Castilleja thompsonii), and deerbrush (Ceanothus integerrimus).

An interesting additional bit of information that came to light as a result of the leafroller survey was the fact that alder was inhabited by quite high numbers of 2 species of sac spider (Clubionidae), Clubiona moesta and C. pacifica. These spiders were often associated with rolled alder leaves and were sometimes found in leafrolls. Clubiona species are in the same family as Cheiracanthium mildei which, observations have shown, may be a very efficient leafroller predator in orchards. Many of the rolled alder leaves showed evidence of predation reminiscent of C. mildei, particularly small holes (2-5 mm in diameter) cut in the leaves. C. mildei cut such holes when attacking leafrollers in their leafrolls during laboratory observations. It would be interesting to determine if the Clubiona species were in fact preying on the alder leafrollers and if they use some of the same specialized predatory behaviors employed by C. mildei, such as cutting holes in leafrolls.

In summary, this research has shown that many of the predatory insects and spiders found in orchards, where they are known or thought to contribute to control of certain orchard pests, also occur on a variety of plants in extra-orchard habitats. Outside the orchard, many of these beneficial arthropods appear to be exploiting alternative prey. Their numbers on some plants can be high, especially around flowering, and colonization of suitable plants by some species occurs rapidly. Presumably, movement from extra-orchard habitat into nearby orchards can occur rapidly as well, if conditions are favorable. Population increase can occur in extra-orchard habitat as some species reproduce on extra-orchard host plants. Since extra-orchard habitats are less subject to human disturbance than orchards, beneficial arthropod populations may be more stable and a principal benefit of extra-orchard habitat may be as a repository of these organisms from which the orchard can draw.

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: 2001-2002

Project total (2 years): $56,130

Year	Year 1 (2001)	Year 2 (2002)
Total	26,620	29,510

Appendix 1: Families and species of extra-orchard host plants sampled for natural enemies in 2001. n = native species, i = introduced species

Sunflower family: Big sage (n), Rigid sage (n), Canada thistle (i), Meadow goldenrod (n), Gray rabbitbrush (n), Green rabbitbrush (n), Annual sunflower (n), Yarrow (n), Spotted knapweed (i), Yellow starthistle (i), Balsamroot (n), Fleabane (Erigeron sp.)(n), Hoary aster (n)
Rose family: Bitterbrush (n), Wild rose (Rosa sp.)(n), Big-leaf maple (n), Ocean spray (n), Himalayan blackberry (i), Goatsbeard (n), Chokecherry (n), Bitter cherry (n)
Mustard family: Tumble mustard (i)
Pea family: White sweet clover (i), Lupine (n), Scotch broom (i), Vetch (i)
Snapdragon family: Common mullein (i), Common toadflax (i)
Willow family: Willow (n), Cottonwood (n)
Milkweed family: Showy milkweed (n)
Goosefoot family: Russian thistle (i), Hopsage (n)
Buttercup family: Western clematis (n)
Honeysuckle family: Snowberry (n), Blue elderberry (n)
Barberry family: Oregon grape (n)
Buckthorn family: Deerbrush (n)
St. John's Wort family: Common St. John's Wort (i)
Buckwheat family: Tall buckwheat (n), unidentified wild buckwheat (n), Curly dock (i)
Dogbane family: Flytrap dogbane (n)
Evening primrose family: Fireweed (n)
Parsley family: Desert parsley (n)
Mint family: Peppermint (n)
Morning glory family: Field bindweed (n)
Currant family: Golden currant (n)
Beech family: Oak (n)
Pine family: Ponderosa pine (n), Douglas fir (n)

Appendix 2: Families and species of extra-orchard host plants sampled for western flower thrips and its predators in 2002. n = native species, i = introduced species

Sunflower family: Linear-leaf daisy (n), Oregon sunshine (n), Shaggy daisy (n), Gray and Green rabbitbrush (n), Arrowleaf and Hooker's balsamroot (n), Yarrow (n), Western groundsel (n), Thread-leaf fleabane (n), Long-leaf and Western hawksbeard (n), Poverty sumpweed (n), Microseris troximoides (n), Rayless goldenweed (n), Bachelor's button (i), Wavyleaf thistle (n), Diffuse knapweed (n), Big sagebrush (n), Unidentified Artemisia (n), Chaenactis (n), Hoary aster (n), Cusick's sunflower (n), Prickly lettuce (i), Ragweed ? (n), Canada goldenrod (n), Canada thistle (i), Western salsify (i), Tidytips (n), False dandelion (n), Spotted knapweed (i)

Rose family: Cinquefoil (n), Bitterbrush (n), Saskatoon berry (n), Wild rose (n), Chokecherry (n), Bitter cherry (n), Ocean spray (n), Strawberry (n), Himalayan blackberry (i), Hawthorn (n)

Mustard family: Tumble mustard (i), Prairie rocket (n), Clasping pepperweed (i), Flixweed (i), Blue mustard (i), Dagger pod (n), Thick-leaf thelypody (n)

Pea family: Lupine (>1 species sampled)(n), White and Red clover (i), White and Yellow sweet clover (i), Alfalfa (i), Milkvetch (n), Scotch broom (i), Vetch (n?), Rabbit-foot clover (i)

Snapdragon family: Lowly penstemon (n), Thompson's paintbrush (n), Common mullein (i), Dalmatian toadflax (i), Small-flowered blue-eyed Mary (n)

Willow family: Slender willow (n)

Milkweed family: Showy milkweed (n)

Goosefoot family: Russian thistle (i), Lambsquarters (i), Kochia (i), Hopsage (n)

Buttercup family: Western clematis (n), Larkspur (n), Buttercup (n)

Honeysuckle family: Orange honeysuckle (n), Snowberry (n), Blue elderberry (n)

Barberry family: Oregon grape (n)

Buckthorn family: Deerbrush (n), unidentified Ceanothus (n), Cascara (n)

St. John's Wort family: Common St. John's wort (i)

Buckwheat family: Heart-leaf, Thyme-leaf, Strict, Parsnip-flowered, Snow, Tall, and Slenderbush buckwheat (all n), Curly dock (i)

Dogbane family: Flytrap dogbane (n)

Evening primrose family: Fireweed (n)

Parsley family: Columbia, Gray's, and Bare-stem desert parsley (all n), Queen Anne's lace (i)

Mint family: Peppermint (n), Purple sage (n)

Currant family: Squaw currant (n)

Purslane family: Miner's lettuce (n)

Madder family: Annual bedstraw (n)

Lily family: Cluster lily (n), Wild onion (n), Death camas (n), Mariposa lily (n)

Waterleaf family: Dwarf waterleaf (n), Silver-leaf and Thread-leaf phacelia (n)

Maple family: Big-leaf maple (n)

Dogwood family: Red osier dogwood (n)

Mallow family: Orange globe mallow (n), gooseberry-leaved globe mallow (n)

Hydrangea family: Mock orange (n)

Borgage family: Fiddleneck (n), Cryptantha (n), Popcorn flower (n), Bluebell (n), Puccoon (n), Great hound's tongue (n)

Phlox family: Collomia (n), Cushion, Showy, and Long-leaf Phlox (n), Scarlet gilia (n)

Saxifrage family: Alumroot (n), Prairie star flower (n)

Sandalwood family: Bastard toadflax (n)

FINAL REPORT
WTFRC Project # AE-01-55 **WSU Project # 13C-3643-3326**

Project title: RAYNOX for suppression of insects in apple and pear

PI: Larry Schrader, Horticulturist
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Co-PIs: Jay F. Brunner, Elizabeth H. Beers and John Dunley
Affiliation: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Objectives:
1. Establish the optimal timing, rates and application frequency for RAYNOX against selected pests of apple and pear.
2. Determine whether RAYNOX has negative effects on natural enemies.
3. Investigate the mechanism(s) by which RAYNOX deters certain pests.
4. Study efficacy of RAYNOX as a stabilizer or protectant for insecticides.

Significant findings:
From 2000:
- RAYNOX significantly suppressed first and second generation codling moth (CM) in apple but did not provide adequate control to stand alone for controlling CM.
- In a rate and timing trial on second generation CM, all rates of RAYNOX provided about 50% control of CM. All RAYNOX treatments were as good as Confirm 2F + Orchex 796 oil.
- In a direct choice bioassay, apples were dipped in RAYNOX and then neonate CM larvae were added. Only 10.6% of CM neonates were found on the apples treated with RAYNOX. Entries by CM into fruit were significantly lower with RAYNOX as compared to the control.
- With leaf disk bioassays, choice tests showed that RAYNOX deterred colonization of Pandemis leafroller (PLR) and lacanobia fruitworm (Ls) larvae. For PLR, only 22% chose RAYNOX-treated disks. For Ls, 17% chose RAYNOX-treated disks.
- RAYNOX was effective against white apple leafhopper (first and second generation). RAYNOX was significantly better than untreated controls for first generation. Results for second generation were less definitive. RAYNOX and Sevin were not statistically different.
- A single application of RAYNOX in early August provided a commercially acceptable level of mite suppression.
- With pears, RAYNOX shows promise for control of psylla and CM.

From 2001:
- RAYNOX was compared at three rates in field trials to Orchex 796 and Assail 70WP for control of CM in apple. RAYNOX at all rates [5%, 10% and 20% (v:v)] significantly reduced first generation CM injury versus untreated control (60% injury in control). However, Orchex and Assail were more effective than RAYNOX.
- At harvest, only the high rate (20%) of RAYNOX had less fruit injury than the untreated control (92% injury in control) and was similar to the Orchex treatment. The Assail treatment had the least CM injury (data not shown). This confirms our earlier conclusion that RAYNOX will not serve as a stand-alone treatment for CM but may be used to augment IPM programs.
- Laboratory bioassays with leafrollers were used to test efficacy of RAYNOX as a spreader/sticker as well as a UV light inhibitor. The efficacy of Deliver (*Bacillus thuringiensis*) mixed with RAYNOX was not significantly different from Deliver alone with PLR or obliquebanded

leafroller (OBLR). This indicates that the Bt product was still accessed by the pest.

- Leaf disk bioassays were used to evaluate RAYNOX as a spreader/sticker as well as a UV light inhibitor with a leafroller granulosis virus (Virosoft OB). Virosoft OB was evaluated alone and in combination with RAYNOX for residual effects on PLR and OBLR. Virosoft OB shows promise as an insecticide for both leafroller larvae. The efficacy of Virosoft OB embedded in RAYNOX was similar to Virosoft OB alone, indicating that the virus product was still accessed by the pest. Both PLR and OBLR bioassays were conducted in late summer so the full potential of RAYNOX as a pesticide protectant was probably not realized.
- Western tentiform leafminers were suppressed about 50% in the bottom half of trees when RAYNOX, Surround and Intrepid were applied to trees before exposing trees to leafminer adults. RAYNOX suppressed the mines per leaf more than 80% in the top half of the trees and was as effective as the other products.
- Neither RAYNOX nor Surround was effective in significantly reducing seasonal nymph populations of *Campylomma verbasci* or in reducing fruit damage by Campylomma. Early season applications of Actara and Carzol were most effective.
- With pears, three prebloom applications of RAYNOX were usually as effective as full-season applications. RAYNOX and Surround were equally effective in suppressing pear psylla. RAYNOX should be used only prebloom to avoid russeting problems.

From 2002:

- A codling moth granulosis virus (Carpovirusine) was evaluated for residual effects on neonate CM larvae. RAYNOX was used a spreader or UV inhibitor, but proved ineffective in increasing the efficacy of the virus.
- In leaf-disk bioassays, *Bacillus thuringiensis* (Dipel DF) was evaluated for residual effects against obliquebanded leafroller neonate larvae. RAYNOX was used as a spreader or UV inhibitor but was not effective in increasing the efficacy of Dipel DF.
- RAYNOX was mixed with Suterra fluorescent microcapsules as a sticker but did not significantly improve adherence of the capsules to leaves.
- Preference tests done with *Typhlodromus occidentalis,* a predatory mite, showed that these mites prefer untreated leaf disks to those sprayed with a 50% (v:v) solution of RAYNOX.

Methods:

Objective 1: Field trials with several cultivars of apple were conducted with several target insects. Initial tests were conducted with high-pressure handgun spray equipment using a spray volume equivalent to a dilute spray application. The results obtained allowed determination of an activity profile for RAYNOX on the target insects. Treatments were replicated with single trees or small blocks of trees for initial trials. Up to three rates of RAYNOX were evaluated for each target insect to determine a rate effect. Time and frequency of applications were studied for selected insects. For entomological evaluations of pests on foliage, populations of insects such as mites, aphids, leafhoppers, pear psylla and leafminers were evaluated pretreatment and at intervals in the posttreatment period to determine efficacy. For pests such as the codling moth that injure fruit, the level of injury to fruit was evaluated at appropriate intervals during the growing season in each treatment by checking at least 25 fruits per tree or replicate.

Objective 2: The effects on natural enemies were evaluated by sampling for their presence. The effects of RAYNOX on certain natural enemies, predatory mites, parasites of leafminer and leafroller, and general predators such as lady beetles and lacewings were evaluated with bioassays. Various natural enemies were exposed to direct sprays of RAYNOX. Their mortality or tendency to leave treated areas was examined after a period of time determined from preliminary tests. More specific details are provided in Results and Discussion for these studies.

Objective 3: The mechanism of action of RAYNOX appears to be different in some respects from an alternative particle film technology. Surround (kaolin) masks an insect's ability to detect its host plant, irritates or repels pests, or actually causes direct mortality of pests. While RAYNOX may mask host plant cues used by some pests, it may also have direct toxic effects on some life stages. The direct and indirect effects of RAYNOX on selected pests were evaluated in laboratory bioassays against leafroller, lacanobia fruitworm, codling moth, leafhopper and leafminer. Various stages were exposed directly to RAYNOX at different concentrations to determine dose-mortality relationships. The behavioral effects of RAYNOX residues were evaluated by treating plant or fruit surfaces to determine suppression of oviposition activities. Specific details on methods are provided with results in Results and Discussion section.

Objective 4: Various insecticides (e.g., IGRs and Bt) were incorporated with varying concentrations of RAYNOX and applied to leaves or fruit to determine if the product is stabilized by RAYNOX and whether the product is still accessed by the pest. Alternatively, the insecticide was applied first and then followed by an application of RAYNOX to determine if RAYNOX protected the insecticides from rain, irrigation water and UV-B radiation. Field-aged studies of RAYNOX-protected insecticide residues were compared with unprotected residues to determine the utility of this product as a surfactant. Standard bioassays developed to test insecticides for leafrollers and lacanobia fruitworm were used in these tests.

Results and discussion:

I. RAYNOX bioassays to evaluate residual effects on neonate codling moth larvae. Brunner and Doerr—2002.

Codling moth granulosis virus (Carpovirusine) was evaluated for residual effects on neonate CM larvae. Two formulations of RAYNOX were tested as a spreader or UV inhibitor. The test was conducted in 10-yr-old Fuji trees on dwarfing rootstocks at the Wenatchee Valley College orchard. Treatments were applied on 23 Jul with a handgun sprayer at 300 psi to the point of drip, simulating a dilute spray of approximately 400 gal per acre. Each treatment was replicated three times with one tree in each replicate. Five fruits were collected from each tree at 1, 7 and 14 days after treatment (DAT). Codling moth eggs were received on waxed paper sheets from the USDA-ARS laboratory in Wapato, WA. A section of waxed paper containing exactly 10 CM eggs was cut and placed on a treated apple. Apples with the waxed paper in the stem bowl were placed individually in clear plastic deli-containers (Anchor Packaging #409CX, Marmaduke, AR). Deli containers were placed in constant temperature growth chambers (72°F, 16:8 L:D). Eggs hatched in approximately 4-5 days. CM entries were evaluated at 21 DAT, were dissected, and larvae were examined to determine survival. Where possible, two entries were dissected per fruit (20 entries per treatment). A theoretical estimation of the number of surviving codling moth larvae (i.e., avg. no. of entries X % live CM larvae) was presented for comparison against an untreated control (Table 1). Statistical analysis: mean separations were determined by Student's Paired *t*-Test (P=0.05) using JMP statistical software (JMP v. 3.2.6, SAS Institute Inc., 1999).

Table 1. Effect of Carpovirusine with and without RAYNOX on neonate CM larvae.

Treatment	Rate (formulation/100 gal)	% Reduction from control-live CM 1	7	14
Carpovirusine	378.0 ml	72.9b	59.7b	66.2b
Carpovirusine + RAYNOX + clay	378.0 ml	60.8b	46.9b	26.4a
Carpovirusine + RAYNOX - clay	378.0 ml	53.9b	0.0a	19.3a

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired *t*-Test).
Means followed by the letter 'a' not significantly different than the untreated control.
RAYNOX mixtures applied at 10% v:v.

II. RAYNOX bioassays to evaluate residual effects of *Bacillus thuringiensis* against obliquebanded leafroller neonate larvae. Brunner and Doerr—2002. Using a leaf-disk bioassay, *Bacillus thuringiensis* var. Kurstaki (Dipel DF, Valent Ag. Products) was evaluated for residual effects against obliquebanded leafroller neonate larvae. Two formulations of RAYNOX were applied as a spreader or UV inhibitor. The test was conducted in 30-yr-old spur type Delicious on dwarfing roots at the Tree Fruit Research and Extension Center. Treatments were applied on 15 Aug with a handgun sprayer at 300 psi to the point of drip, simulating a dilute spray of approximately 400 gal per acre. Each treatment was replicated three times with one tree in each replicate. Ten leaves were collected from the interior canopy of each tree at 1, 4, 7, and 14 days after treatment (DAT). Two punches (2.3 cm diameter) were taken from each leaf. Four punches were placed in a petri dish (Falcon 1006, 50x9 mm), keeping the leaves from each replication separate. Petri dishes were chosen randomly, and five 1- to 2-day-old leafroller larvae were placed on the leaves. Five petri dishes were prepared for each tree and each leafroller species (75 larvae per treatment). Petri dishes were placed inside a food storage container and kept at 75°F (±2°F) constant temperature and 16:8 photoperiod. Petri dishes were examined after 7 days and larval survival recorded. Statistical analysis: mean separations were determined by Student's Paired t-Test (P=0.05) using JMP statistical software (JMP v. 3.2.6, SAS Institute Inc., 1999).

Table 2. Residue effects of *Bacillus thuringiensis* against obliquebanded leafroller neonate larvae in presence of Dipel DF with and without RAYNOX.

Treatment	Rate (formulation/100 gal)	Avg. corr. % mort (DAT[1]) 1	4	7	14
Dipel DF	113.5 g	100.0c	98.6c	79.1c	31.6b
Dipel DF + RAYNOX - clay	113.5 g 10% v:v	72.1b	64.3b	34.3b	36.8b
Dipel DF + RAYNOX + clay	113.5 g 10% v:v	66.2b	55.7b	40.3b	29.8b

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired *t*-Test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1] DAT- Days after treatment.

III. Effect of different stickers on adherence of Suterra microcapsules to leaves. Brunner and Doerr—2002.

Suterra microcapsules (100 ml of formulated capsules or 115 g) were mixed in 10 gal of water and applied at a rate of approximately 1 gal of water per tree. For each treatment, capsules were loaded with fluorescent dye, and the fluorescent capsules were applied with Nu-Film 17, 5% RAYNOX or No Sticker to five trees. Trees were washed with approximately 800 gal of water per acre, and washing was repeated for a total of three washes. Samples of 10 leaves per tree were taken at each evaluation: at Day 0 (immediately following application), on Day 1 (immediately following washing), and on Day 7 following a rain event. Since this was the initial test using this product, the best light source and means of seeing the capsules was experimental. A black spotlight was used initially. At Day 0 (pre-wash), this light source seemed sufficient. However, the capsules were difficult to see at Day 1. It is not evident if the 90% reduction in capsules was due to the washing or to reduced fluorescence of capsules. Thus, the trees were re-sampled using a more powerful UV spotlight. This light source worked much better, as seen by the higher numbers of capsules found post-wash with UV rather than black light. The average number of capsules found at 7 DAT was down significantly from the post-wash sample. It is not evident if the rain washed capsules off or if fluorescence was reduced due to 7 days of exposure.

Summary: The initial pre-wash sample had significantly more capsules in the RAYNOX treatment than the No Sticker treatment (Table 3). However, at all post-wash evaluations no significant effect of adding RAYNOX to the trees was noted. Neither Nu-Film 17 nor RAYNOX was an effective sticker, as the capsules were easily dislodged by shaking or blowing on the leaf.

Table 3. Effect of stickers on adherence of Suterra capsules to leaves.

		Avg. capsules/leaf			
		Black light		UV light	
Sticker	Rate/100	Pre-wash	Post-wash	Post-wash	Post-rain (7DAT)
No Sticker		9.9b	0.7a	14.0b	3.3b
Nu-Film 17	8 fl. oz.	5.7a	0.7a	7.4a	1.6a
RAYNOX	5 gal.	14.8c	1.2a	11.5b	2.3ab

Means in the same column followed by the same letter not significantly different (p=0.05, Student's paired t-test).

IV. Repellency of *Typhlodromus occidentalis* by RAYNOX. Beers-Peryea—2002.
There is extensive anecdotal evidence that Surround causes mite outbreaks when used on apple foliage. There is little evidence for toxicity to *Typhlodromus occidentalis* (Nesbitt) and some direct mortality of phytophagous mites. Repellency of particle films is thought to be a factor in mite population regulation, but no studies have been done to examine this rigorously. This experiment was conducted to see if the clay-based RAYNOX is repellent to *T. occidentalis.*

Leaves were collected from an unsprayed apple orchard at WSU-TFREC and cut into 40 mm diameter leaf disks. Leaf disks were cut so the mid-vein divided the disk into two equal halves. Eighteen leaf disks were cut and placed with the abaxial surface facing up on cotton pads saturated with water in clear plastic cups. Leaf disks were kept stationary throughout the experiment; each half was designated A or B with permanent marker on the rim of the cup. *T. occidentalis*, a predatory phytoseiid, were taken from a colony reared on waxed paper islands. The eggs of two prey species (twospotted spider mite, *Tetranychus urticae* Koch, in test 1 and European red mite, *Panonychus ulmi* (Koch), in test 2) were used to provide food for the predators during the test. Forty adult female mites of the prey species were transferred to each disk with a camel's-hair brush and allowed to oviposit for 48 h before removing them. The three treatments were designated by the material applied to the disk halves (A and B): A=RAYNOX, B=DW (distilled, deionized water, ddH_2O) (the choice test); A=RAYNOX, B=RAYNOX (the no-choice test); and A=DW, B=DW (check). All RAYNOX treatments were a 1:1 dilution of the concentrate. Treatments were applied to six replicate disks using a hand-pressurized aerosol spray can. When spraying the A=RAYNOX, B=DW treatment, the opposite side of the leaf was protected from the spray with a piece of paper held flush against the mid-vein. After application, the disks were allowed to dry, and Tanglefoot (Tanglefoot Co., Emeryville, CA) was applied in a ring around the edge of the disk to prevent the *T. occidentalis* from running off the disk. After treatments had dried, 20 *T. occidentalis* females per disk were applied with a camel's-hair brush, initially positioned on the mid-vein. Treatments were evaluated every two hours the afternoon of application and the following morning and afternoon, for a total of six to seven evaluations per test. Sides A and B of each leaf disk were evaluated separately for presence of phytophagous mite eggs and *T. occidentalis* eggs and adults. Runoff (into the Tanglefoot barrier) was also recorded at each evaluation.

Data were analyzed using the Statistical Analysis System (SAS 1988). The variable analyzed was the sum of live *T. occidentalis* females found over the course of the experiment. Two approaches were used. The first compared the percentage of *T. occidentalis* on each side given a theoretical 50:50 distribution as a comparison. For this approach, the χ^2 and probability values were calculated during the data step. The second approach used the actual distribution in the control treatment as a comparison (PROC FREQ). The choice and no-choice treatments were compared separately to the control distribution.

Results—Test #1: Initial plans were to assess the prey consumption rate by counting the eggs at each evaluation. However, in the RAYNOX treatment the large decrease in the number of eggs 2 hours after treatment was attributed to the difficulty in discerning the pale green eggs. This introduced a bias that made this parameter unusable. There was a clear tendency on the part of the female predators to avoid resting on the side of the disk with RAYNOX (Table 4, Fig. 0225.1), which was highly significant by either analytical method used. In this test, however, there was also a difference between sides A and B when both were treated with RAYNOX and compared to a theoretical 50:50 distribution, although the χ^2 value was much smaller in magnitude than for the previous comparison. The DW:DW treatment was not significantly different than a 50:50 distribution. In the second analysis, the RAYNOX/RAYNOX treatment did not differ from the DW/DW check.

Results—Test 2: The same trend of RAYNOX avoidance was demonstrated in the second test (Table 5, Fig. 0225.2). In this test (type 1 analysis), all three treatments were different than the 50:50 distribution. However, the RAYNOX/DW treatment had a much higher χ^2 value. In the second analysis, both treatments were different than the distribution in the check, with the RAYNOX/DW treatment having a much higher χ^2 value. This test strongly supports the hypothesis that the dilution of RAYNOX used in this test is highly repellent to the predatory mite *T. occidentalis*. In the absence of evidence of direct toxicity, this mechanism is proposed as being a significant factor in disruption of biological control by a particle film.

Table 4. Behavioral response of twospotted spider mite to a choice and no-choice test with RAYNOX (1:1 dilution), 2002.

	Cum. live mites		Theoretical 50:50 distribution		Comparison to DW/DW check	
Treatment	Side A	Side B	χ^2	*P*	χ^2	*P*
A=RAYNOX, B=DW	102	472	40.25	0.0000***	154.644	0.001***
A=RAYNOX, B=RAYNOX	336	274	6.30	0.0121**	0.314	0.575NS
A=DW, B=DW	281	245	2.47	0.1164NS	----	---- ----

Table 5. Behavioral response of European red mite to a choice and no-choice test with RAYNOX (1:1 dilution), 2002.

	Cum. live mites		Theoretical 50:50 distribution		Comparison to DW/DW check	
Treatment	Side A	Side B	χ^2	*P*	χ^2	*P*
A=RAYNOX, B=DW	87	277	99.18	0.0000***	92.753	0.0010***
A=RAYNOX, B=RAYNOX	252	205	4.87	0.0279**	2.87	0.0900*
A=DW, B=DW	171	107	14.74	0.0001***	----	---- ----

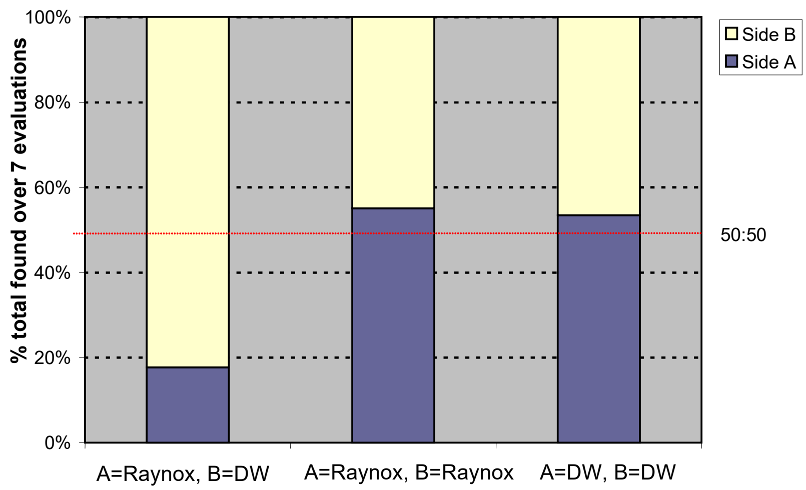


Fig. 0225.1. Distribution of live mites (twospotted spider mite) on treated or untreated apple leaf disk halves, 2002.

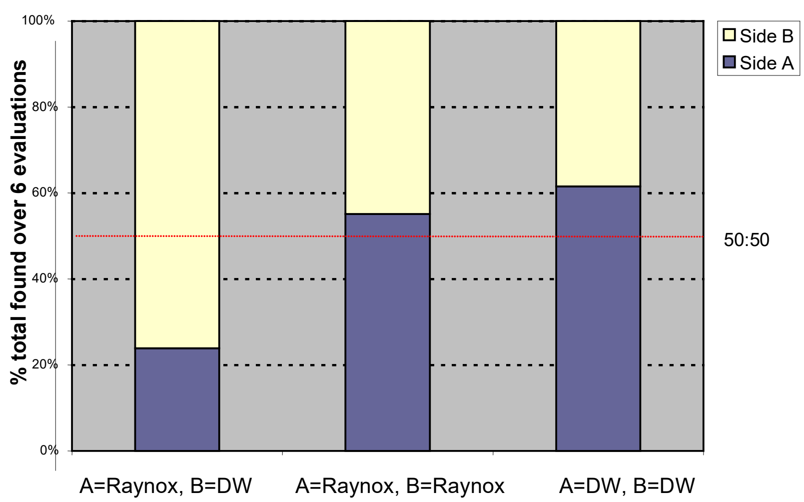


Fig. 0225.1. Distribution of live mites (European red mite) on treated or untreated apple leaf disk halves, 2002.

V. Bioassays with RAYNOX and Deliver on mortality of Pandemis leafroller (PLR) (*Pandemis pyrusana* Kearfott) and obliquebanded leafroller (OBLR) (*Choristoneura rosaceana* Harris). Brunner and Doerr—2001.

Using a leaf-disk bioassay, *Bacillus thuringiensis* var. Kurstaki (Deliver, Certis USA Crop Protection) and RAYNOX were evaluated alone and in combination for residual effects on PLR and OBLR neonate larvae. RAYNOX was added to the Deliver treatment to test its efficacy as a spreader/sticker and as a UV light inhibitor. The test was conducted on 15-yr-old spur-type Delicious on dwarfing roots. Treatments were applied on 6 September with a handgun sprayer at 300 psi to the point of drip, simulating a dilute spray of approximately 400 gal per acre. Ten leaves were collected from the interior canopy of each tree at 1, 4, 7, and 14 days after treatment (DAT). Two disks (2.3 cm diameter) were taken from each leaf. Four disks were placed in a petri dish (Falcon 1006, 50x9 mm), keeping the leaves from each replication separate. Five 1- to 2-day-old leafroller larvae were placed on the leaves. Five petri dishes were prepared for each tree and each leafroller species (75 larvae per treatment). Petri dishes were examined 7 DAT and larval survival recorded.

Significant mortality was noted in the Deliver treatment against both PLR and OBLR neonate larvae at 1 DAT (Table 6). The level of mortality caused by the Deliver treatment in this bioassay was moderate relative to that seen in field-aged bioassays of other Bt products. Low mortality levels from the Deliver treatment were noted at 4 DAT and 7 DAT against both species. The 4 DAT evaluation against OBLR and the 7 DAT evaluation against PLR were not significantly different than the untreated control. No significant mortality was noted against either species at 14 DAT. The RAYNOX-only treatment resulted in a significant but low level of mortality against PLR neonate larvae and no significant mortality against OBLR neonate larvae at 1 DAT. No significant mortality was noted against either species at 4 DAT or 7 DAT. The RAYNOX-only treatment was not evaluated at 14 DAT. The addition of RAYNOX to Deliver did not significantly increase efficacy or residual activity of the Bt product. There was no apparent difference in susceptibility of either leafroller species to Deliver or RAYNOX.

Table 6. Effect of RAYNOX and Deliver on mortality of leafrollers.

	Rate/	Avg. corr. % mortality- PLR			
Treatment	100 gal	1 DAT[1]	4 DAT	7 DAT	14 DAT
Deliver	113.5 g	60.9bc	16.7b	13.0ab	4.1a
Deliver + RAYNOX	113.5 g +10% v:v	82.6c	22.2b	24.6b	28.0
RAYNOX	10% v:v	36.2b	1.4a	0.0a	---

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1]DAT = days after treatment.

	Rate/	Avg. corr. % mortality- OBLR			
Treatment	100 gal	1 DAT[1]	4 DAT	7 DAT	14 DAT
Deliver	113.5 g	57.5b	8.8a	16.7b	4.5a
Deliver + RAYNOX	113.5 g + 10% v:v	38.4b	20.6a	22.2b	4.5a
RAYNOX	10% v:v	10.9a	4.4a	1.4a	---

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1]DAT = days after treatment.

VI. Bioassays with Virosoft OB (leafroller granulosis virus) and RAYNOX on Pandemis leafroller (PLR); *Pandemis pyrusana* Kearfott and obliquebanded leafroller (OBLR); *Choristoneura rosaceana* (Harris). Brunner and Doerr—2001.

Using a leaf-disk bioassay, a leafroller granulosis virus (Virosoft OB, BioTepp, Inc., Charlesbourg, Qc, Canada) and RAYNOX were evaluated alone and in combination for residual effects on PLR and OBLR neonate larvae. RAYNOX was added to the Virosoft OB treatment to test its efficacy as a spreader/sticker as well as a UV light inhibitor. Methods were as described above for Bt tests. Petri dishes were examined up to 14 DAT and larval survival recorded.

Very little neonate larval mortality was noted after 7 days exposure to the virus treatments, and thus only the 14-day exposure data are presented (Table 7). Significant mortality was noted with both PLR and OBLR neonate larvae at each evaluation date with the exception of the 7 DAT bioassay against OBLR. However, significant OBLR neonate larval mortality was again noted at 14 DAT. It appeared that the relative efficacy of Virosoft OB declined at the 7 DAT evaluation but did not continue to decline through the 14 DAT evaluation. The RAYNOX-only treatment resulted in a significant but low level of mortality against PLR neonate larvae and no significant mortality against OBLR neonate larvae at 1 DAT. The RAYNOX-only treatment was not evaluated at 14 DAT. The addition of RAYNOX to Virosoft OB did not significantly increase efficacy or residual activity of the Bt product, but RAYNOX did not affect access of the pest to Virosoft OB. There was no apparent difference in susceptibility of either leafroller species to Virosoft OB or RAYNOX. These bioassays indicate that the Virosoft OB formulation is a promising insecticide for the management of both PLR and OBLR larvae. It appeared that at least 10 days were needed before larval mortality could be detected. This test was conducted relatively late in the growing season and thus the growth of new foliage was minimal and UV exposure was less than what would be observed when the product would be used for pest management.

Table 7. Effect of RAYNOX and Virosoft on mortality of leafrollers.

Treatment	Rate/ 100 gal	Avg. corr. % mortality- PLR (14 d)			
		1 DAT[1]	4 DAT	7 DAT	14 DAT
Virosoft OB	23.7 fl oz	78.6c	86.6b	50.0b	75.9b
Virosoft OB + RAYNOX	23.7 fl oz 10% v:v	92.9c	93.3b	63.0b	62.5b
RAYNOX	10% v:v	36.2b	1.4a	0.0a	---

Means within the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1]DAT = days after treatment.

Treatment	Rate/ 100 gal	Avg. corrected % mortality—OBLR (14d)			
		1 DAT[1]	4 DAT	7 DAT	14 DAT
Virosoft OB	23.7 fl oz	70.8b	90.5b	17.4ab	65.0b
Virosoft OB + RAYNOX	23.7 fl oz 10% v:v	81.3b	88.1b	50.0b	55.0b
RAYNOX	10% v:v	10.9a	4.4a	1.4a	---

Means within the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1]DAT = days after treatment.

VII. Control of pear psylla with particle film materials and conventional compounds. Dunley,

Greenfield and Bennett—2001.
These tests were performed to compare different timings and combinations of particle film technologies [Surround (Kaolin), RAYNOX] and conventional treatments to give seasonal control of pear psylla on pear. Treatments were applied to deliver 200 gpa on prebloom applications and 100 gpa on postbloom applications. All insect counts were made on the middle tree of the middle row of each plot or, in the case of the RAYNOX treatments, on each of the treated trees. The conventional prebloom treatments were followed either by conventional postbloom sprays or applications of either RAYNOX or Surround. Conventional postbloom treatments were preceded by conventional prebloom treatments or prebloom applications of either RAYNOX or Surround. Pear psylla adult counts were made by using a beating tray and counting the number of adults on four trays. Pear psylla egg and nymph counts up through 19 April were made by collecting five spurs per plot, examining them under magnification and counting the number of eggs and nymphs. Beginning 28 April, 25 leaves were collected from each plot and brushed with a standard mite-brushing machine onto a glass plate. Plates were examined under magnification and the number of eggs and nymphs counted and recorded.

In general, the particle film technologies (PFT) were successful in controlling pear psylla nymphs (the damaging stage) when compared to a conventional pear psylla management program. Prebloom applications of Surround or RAYNOX followed by a conventional spray program appear to provide adequate control of pear psylla eggs (data not shown) and nymphs (Table 8). The half rate of RAYNOX followed by a conventional program provided similar control to the other prebloom PFT treatments. The seasonal Surround program also maintained pear psylla at less than damaging levels, as did the conventional treatment. The postbloom treatments, with conventional prebloom treatments did not perform as well as those treatments that had PFT prebloom. It appears that the prebloom timing of PFT applications is important in reducing pear psylla for the postbloom period.

Table 8. Mean pear psylla nymphs in samples collected from particle film technology treatments.

	Nymphs/spur		Nymphs/leaf						
	20-Apr	27-Apr	7-May	16-May	30-May	11-Jun	22-Jun	12-Jul	27-Jul
Check	3.22a	13.40ab	0.88ab	1.40a	0.72a	0.38a	1.12a	7.08a	2.34a
Surround + Conventional	0.15b	0.80b	0.16b	0.14c	0.12b	0.10a	0.12b	2.20b	1.50a
Conventional + Surround	0.50b	3.55ab	0.58ab	0.94ab	0.28b	0.16a	0.14b	1.88b	1.22a
Surround	0.05b	3.35ab	0.08b	0.06c	0.02b	0.10a	0.10b	1.38b	1.06a
Conventional	0.45b	18.60a	1.24a	0.34bc	0.32b	0.18a	0.32b	2.56b	1.68a
RAYNOX + Conventional	0.10b	0.95b	0.00b	0.12c	0.16b	0.28a	0.24b	2.68b	1.70a
Conventional + RAYNOX	0.25b	16.80a	1.02ab	0.68bc	0.18b	0.30a	0.36b	1.90b	1.92a
RAYNOX	0.00b	1.10b	0.12b	0.02c	0.06b	0.14a	0.46b	2.68b	1.82a
RAYNOX rate + Conventional	0.15b	9.20ab	0.16b	0.28bc	0.00b	0.02a	0.34b	3.32b	1.80a

Means within the same column followed by the same letter are not significantly different (Student-Newman-Keuls, *P*=0.05).

Budget:
Project title: RAYNOX for suppression of insects in apple and pear
PI: Larry Schrader
Project duration: 2000-2002
Project total (3 years): $60,000

Year	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Total	20,000	20,000	20,000

Current year breakdown

Item	Year 1 (2000)	Year 2 (2001)	Year 3 (2002)
Salaries	0		
Benefits (%)	0		
Wages	13,800	13,800	13,800
Benefits (16 %)[1]	2,200	2,200	2,200
Equipment	0	0	0
Supplies	3,000	3,000	3,000
Travel[2]	1,000	1,000	1,000
Miscellaneous	0	0	0
Total	20,000	20,000	20,000

[1]Fringe benefits are 16% for time-slip employees.
[2]Travel related to data collection for project.

Other support: We received $23,500 for 2002 from the Washington State Commission on Pesticide Registration.

Summary of total cost: $60,000

FINAL REPORT
WTFRC Project #AE-98 **AAFC Project #A001063**

Project Title: Use of field wind tunnels to understand mechanisms of communication disruption and improve delivery and development of multiple-species mating-disruption systems

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

Objectives:
1. To examine the role of sensory overload and camouflage as mechanisms of mating disruption in codling moth and leafrollers, and there importance in different pheromone delivery systems.
2. Correlate mating and flight behaviour of insects with airborne pheromone concentration data.
3. Measure concentrations of airborne pheromone and correlate with EAG measurements.

Significant Findings:
- Demonstrated the long-term (2-h) behavioural effects of pheromone sensory overload on codling moth and its *absence* in oblique-banded (OBLR) and *Pandemis* leafroller (PLR)
- Characterized the time, dose, and time-dose interaction to achieve this effect and for recovery
- Showed that long-term antennal adaptation, not central nervous system habituation, is the mechanism by which sensory overload causes communication disruption in codling moth
- Demonstrated a dose-dependent disruption effect with 3M-LRX sprayable micro-encapsulated (MEC) pheromone on OBLR and PLR in laboratory flight tunnels developed under this project
- Characterized the four-week disruption activity of 3M-LRX sprayable against OBLR and PLR in unique field flight tunnels and its six-week longevity in orchard trials and commercial utility
- Showed in field flight tunnels that one application of 3M-LRX sprayable (40 g ai/acre) was equivalent to Isomate CM/LR (200 dispensers / acre) as a leafroller disruptant, for first four weeks
- Demonstrated a clear relationship between airborne pheromone concentration delivered by 3M-LRX MEC sprayable and disruption of OBLR and PLR in the laboratory flight-tunnel system
- Correlated EAG detection of pheromone plumes in backgrounds of air laden with sprayable pheromone disruptant and showed that camouflage of EAG's was predicted by background levels
- Showed that contact with treated leaves was important in seeing the full impact of sprayable pheromones on disruption of leafrollers; reconciling differences between lab and field assays
- Demonstrated that Suterra CM-F sprayable (20 g ai/acre) disrupted codling moth activity for ca. 7-10 days, whereas 3M-CM Phase IV-e^{+} (20 g ai/acre) had no disruption effect in field flight tunnels
- Showed that airborne concentration of codlemone released from Suterra CM-F (151.8 pg•h•cm^2 day 2) was 110*x* higher than from 3M-CM sprayable (1.38 pg•h•cm^2) when applied at 20 g ai/acre
- Contact with 3M-CM MEC sprayable-treated leaves had no effect on disruption of codling moth
- Using leaf tissue extraction and GC-MS determined that leaf surface residues of codlemone were ca. 250 ng•cm^2 24 h after applying 3M-CM Phase IVe^{+} MEC (20 g ai/acre) with an

airblast sprayer

- Demonstrated a clear lack of disruption efficacy with 3M-CM Phase IV-s, IV-e & IV-e$^+$ MEC sprayable formulations against codling moth in laboratory and field flight-tunnels assays
- Showed that current MEC sprayable pheromone formulations are inadequate for control of codling moth because they release too little pheromone to cause sensory overload or camouflage

Materials and Methods:

Objective 1. Pheromone *sensory overload* was studied in codling moths and leafrollers (OBLR & PLR) using static and dynamic air flow delivery systems. In the static air system, male moths were held for various lengths of time, in sealed 1-L glass mason jars containing various amounts of synthetic pheromone released from filter paper discs. Concentration of pheromone in mason jars was measured using a solid phase micro-extraction system (SPME) and gas-chromatographic (GC) analysis. In a dynamic airflow system moths were held in screen cages in a miniature (60 cm *x* 15 cm *x* 15 cm) wind tunnel downwind of Conrel® fibres delivering varied amounts of pheromone (1 – 20 ug•h).

One experiment examined sensory overload of codling moths under field conditions. Moths were placed in metal screen cages and hung about mid-canopy height for 24 h between adjacent apple trees of an orchard (10 *x* 15 ft. tree-row spacing) that had been treated with 400 Isomate-C$^+$ dispensers per acre one week earlier. After all exposure treatments flight of pre-exposed moths to synthetic pheromone lures, or live females, was compared to untreated moths. Depending on the specific experiment being conducted or behaviour being examined, moths were flown individually or in groups of moths in a laboratory flight tunnel.

Based on results of behavioural experiments antennal responses of pre-exposed and naïve male codling moths to a broad dose range of codlemone was measured using a Syntech EAG system. Moth antennae were excised, mounted with electrolytic gel to silver electrodes, and challenged with a series of pheromone puffs of increasing and then in reverse order, decreasing concentrations. All pheromone puffs were preceded and followed by puffs of the green leaf volatile *E*2-hexanal, responses to which were used to normalize pheromone responses. Single antennae from each of 10 pre-exposed and 10 naïve moths were analyzed and compared.

Objective 2. Pheromone researchers have long sought to measure airborne concentrations of disruptant pheromone and correlate this concentration with observed disruption data, be that trap catches, mating, or crop damage, in order to provide predictability to pheromone technology as a pest-control tool. The intermittent, dynamic nature of pheromone plumes generated by delivery systems like hand-applied dispensers or puffers makes the expression of pheromone concentration per volume of air dubious. In order to address objective 2 a decision was made in Year 3 to focus on the mode-of action of sprayable pheromones, because of all the current pheromone delivery systems, the physical nature of sprayable pheromones, with their uniformity in distribution, lack of definable plumes, lack of attraction (eliminating false-trailing effects), and potential to *camouflage* natural pheromone plumes causing sensory overload should make its efficacy more directly related to airborne concentration levels than any other system.

Laboratory Camouflage Studies: A pulling-type (35 cm•sec) laboratory flight tunnel (1.8 m long *x* 0.75 m wide *x* 0.75 m high) with disposable outer "skin" was constructed from plastic acetate sheeting and a steel frame to test the *efficacy x concentration* relationship of MEC pheromones. Uniform clouds of MEC pheromone were created within the tunnel flight section by spraying known amounts of MEC pheromone on stainless steel slats, or 3M Filtrete® fibre cloth that formed the upwind end of the tunnel through which air was drawn. This experimental design mimicked conditions thought to be typical of MEC formulations in the field. Airborne concentrations of pheromone were varied by spraying different amounts of active ingredient (ai) on the upwind surfaces and by aging sprayed materials before testing. Moths were flown in groups of 5-15, 4 h after applying a treatment and at 24-h intervals thereafter, until response in the pheromone tunnel equalled response of reference flights done in a clean air tunnel simultaneously. Moth response was measured by catches in traps baited with "calling females", female extracts or synthetic lures. Overlaying time sequence behavioural observations on pheromone loss curves provided by 3M engineers, the airborne concentration at which MEC pheromone camouflaged and prevented location of females, female extract or synthetic lures was described.

Pheromone Dose Response Flight Curves. Using a peso-electric micro-sprayer (El-Sayed *et al*. 1999) that delivers pheromone plumes of precisely known concentrations, the optimal concentration for male moth attraction and source contact was determined for codling moth and leafrollers. By varying plume emission rate it was possible to mimic pheromone release by female moths and indirectly test the hypothesis that when airborne pheromone disruptant concentrations, as predicted from 3M pheromone loss curves, were above levels of attractive plumes behavioural responses were camouflaged and disrupted.

Field Wind-Tunnel Studies: While the laboratory flight tunnel proved useful for evaluating effects of sprayable pheromone concentration, ultimately formulations had to be tested under more natural conditions. Following the example of Cardé et al. (1998), 3 field flight tunnels were built that enabled application of MEC pheromone to trees while providing control over other experimental conditions. Behavioural tests to assess levels of mating disruption were conducted within field wind tunnels (6.4 m long *x* 4.4 m wide *x* 2.4 m high) enclosing 18-36 potted apple trees that were removed from tunnels and sprayed at recommended field rates using either backpack, handgun, or airblast sprayers, air dried and returned to tunnels. Water-treated trees in a separate tunnel served as a control for all experiments. Marked moths were released in trees from the downwind end of tunnels. Levels of disruption were assessed by moth recapture in 2-4 traps baited with females, or synthetic lures hung in trees at the upwind end. Tunnels were designed to allow simulated rainfall events using overhead irrigation to test effects of sticking agents. During this project experiments were conducted on several different MEC formulations for codling moth (3M-CM Phase IV-s, IV-e, IV-e$^+$ and Suterra CM-F) and leafrollers (3M- LRX Phase I and V). Isomate-C$^+$ and Isomate-CM/LR "Dual" were tested as standards for comparison.

Orchard Studies: 3M sprayable pheromones were tested in non-replicated trials in a 1-ha apple orchard at the Summerland Research Centre. 3M-CM (Phase IV-e) was applied at 20 g ai/acre and tank mixed with leafroller LRX (Phase V) applied at 30 g ai/acre in 2001 and 2002. An adjacent untreated block served as a control each year. Codling moth was monitored in each block with two, 1 and 10 mg codlemone-baited traps and two virgin female-baited traps. Each leafroller species (OBLR & PLR) was monitored with two, 3-mg synthetic pheromone-baited traps in each block. Wild moth populations were augmented with weekly releases of 100 marked sterile codling moths and 50 marked laboratory-reared leafrollers (OBLR & PLR) in each block.

Objective 3. Air-capture techniques proved ineffective for measuring airborne concentrations of MEC pheromone at levels behaviourally active in the lab so a somewhat simpler, though not simple approach was needed to address objective 3. Correlating airborne concentrations of pheromone with EAG measurements was examined indirectly using a micro-sprayer (El-Sayed *et al*. 1999) that delivers precise amounts of pheromone as a plume over a broad range of rates (pg to mg•min). Moths were challenged to respond behaviourally and physiologically in a background of MEC pheromone disruptant. Knowing the concentration of the plume delivered and examining the EAG that resulted, allowed us to record EAG responses relative to given background concentrations. Using MEC pheromone-release rates calculated from 3M pheromone loss curves a correlation between EAG detection of the known sprayer-delivered pheromone concentration and background was established. Using a plume delivery rate mimicking female moths it was possible to determine the concentration necessary to achieve camouflage of antennal response, i.e. disruption, with MEC pheromone, at least under laboratory conditions.

Results and Discussion:

Objective 1. Codling Moth**.** Pre-exposure of male codling moth to codlemone in static air reduced subsequent pheromone-mediated flight behaviour in a dose- (Fig. 1) and time-dependent manner (Fig. 2). Pre-exposure of moths to 5 and 50 ug of codlemone for 30 min. had little influence on behaviour, but a 10-minute exposure to 500 ug almost completely shut down flight for 2 h. Exposing moths to identical *time x dose* pheromone loads by adjusting time or dose independently, did not produce similar behavioural effects (Fig. 3). This result suggests there is an optimal pheromone pre-exposure dose and time that operate to produce behavioural insensitivity and cause disruption of communication.

Sensory overload in codling moth was more pronounced when codlemone was presented in a dynamic airflow system as occurs in orchards. A 10 min. 20-ug•h exposure (3.3 ug *time x dose* load) caused 99% disruption, and even a 1-ug•h exposure (0.16 ug *time x dose* load) caused 75% disruption (Fig. 4). Putting these exposure rates in perspective, the highest exposure is equivalent to a new Isomate-C$^+$ dispenser and some 300,000*x* greater than the near optimal codlemone release rate (1000 pg•min) needed to elicit attraction (Fig. 5). The lowest exposure was 17*x* greater than this optimal rate. In practical terms codling moths might encounter exposure to these levels throughout the season when ever landing near most hand-applied dispensers; the greater the number of dispensers in an orchard the greater the probability of encountering these pre-exposure conditions. Using fewer, even higher emission sources, like "puffers" (20,000 ug•h), would not necessarily increase exposure rates because too high an emission rate prevents upwind flight and contact (Fig. 5) and fewer dispensers lowers probability of exposure. The ideal delivery system would be one that emits a rate that maximizes upwind flight and landing, and thus, exposes moths to rates sufficient to cause sensory overload; Isomate-C$^+$ appears near this optimum.

Decline in behavioural activity of codling moth following pre-exposure was correlated with reduced antennal sensitivity to codlemone. Pre-exposed moths were insensitive to increasing doses of pheromone that elicited strong responses from control moths (Fig. 6). Experiments demonstrated a form of "long-term" antennal adaptation not previously known to occur. Complete recovery from antennal adaptation took ca. 2 h (Fig. 7); this is the first evidence of this disruption mechanism.

Leafrollers. Both OBLR and PLR were unaffected by pre-exposure to their complete pheromone or its components in amounts of 3000 ug for periods as long as 24 h (Fig. 8). When leafroller moths were physically removed from pheromone disruptant, which would occur in nature by flying above the canopy for example, they quickly recovered and were able to respond to female pheromone when encountered. The reason for this basic physiological difference in closely related moths is unknown, and challenging in terms of finding a mating-disruption approach that fits all species. Differences may be as basic as the chemical difference between the codling moth alcohol pheromone and leafrollers' acetate pheromones. The effect of pre-exposure on long-term antennal adaptation in leafrollers was not tested because it was not anticipated given the lack of behavioural activity. Short-term antennal adaptation could still be acting as a disruption mechanism in leafrollers while they are in the presence of high levels of pheromone.

Objective 2. Codling Moth. All attempts to demonstrate disruption of codling moth with 3M-CM sprayable pheromones in the lab flight tunnels failed. The efficacy of several codling moth 3M-CM MEC formulations (Phase IV-s, IV-e & IV-e^+), with and without Nufilm-17 sticker, UV shaded and nonshaded, before and after rain events, was examined in field flight tunnels in 2000, 2001 and 2002. None of these formulations, under any conditions, showed any activity 48 h after application at 20 g ai/acre.

In 2002 side-by-side comparison of the 3M-CM Phase IV-e^+ experimental formulation and Suterra CM-F commercial formulation both applied at 20 g ai/acre with Nufilm-17 sticker (1 pint /100 gallons/acre) was performed. Once again the 3M-CM sprayable was ineffective at 48 h and not-significantly different from the water-sticker control treatment (Fig. 9). Suterra CM-F however, disrupted trap catches for ca.1 week, yet the level of disruption was only 61% after 7 days. Under similar conditions Isomate-C^+ caused 100% disruption. This lack of efficacy from sprayable formulations against codling moth made it impossible to correlate pheromone concentrations with observed disruption and answer objective 2. Chemical analysis was performed to determine exactly what amount of MEC pheromone was reaching leaves and what its release rate was relative to products known to work; information useful to engineer future MEC products.

Extraction and GC analysis of pheromone residues 4 h after application of 3M sprayable at 20 g ai/acre to apple leaves using an airblast sprayer found codlemone concentrations of only 250 ± 35 ng•cm^2 of leaf surface. Using this amount as the starting point (100%) and the pheromone loss curve provided by 3M (Fig. 10), calculations predicted a release rate of 6.6 pg•min•cm^2, which is less than amounts males are responsive too (Fig. 5), much less than amounts coming from females (≈ 166 pg•min), and far less than the microgram (ug) amounts necessary to cause sensory overload (Fig. 4). Therefore, it seems understandable why 3M-CM sprayable did not cause any disruption, as it could not camouflage antennal detection of plumes (Fig. 11), nor cause any sensory overload. Disruption of codling moths was not improved even when moths were held in contact with treated leaves, suggesting the 3M-CM Phase IV-e^+ MEC formulation encapsulates codlemone too tightly and releases it too slowly to be biologically effective.

Empirical measurement of codlemone release from both 3M and Suterra formulations was performed in the laboratory using Porapak-Q trapping and GC-MS. This analysis showed actual release may be lower than predicted. 3M-CM sprayable was releasing 0.023 pg•min•cm^2 during day 2, and the Suterra product was releasing 2.53 pg•min•cm^2, i.e. 110*x* more codlemone. This difference alone explains the relative efficacy of the two products (Fig. 9) and gives an idea of the concentration of codlemone necessary to cause disruption. By comparison an Isomate-C^+ dispenser might release 15,000 – 30,000 pg•min•cm^2.

Leafrollers. Disruption of leafrollers with the 3M-LRX MEC sprayable (Phase I capsules) was shown to be dose- and time-dependent in laboratory flight tunnels (Fig. 12), however disruption was short-lived. At a recommended field rate of 40 g ai/acre disruption of calling females lasted just 1 day in OBLR and 2 days in PLR under lab conditions. The same application rate in field flight tunnels (Fig. 13) and small orchard plots (Fig. 14) caused near 100% disruption of both OBLR and PLR for about 21 days, declining to 33% by 35 days. A 2nd gen. application in the field produced 100% disruption for 42 days (Fig. 14).

These conflicting results from laboratory and field flight-tunnel assays led to the hypothesis that contact with pheromone sprayed leaves might improve disruption in the field. Follow-up laboratory flight-tunnel experiments showed that the disruption effect of sprayable pheromones in orchards likely results from the combined effects of airborne pheromone concentrations and pheromone on or near the leaf surface boundary layer when moths are stationary. PLR moths placed on MEC-treated apple leaves brought in from the field at various times after treatment with an airblast sprayer, and then exposed simultaneously to airborne pheromone in the laboratory flight tunnel, were disrupted as long as moths tested under field conditions (Fig. 15). Exposure to leaves led to congruency of lab and field disruption longevity results. These results suggest pheromone concentrations at the leaf surface interface are likely more important in disruption than the airborne concentrations of pheromone researchers have long sought to quantify. This is supported by the fact that codling moth exposed only to airborne pheromone while held in screen cages in orchards treated with Isomate-C^+ showed no effects of sensory overload. The issue of measuring air concentrations of pheromone to predict efficacy of mating disruption may need to be reconsidered.

Although leafroller moths did not fly to low concentration plumes while they were sitting on treated leaves, however, when the concentration of a pheromone plume was increased above the predicted release rate from the leaf surfaces, moths began to fly to the source. This result indicates that the leafrollers' CNS was not habituated to pheromone, consistent with sensory overload experiments (Fig. 8), but was likely experiencing short-term antennal adaptation to pheromone concentrations experienced on the leaf. Therefore, the main mechanism of disruption with sprayable MEC pheromone is one of camouflage, likely brought about by antennal adaptation to pheromone-treated leaves and air. Adaptation leads to physiological camouflaging of the plume, as long as the MEC concentration is above the plume concentration, and as long as moths are in contact with pheromone-treated surfaces disruption occurs. Taking flight (= removal from leaves) exposes moths to pheromone-laden air only, and these moths are disrupted much less effectively than those exposed to leaf and airborne pheromone together (Fig. 15).

Objective 3: Leafrollers. By converting pheromone loss curves for 3M-LRX, as provided by 3M, into release rates per cm^2, it was possible to show that leafrollers became responsive to pheromone plumes in the laboratory tunnel when the predicted release of MEC pheromone dropped below that of the plume source; this occurred at about 1 day for OBLR and 2 days for PLR (Fig. 16). For example, when 3M MEC pheromone was releasing above 50 pg•min•cm^2 female PLR would be camouflaged, whereas for OBLR the release rate must be above 500 pg•min•cm^2 or OBLR females known to release this amount are detectable and males respond (Fig. 16). EAG measurements in laboratory flight tunnels treated with MEC pheromone disruptant, confirmed that antennal detection of a plume occurred as predicted when MEC release rate dropped below the plume concentration moth antennae were challenged with. Therefore, when the release rate of MEC sprayable pheromone is above a certain concentration, female plumes would clearly be *physiologically camouflaged*. This concentration will vary for different species depending on the females release rate. While these EAG results explained and correlated well with the short-lived disruption periods (1-2 days) seen in laboratory assays (Fig. 12), it did not reconcile the observed longevity (21-35 days) of leafroller disruption when MEC pheromone was applied to trees in field wind tunnels (Fig. 13), or orchards (Figs. 14). EAG measurement with moths sitting on treated leaves has not been possible, but from previous behavioural tests it is expected EAG's would be dampened. These studies confirmed that EAG detection of a plume in a background of disruptant is possible, and they are correlated with flight responses in that they occurred when the predicted MEC release rate dropped below the plume concentration.

Codling Moth. Similar EAG and behavioural correlation could not be done with codling moth because disruption could not be demonstrated. However, EAG's showed that detection of plumes near the optimal release rate for attraction (1000 pg•min•cm^2) was possible in a background of laboratory flight-tunnel air treated at 20 g ai/acre with 3M-CM sprayable (Fig.11), explaining the lack of disruption with this product.

Project Title: Use of field wind tunnels to understand mechanisms of communication disruption and improve delivery and development of multiple-species mating-disruption systems

PI: Gary Judd

Project Duration: 1998 – 2002: This project has lasted five years and terminates with this report.

Year	Year 1 (1998)	Year 2 (1999)	Year 3 (2000)	Year 4 (2001)	Year 5 (2002)	TOTAL
Total	18,788	18,788[a]	0[a]	17,880	0[a]	55,546

Breakdown for WTFRC Funding Only

Item	Year 1 (1998)	Year 2 (1999)	Year 3 (2000)	Year 4 (2001)	Year 5 (2002)	1998 – 2002
Salaries	15,287	15,287	0	15,287	0	45,861
Benefits %	611	611	0	611	0	1,833
Travel	500	500	0	500	0	1,500
Supplies	1,590	1,090	0	1,082	0	3,762
Equipment	800	1,300	0	400	0	2,500
Total	18,788	18,788	0	17,880	0	55,456

[a] Money awarded by WTFRC in 1999 was received but not spent until early 2000 because of difficulties in getting wind tunnels built. Commissioners granted permission to roll remaining 1999 dollars into 2000. No money was requested of WTFRC in 2000, but in this year AAFC matched the 1999 WTFRC grant. Money received from WTFRC in 2001 was inadvertently returned by PARC administration, by the time this issue was corrected and money received late in 2001 it was too late to spend matching money from AAFC, so it was received in 2002 and the project was completed in 2002 even though no WTFRC funding was requested or remained.

References Cited:

Cardé, R.T., R.T Staten, and A. Mafra-Neto. 1998. Behavior of pink bollworm males near high-dose, point sources of pheromone in field wind tunnels: insights into mechanisms of mating disruption. Entomologia Experimentalis et Applicata 89: 35-46.

El-Sayed. A., J. Gödde, and H. Arn. 1999. Sprayer for quantitative application of odor stimuli. Environ. Entomol. 28: 947-953.

Fig. 1. Dose-Dependent Pre-Exposure Effect

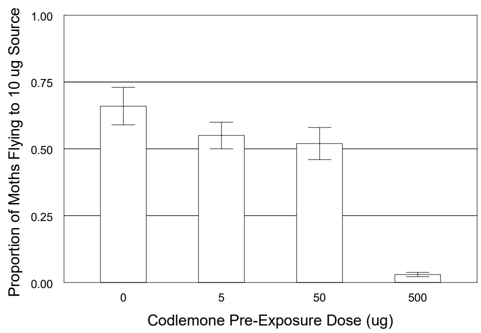


Fig. 2. Time-Dependent Pre-Exposure Effect

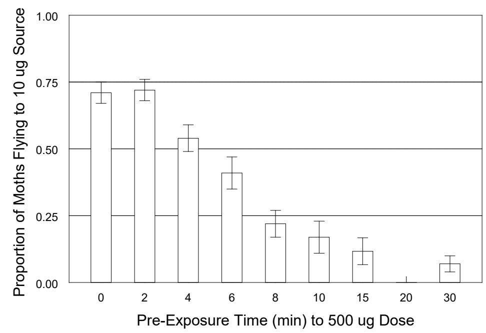


Fig. 3. Time x Dose Pheromone Product Effect

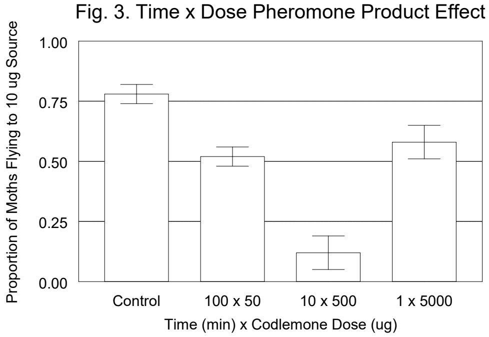


Fig. 4. Dynamic Airflow Pre-Exposure Effect

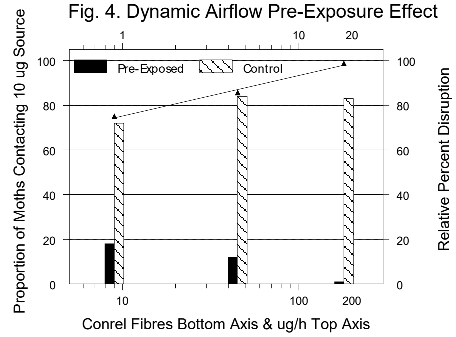


Fig. 5. Codling Moth Attraction Dose Range

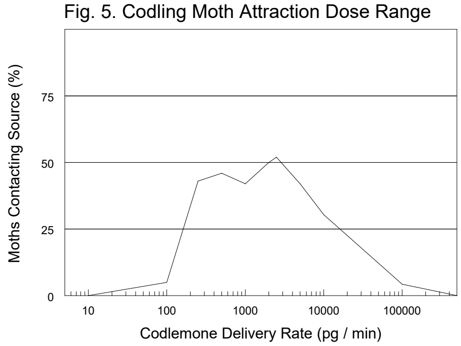


Fig. 6. Codling Moth EAG Dose Response

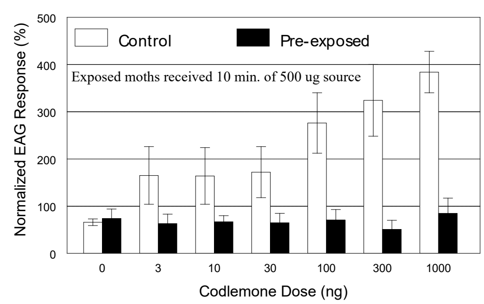


Fig. 7. Time-Dependent EAG Recovery

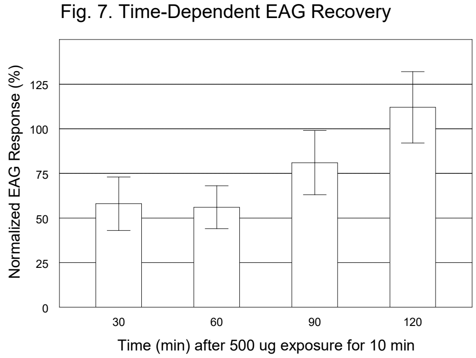


Fig. 8. Leafroller Response After Pre-Exposure

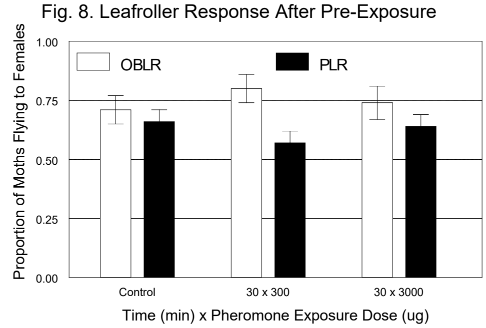


Fig. 9. Codling Moth Disruption With Sprayables

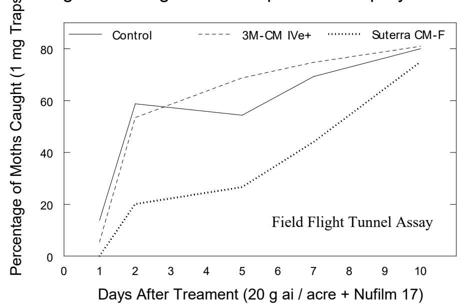


Fig. 10. 3M-CM MEC Codlemone Loss Curve

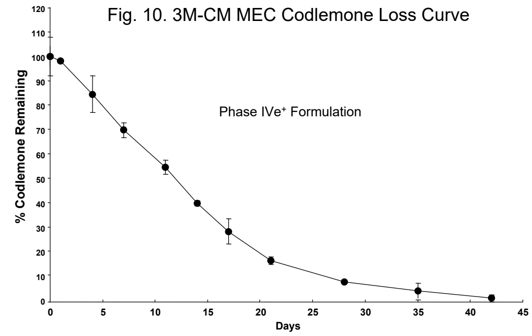


Fig. 11. EAG of Codling Moth Before and After MEC Application in Lab Tunnel Showing No Effect

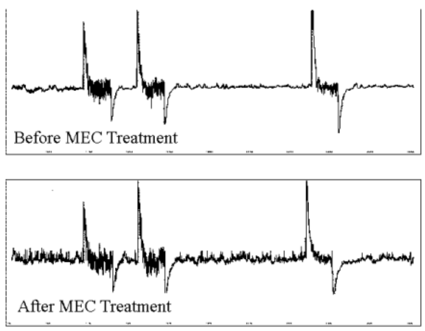


Fig. 12. Dose-Dependent Disruption of PLR With 3M-LRX Sprayable in Lab Flight Tunnel

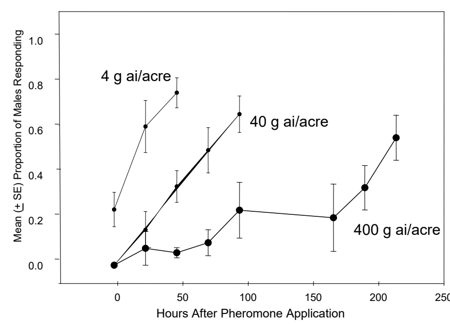


Fig. 13. Disruption of PLR in Field Flight Tunnels With 3M-LRX MEC @ 30 g ai/acre

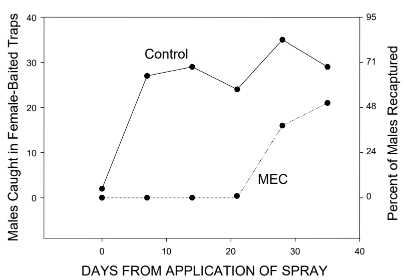


Fig. 14. Disruption of PLR in Small Plot Orchard Trial With 3M-LRX MEC @ 30 g ai/acre

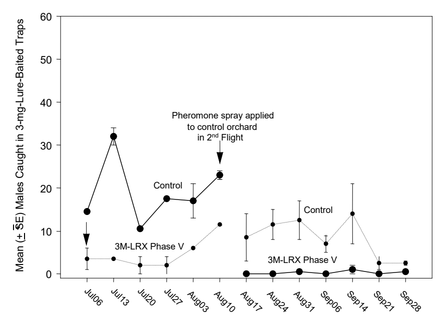


Fig. 15. Disruption of PLR Exposed To Air, Leaves, and Air + Leaves Treated with 3M-LRX MEC @ 40 g ai/acre

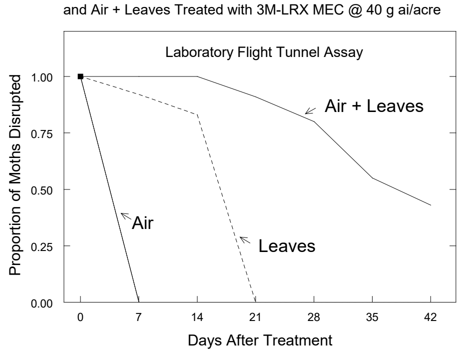
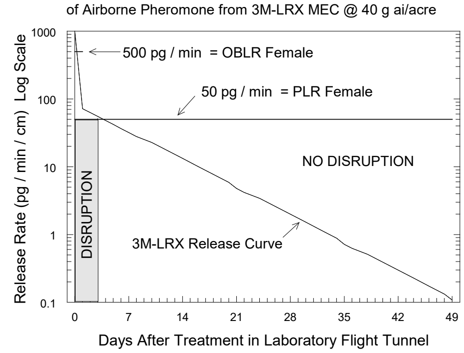


Fig. 16. Disruption of Leafrollers in Response to Release of Airborne Pheromone from 3M-LRX MEC @ 40 g ai/acre



FINAL REPORT
WTFRC PROJECT # AE-02-218

TITLE:	Acquisition of a Agilent 5973N Bundled Gas Chromatograph with Chemical Ionization and Mass Selective Detector
PRINCIPAL INVESTIGATOR:	Vincent R. Hebert, Residue Laboratory Director, Food and Environmental Quality Laboratory, WSU TriCities
COLLABORATORS:	Vincent P. Jones, Associate Entomologist and Jay F. Brunner, Entomologist, WSU-TFREC, Wenatchee

OBJECTIVES:
The acquisition of the Agilent 5973N gas chromatograph with chemical ionization and mass selective detector (GC/MS/CI) will:

1) Provide benefits to tree fruit growers by making available an essential tool needed for evaluating the performance (i.e., pheromone release rates) of mating disruption systems.
2) Provide analytical support for tree fruit IPM field research in the foreseeable future.
3) Assist in the timely registration of current and emerging pesticide chemistries.

The Agilent GC/MS/CI system was purchased in July 2002. The instrument was received and put on-line for analytical use in electron impact mode (EI) August 2002. Final system checkouts for the chemical ionization unit were completed in December 2002.

SIGNIFICANT FINDINGS:
Conventional chemical analyses for volatile insect pheromones usually involve the use of a gas chromatograph (GC). The GC separates individual chemical components in the complex mixture. Each separate compound is then usually quantified using a flame ionization detector (FID). This detector basically burns and ionizes all the carbon in each separated chemical component. This method of analysis requires a relatively large chemical concentration to acquire a good signal for quantitation. Besides being insensitive, this form of ionization detection is nonspecific and not useful for assessing very low but bioactively significant pheromone concentrations that result in mating disruption in apple and pear orchard air.

Our preliminary method development findings using the GC/MS/CI show that both the electron impact (EI) and chemical ionization (CI) modes on the mass spectrometer have greatly enhanced sensitivity for codlemone quantitation compared to conventional GC/FID techniques. We are currently in the process of quantifying low pico-to-fentogram concentrations per cubic meter of air. In concert with trapping codlemone from air using high-volume air samplers, we fully anticipate to achieve sensitivities for chemically mapping the atmospheric release behavior of codlemone from two distinct mating disruption systems in the 2003 orchard growing season.

DISCUSSION OF ANTICPATED GC/MS USES IN 2003

In 2003, we plan on sampling orchard air at regular intervals to determine chemical dissipation behavior in both sprayable and hand-applied dispenser products (2003 WTFRC new proposal submission). The overall goal of this submission will be to provide environmentally relevant air concentration data that can be used in combination with dispenser release rate and pest monitoring data for assessing the performance and efficacy of mating disruption products.

This instrument will also see use in our on-going air monitoring program evaluating off-target movement of auxin agonist herbicides to wine grape vineyards. 2,4-D-type herbicides can be detected at extremely low air concentrations using the GC/MS in negative chemical ionization (NCI) mode. This instrument will also provide needed specificity when sampling complex mixtures of organic substances from the ambient air. Additionally, this instrument will be used to provide USDA IR-4 *Magnitude of the Residue* information in the fall of 2003 for supporting a lambda-cyhalothrin (Karate) pesticide registration in/on asparagus.

2002 Budget:

TITLE: Acquisition of a Agilent 5973N Bundled Gas Chromatograph with Chemical Ionization and Mass Selective Detector

PRINCIPAL INVESTIGATOR: Vincent R. Hebert

Expenditure	WTFRC	Funds (cash)			Total Instrument Cost
		Source: WSCPR[1]	Source: WWC[2]	Source: WSU[3]	
Salary	0	0	0	0	0
Travel	0	0	0	0	0
Equipment	13,000	23,245	10,000	46,245	92,490
Other (specify)					
Total	**13,000**	23,245	10,000	46,245	92,490

1. Funding provided from the Washington State Commission of Pesticide Registrations
2.Funding provided by the Washington Wine Commission.
3. Funding provided by the Washington State University.