## 2005 Apple Entomology Thursday, January 27

| Time | Page | PI | Proposal Title | Funding Period |
|---|---|---|---|---|
| 8:00 | | McFerson | Introduction | |
| 8:15 | 1 | Brunner | Stink bug behavior and control in orchards | **02-04** |
| 8:30 | 11 | Beers | Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci* | **02-04** |
| 8:45 | 21 | Walsh | Lygus bug thresholds | **04** |
| 9:00 | 28 | Knight | Management of Codling Moth | **04** |
| 9:15 | 37 | Landolt | Codling moth selection of pupation and hibernation sites: enhancement of tree banding. | **04** |
| 9:30 | 42 | Brunner | Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm. | **02-04** |
| 9:45 | 51 | Miliczky | Identification of extra-orchard host plants and habitats for key natural enemies of pome fruit pests | **03-04** |
| 10:00 | | **Break** | | |
| 10:15 | 61 | Unruh | Effects of new insecticides - no report submitted | 04 |
| 10:30 | 62 | Felsot | Alternative sprayer technology (Technology Committee) | **02-04** |
| 10:35 | 73 | Brunner | Sensor webs (Technology Committee) | 03-05 |
| 10:40 | 74 | Barcenas | Development of genetic markers to identify problematic pests | 02-04 |
| 10:45 | 79 | Yee | Alternative hosts of apple maggot as threat to apple | 04-06 |
| 10:50 | 85 | Yee | Control of apple maggot using bait spray insecticides and traps | 04-06 |
| 10:55 | 92 | Beers | Biology, migration, and management of Western flower thrips in apple orchards | 03-05 |
| 11:00 | 98 | Unruh | Biological control of leafrollers through habitat modification | 03-05 |
| 11:05 | 103 | Lacey | Optimizing the use of the codling moth granulovirus | 04-05 |
| 11:10 | 109 | Landolt | Field testing of multi-component host plant kairomones for the codling moth | 03-05 |
| 11:15 | 112 | Judd | Evaluation of a codling moth larval aggregation pheromone as an IPM tool | 03-05 |
| 11:20 | 117 | Jones | The importance of dispersal in biological control and IPM | 04-06 |
| 11:25 | 121 | Jones | Mechanisms underlying mating disruption | 04-06 |
| 11:30 | 126 | Pszczolkowski | Feeding stimulants to increase efficacy of insecticides | **03-05** |

**FINAL REPORT**
**WTFRC Project #AE-02-221** **WSU Project #13C-3643-4094**

**Project title**: Stink bug behavior and control in orchards

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**Objectives:**
1. 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.
2. Determine the suitability of orchard cover crop plants as hosts that will mature stink bugs.
3. Determine if control programs directed at orchard cover crops would be a practical management strategy for stink bugs without disrupting integrated mite management.
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:**
1. Pyrethroid insecticides were found to be most effective against stink bugs in previous research; however, when they were applied to orchard borders they failed to reduce injury relative to an untreated control. Overall injury in the plots was only about 2% on the border row, which was much lower than other untreated plots (16%) suggesting that check plots in the insecticide trial were too small to reveal differences in treatments.
2. 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.
3. Stink bugs were able to complete development on mullein, common mallow and white clover but not on grass, lamb's quarter or dandelion.
4. D-Vac collections from the orchard in 2002 and 2003 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. Damage peaked late in summer, and stink bugs were found to feed the most during hours of darkness.
5. ***Pyramid traps:*** No difference was found relative to trap size (height) between 2, 3 and 4 feet tall. Traps baited with an aggregation pheromone captured four times the number of unbaited traps.

***Pheromone lures:*** There was no difference in the attraction of lures provided by two pheromone companies. Lures lasted for at least three weeks, but captures declined after six weeks.

6. Danitol-treated pyramid traps were effective at attracting and killing stink bugs, but no significant reduction in damage was noted along orchard borders.

**Methods**:

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

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

**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. In addition, we conducted D-Vac or vacuum samples of orchard ground cover 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. Damage was counted on borders weekly beginning in July, and stink bugs were observed in the laboratory at hourly intervals to determine daily feeding patterns.

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

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

**Danitol-treated traps:** Undiluted Danitol was applied to 3-foot pyramid trap surfaces using a paintbrush. Traps were placed at 20-foot intervals between trees along orchard border rows from early August through late September. Numbers of dead and live bugs in traps were counted twice weekly and fruit damage recorded at harvest. The study was replicated in two orchards, and each area (treated vs. untreated) was 200 feet in length.

**Results and discussion:**
In light of extensive research in 2002 indicating that stink bug damage occurs primarily on orchard border rows, we confined insecticide treatments to these rows only. Counts of damaged fruit indicated no significant differences between any of the treatments and the unsprayed control blocks (Fig. 1). It is possible that the untreated areas were not large enough and that stink bug populations in check plots were affected by the insecticide-treated areas. Studies in previous years have demonstrated that all of the synthetic pyrethroids used in these trials have high acute toxicity to stink bugs; however, their residual toxicity may not be sufficient to protect the crop at the intervals tested (14 days).

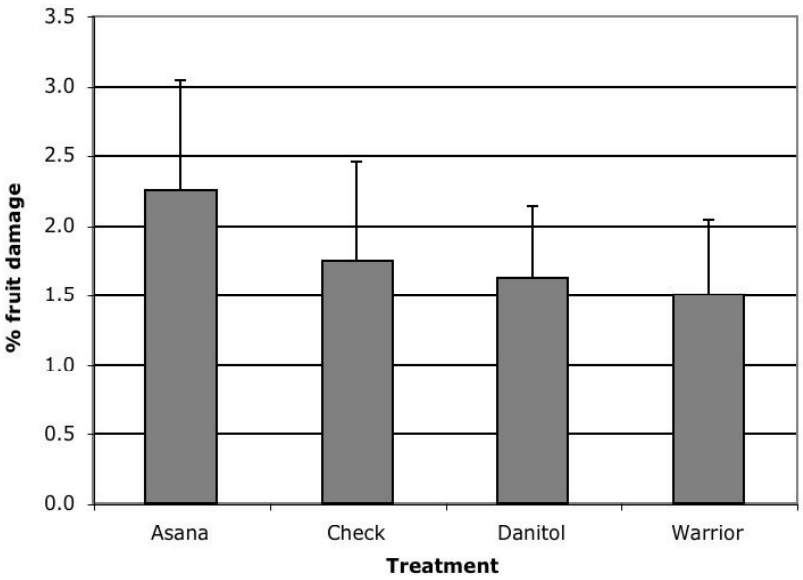


Figure 1. By-treatment distribution of stink bug injury in pyrethroid-treated blocks at harvest; data represent pooled results of four orchards surveyed. No significant differences detected.

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

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

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

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

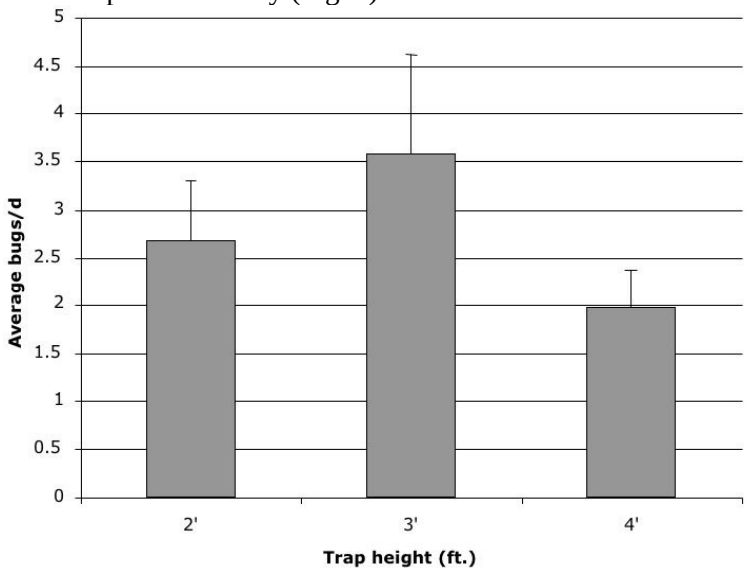


Figure 2. Mean capture of *E. conspersus* in pheromone-baited pyramid traps of three heights. No significant differences detected.

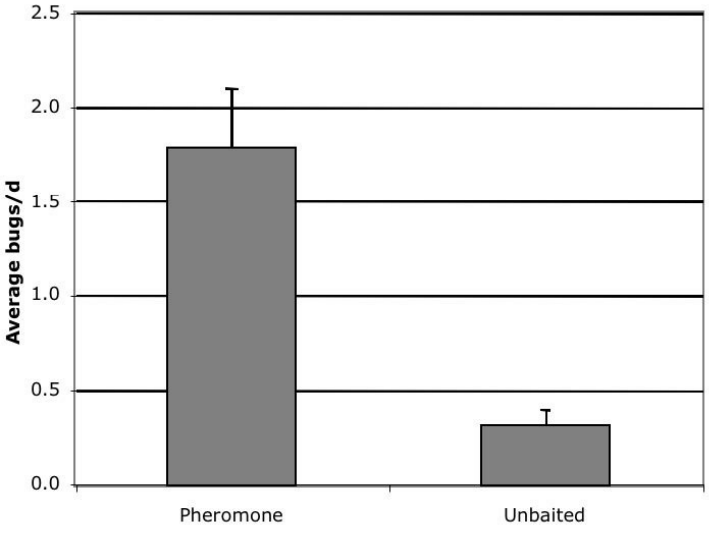


Figure 3. Mean capture of *E. conspersus* in pheromone-baited and unbaited pyramid traps.

In 2003 we continued to test pheromone release devices to develop an optimal lure type for use in monitoring and trapping programs. Two commercial lures were tested, the bubble lure produced by PheroTech Inc. and a polyethylene vial produced by IPM Technologies Inc. Both lures exhibited similar attractiveness when placed on mullein plants (Fig. 4), with a decline in attractiveness of both lures between three and six weeks. Either of these lures would be suitable for a management/monitoring application, such as combination with pyramid traps for in-orchard monitoring or for use in mass trapping initiatives.

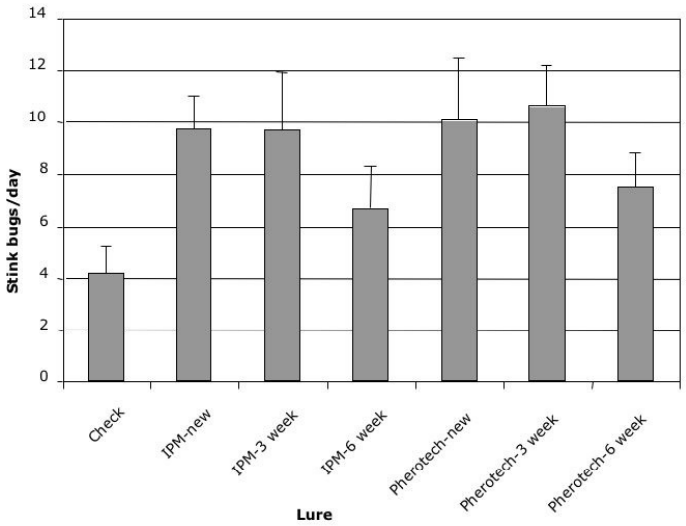


Figure 4. Comparison of field attractiveness of two different lure types placed on mullein plants and comparison with unbaited control plant. No significant differences detected.

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 2), and damage counts conducted in the orchard once again revealed a trend of decreasing damage away from border rows (Fig. 5).

Table 2. 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<br>Border vegetation | 0.11<br>1.00 | $2^{nd}$<br>$2^{nd}$ |
| 07/09/02 | In-orchard<br>Border vegetation | 0<br>0.55 | N/A<br>$2^{nd}$-$4^{th}$ |
| 08/01/02 | In-orchard<br>Border vegetation | 0<br>0.5 | N/A<br>$4^{th}$-adult |
| 08/14/02 | In-orchard<br>Border vegetation | 0<br>0.88 | N/A<br>$5^{th}$-adult |
| 08/31/02 | In-orchard<br>Border vegetation | 0<br>0.33 | N/A<br>4th |

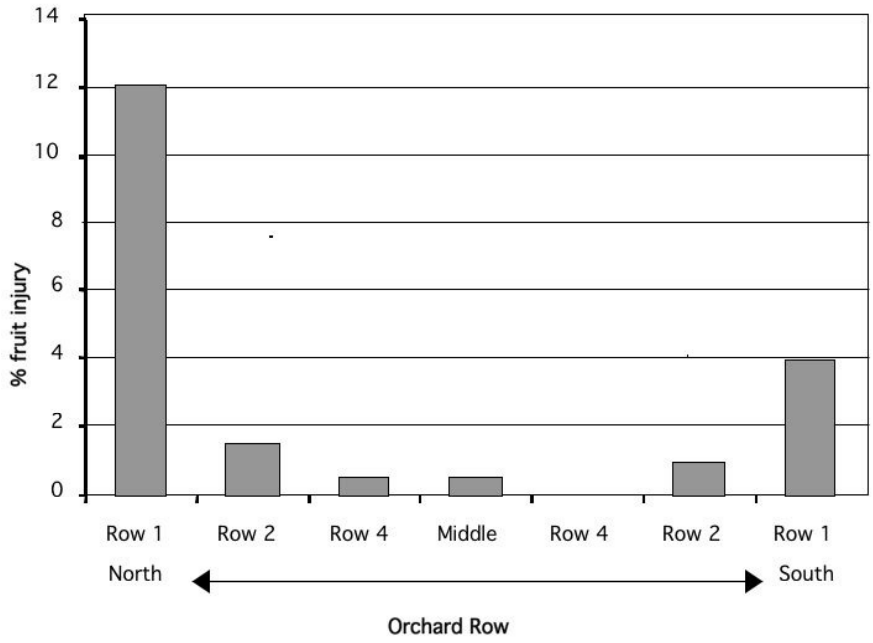


Figure 5. The percent fruit injury by stink bugs on different rows relative to the orchard border, row 1 on each side is the border row.

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 3). These plants could be managed with effective broadleaf weed control. Since previous experiments have shown that stink bugs are unable to develop on apple, this may represent an ideal way to restrict stink bug populations to areas outside orchard borders.

Table 3. Percent 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 on 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 4), the emphasis of management efforts may be better confined to orchard borders.

Table 4. 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 |

Applications of Danitol in 2001 had a marked negative effect on 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 5). These orchards were sprayed with a miticide on July 31, 2002, to prevent economic loss due to these heavy mite infestations. This disruption of integrated mite control is a serious drawback 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 5. Average mites per leaf in Danitol-treated orchards compared to orchards treated with Phosphamidon or left untreated.

| Orchard | Treatment | ERM per leaf | | Pred./leaf | ERM per leaf | | |
|---|---|---|---|---|---|---|---|
| | | 2001 | | | 2002 | | |
| | | 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 (Fig. 6). 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. In addition, lab and greenhouse studies revealed that stink bug feeding occurs mainly late in the afternoon and during the night (Fig. 7), so late afternoon or early morning spray applications may be preferable wherever possible.

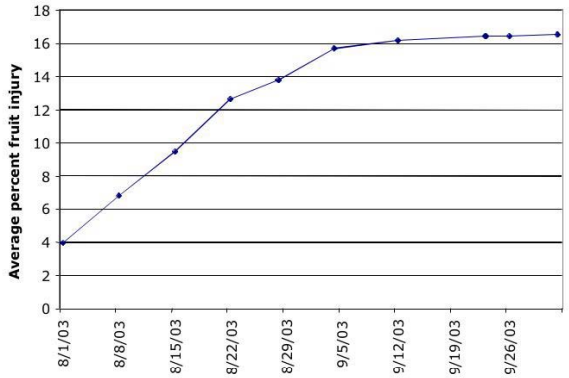


Figure 6. Average percent fruit injured by stink bug feeding on border trees.

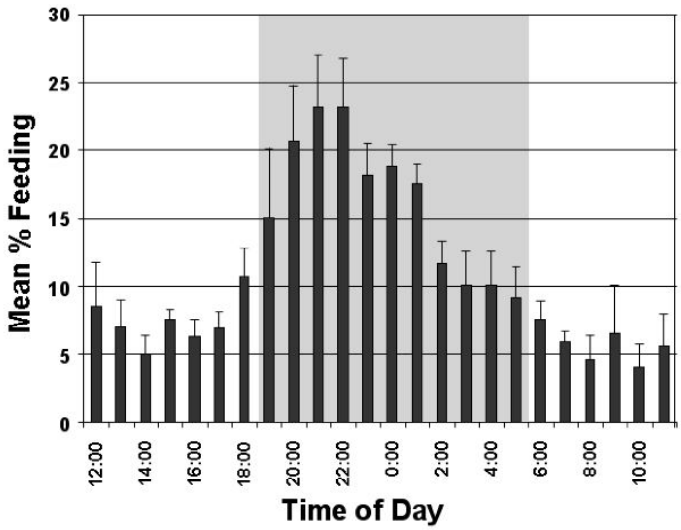


Figure 7. Average percentage of adult stink bugs feeding over 24-h period. Shaded area indicates hours of darkness.

Toxic pyramid traps were found to capture and kill significant numbers of stink bugs when placed along orchard borders (Fig. 8), indicating that the presence of Danitol on the trap surface did not deter the insects from being attracted to the trap and crawling on its surface. However, there were no significant differences in fruit injury at harvest (Fig. 9), indicating that either the traps were not on borders for a long enough period or that the number of toxic traps used was not sufficient.

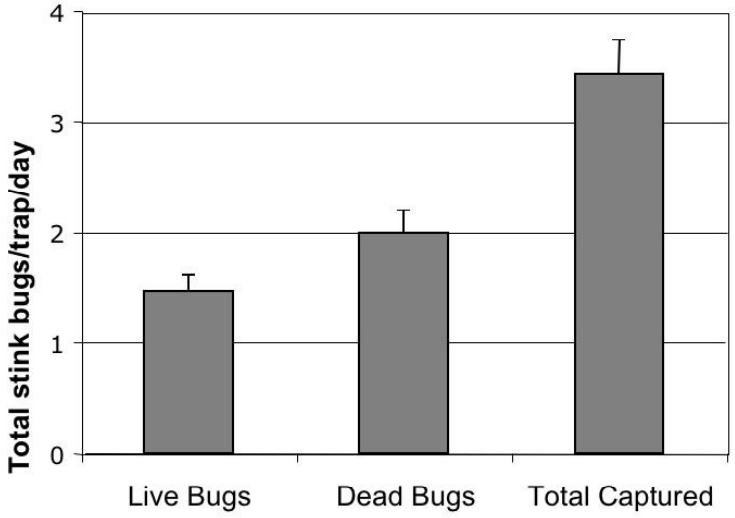


Figure 8. Numbers of live and dead bugs collected from Danitol-treated pyramid traps placed along orchard borders.

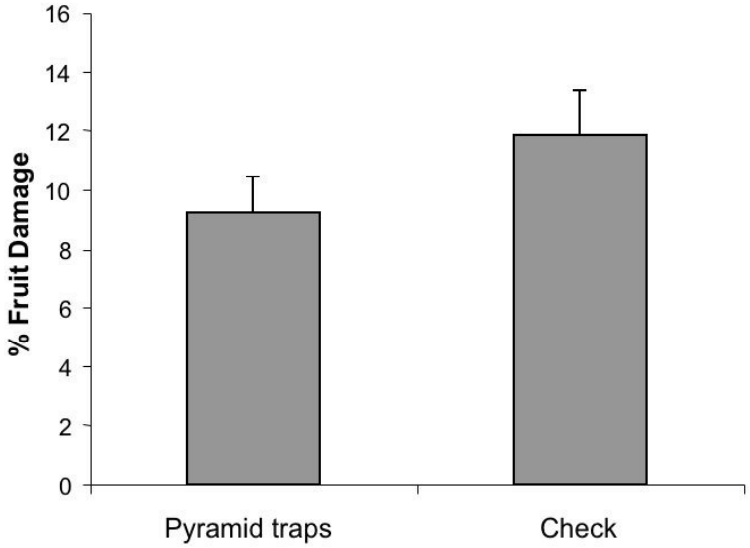


Figure 9. Comparison of fruit damage at harvest on border rows between areas with Danitol-treated pyramid traps and "check" areas with no traps. No significant differences detected.

**Budget**:

**Project title**: Stink bug behavior and control in orchards
**PI**: Jay F. Brunner
**Project duration**: 3 years (2002-2004)
**Project total (3 years)**:

| Year | Year 1 (2002) | Year 2 (2003) | Year 3 (2004) | Total |
|---|---|---|---|---|
| Total from WTFRC | $28,197 | $26,847 | $27,847 | $82,731 |
| From IFAFS/RAMP | $15,000 | $15,000 | $15,000 | $45,000 |

**FINAL REPORT**
**WTFRC 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
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**Co-PIs and affiliations:** Stephen D. Cockfield, Research Associate,
WSU Tree Fruit Research and Extension Center, Wenatchee

**Contract administrator:** Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu); (509) 335-3691


**Objectives:**

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

**Significant findings:**

1. Pheromone trapping of adult *C. verbasci* in the fall was successful in determining risk of nymph infestation the following spring.
   - ◊ The Pherocon IV and the smaller Pherocon II trap caught significantly fewer *C. verbasci* than did the nearly obsolete Pherocon1C, and the efficiency of the Pherocon IV was approximately half that of the 1C. However, capture in the Pherocon IIB trap was not significantly different from that of the 1C.
   - ◊ Risk assessments were most reliable in 'Delicious' orchards. All the orchards determined to be low risk had undetectable nymph populations. In one case, traps gave advance warning of an extremely severe spring infestation. All errors were false positives.
   - ◊ Risk assessments were less reliable in 'Golden Delicious' orchards. Although most low trap catches resulted in below-threshold nymph populations, injury was often evident at harvest. Because of the high sensitivity of 'Golden Delicious', there was an inherent problem in the reliability of tap samples (all errors, 5/16 observations, were false negatives).
2. Most common cultivars are susceptible to *C. verbasci* injury.
   - ◊ In field assays evaluated before June, the susceptibility of 'Gala', 'Granny Smith', 'Fuji', and 'Cameo' to feeding injury appeared to be intermediate between that of 'Delicious' and 'Golden Delicious'.
   - ◊ 'Braeburn' appears highly resistant to *C. verbasci*.
3. The economic injury level for *C. verbasci* on 'Gala' is ca.1 nymph per tap. In 2003 and 2004, about half to two-thirds of the injury on 'Gala' was minute and would not cause culling. Some injury appeared atypical (russet or tiny dark spots), and some typical (a combination of bumps and dimples).

**Methods:**

**Validation of pheromone trap sampling:**
***Comparison of pheromone trap designs:*** Beginning in August, two Pherocon 1C traps and two Pherocon IV traps loaded with *C. verbasci* pheromone flex lures (Phero-Tech, LTD) were placed in

four 10-acre blocks (replicates) in Brewster, WA. Traps were checked weekly. Each trap station, four per block, was at the center of a 2.5-acre quadrant. Individual traps were re-randomized among the four trap stations after each weekly count. Traps were collected after 5 weeks (the recommended lure replacement interval). Data analysis consisted of an analysis of variance with LSD mean separation for the total number of males captured per trap over the 5-week study.

Four trap designs (Pherocon 1C, Pherocon IV, Pherocon II, and Pherocon IIB) (Trécé, Inc.) were compared in an Orondo orchard with a high population of *C. verbasci* adults. Four replicate blocks of the four trap types were placed in a single row of the orchard. Traps within blocks were placed every other tree, and blocks were separated by 10 trees. After 1 week, the number of males caught in the Pherocon IV, II, and IIB traps was compared to the number caught in the 1C traps (the current standard). Differences in trap catch were tested for significance with Dunnett's test.

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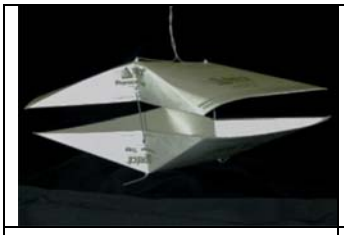
|  |  |  |  |
| Plate 1a. Wing trap, Pherocon 1C. | Plate 1b. Large delta trap, Pherocon IV, commonly used for codling moth. | Plate 1c. Small diamond trap, Pherocon II. | Plate 1d. Large diamond trap, Pherocon IIB, designed for codling moth. |

***Validation of risk thresholds:*** Ten 'Delicious' and 10 'Golden Delicious' orchards with a history of *C. verbasci* damage were selected in north-central Washington to serve as test subjects of the fall pheromone trapping technique. Each 10-acre block was divided into four 2.5-acre quadrants. Pheromone trap procedures followed those of Reding (2000). One Pherocon IV trap was placed 2 m above the ground in the center of each quadrant. *C. verbasci* pheromone lures were suspended in the center of each trap. Trap liners and lures were changed every 4-6 weeks from the last week of July to the first week of November. The risk of encountering a high spring population of nymphs was calculated according to the thresholds modified for the large delta trap (see Results and Discussion). We based our thresholds on those proposed by Reding (2000) for the "long-fall" trapping period in untreated orchards. Of his thresholds offered, one set for commercial and one set for untreated orchards, the ones for untreated orchards are the most conservative. The delta-trap threshold was 175 nymphs per trap for 'Delicious' and 125 for 'Golden Delicious'. Densities of secondary pests that serve as prey for *C. verbasci* were assessed in November. In 2002, about 25 spurs were randomly selected and examined under a microscope for European red mite (ERM) eggs. One hundred shoots were examined in the field for signs of aphid colonies (aphid parts, honeydew, or sooty mold). In 2003, 100 spurs and 50 shoots were inspected from each block.

The following spring, 25 tap samples were taken around the location of each trap, for a total of 100 tap samples per block. Samples were taken as far into the egg hatch period as possible during the time of fruit susceptibility, which ends at petal fall (Reding 2000). Most samples on 'Delicious' were taken at petal fall while most on 'Golden Delicious' were taken the day before the blocks were sprayed for *C. verbasci*, usually between full bloom and petal fall. Each block was determined to be above or below threshold according to the current practice: 1 nymph per tap for 'Golden Delicious' and 4 nymphs per tap for 'Delicious'. Fruit injury was assessed by examining 400 fruits per block *in situ* before harvest.

***Apple cultivar susceptibility:*** Trees of the cultivars 'Golden Delicious', 'Delicious', 'Fuji', 'Gala', 'Granny Smith', and 'Cameo' were selected in orchards in north central Washington in 2002 and 2003. At king bloom, *C. verbasci* nymphs were collected in a heavily infested TFREC orchard near Orondo, WA. Second instars were placed in 15 × 20 cm sleeve cages placed over flower clusters at each site and nymphs were allowed to feed for 1 week. Flowers in the cages were pruned to a single king bloom and two leaves and pollinated before closing the cage. Each cultivar had about 20 cages containing one (2002) or two (2003) *C. verbasci* nymphs and a corresponding number of check (empty) cages. Fruit injury was assessed at petal fall and the number of *C. verbasci* nymphs recorded. A sample of flowers from each cultivar was photographed to compare surface structures, and damaged fruit were photographed at harvest. Data were analyzed using two-way analysis for comparisons of damage/no damage frequency. Data for each cultivar were compared with 'Golden Delicious' and 'Delicious'.

***Economic threshold for 'Gala':*** In 2003, six 'Gala' trees were selected from a block at the TFREC, Wenatchee, WA, and used for artificial infestation with *C. verbasci* nymphs. Cages consisting of a fabric tube were slipped over a branch of blossom clusters at approximately full bloom. Cages fit over whole sections of branches the width of a beating tray (45 cm), and blossoms were pollinated with crabapple flowers before closing. The treatments were different nymph densities (0, 10, 20, 30, or 40 nymphs/cage), with four replicate cages per treatment. Cages were removed at petal fall, and tap samples for nymphs were taken for each branch. No chemical thinning agents were used; however, trees were hand thinned outside the caged areas. Fruit were counted and evaluated for *C. verbasci* feeding injury at the end of May and after June drop (July). In May, all injury was counted; by July, much of the injury was barely visible and only economically significant damage was counted. A final evaluation was done at harvest in August.

The procedure was repeated in 2004 with the same group of trees. Treatments were 2, 5, 10, and 20 nymphs per cage, with the same number of replicate cages per treatment. At petal fall, tap samples revealed a native nymph population in addition to those added. Fruit were evaluated about a week after petal fall, then before and after June drop, and again at harvest. A linear regression was done for each fruit evaluation period of the proportion fruit damaged and the tap count at petal fall. No intercept was used in the linear regression, forcing the line through the origin.

**Results and discussion:**

**Validation of pheromone trap sampling**

***Comparison of pheromone trap designs:*** In the Brewster experiment, the Pherocon 1C caught an average of 26.3 males per trap, whereas the Pherocon IV caught an average of 13.5. The catch of the Pherocon IV was significantly lower (about half) than that of the Pherocon 1C (LSD=8.46, df=3, $\alpha$=0.05). In the second experiment at Orondo, the average catch in the Pherocon 1C trap was 100 males/trap, whereas the average caught in the Pherocon II, Pherocon IIB, and Pherocon IV was 36.0, 83.0, and 54.8, respectively. The catch of the Pherocon II and the Pherocon IV was each significantly lower than that of the Pherocon 1C, whereas the catch of the Pherocon IIB was not significantly different from that of the Pherocon 1C (Dunnett's test, df=3,9, $\alpha$=0.05).

In both trials, the catch of males in the Pherocon IV was about one-half the catch in the Pherocon 1C. The area of the liner for the Pherocon 1C trap was 63 inches$^2$, whereas the area of the liner for the Pherocon IV was 47 inches$^2$. Therefore, the area of the sticky trap surface did not explain the difference in catch for the two trap types. Smith (1989) reached the same conclusions about these traps and speculated that the differences were largely the result of the lower "lip" on the delta style trap (same as Pherocon IV) inhibiting entry of flying males. However, this does not invalidate the use

of the Pherocon IV, which is easier to handle and maintain. Lowering the threshold for a delta trap by one-half should detect approximately the same risk as a Pherocon 1C trap. The low risk thresholds for Reding's "long-fall" trapping period, which runs from 1 August to 31 October, is 250 males/trap for 'Golden Delicious' and 350 for 'Delicious' (Reding 2000). Thus, the Pherocon IV thresholds would be 125 for 'Golden Delicious' and 175 for 'Delicious'.

***Validation of risk thresholds:*** The trap technique was designed to indicate nymph density the following spring rather than fruit injury. For 'Delicious', all orchards below the 175 adults/trap threshold in the fall produced nymph densities <4/tap (the current threshold); in fact, most in this

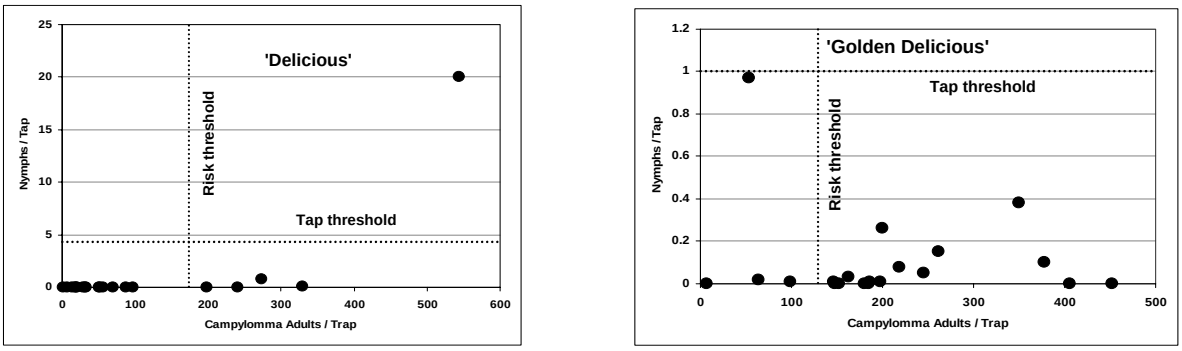



**Fig 1**. Results of trap catch and tap samples for 10 "Delicious' and 10 'Golden Delicious' orchards over two seasons. The thresholds for 'Delicious' are 175 adults/trap and 4 nymphs/tap. Thresholds for 'Golden Delicious' are 125 adults/trap and 1 nymph/tap.

group were zeros (Fig. 1).

Four out of 20 cases were false positives, and one case was a true positive (high trap catch resulted in high nymph populations). For 'Golden Delicious', four cases fell below the threshold of 125 adults/trap, and three out of four produced low (near zero) nymph densities. However, there was one false negative, where a trap catch of 54 adults/trap resulted in a near-threshold (1/tap) nymph density for this cultivar. This site had greater than 1% fruit injury at harvest. The remaining cases were false positives. Of these 12 sites, four had fruit injury above 1% at harvest.

The relationship between nymph densities and fruit damage was poor for 'Golden Delicious' (Fig. 2); however, it must be emphasized that all of these orchards were treated for *C. verbasci*. Thus, the resulting fruit damage reflects the efficiency of the control rather than the inherent damage potential on this cultivar. Despite treatment, five of the 16 cases had >1% fruit damage. It appears that the threshold of 1/tap is too high to avoid significant fruit damage (Fig. 2). Indeed, the tap sample threshold (Thistlewood 1986, Thistlewood et al. 1989) for 'Golden Delicious' assumes a higher tolerance of fruit injury. The threshold corresponds to 1% injury for 'Red Delicious' and actually 3% for 'Golden Delicious'.

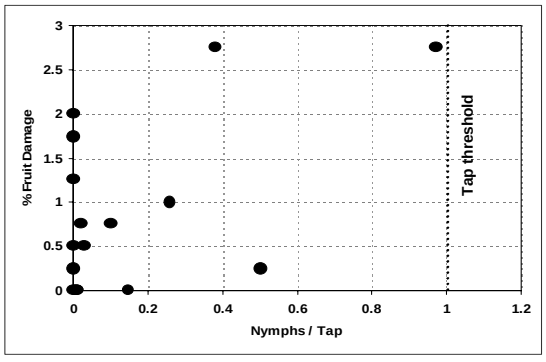



**Fig. 2.** Nymph samples and fruit damage in 10 'Golden Delicious' orchards over two seasons

*C. verbasci* females may selectively lay eggs on branches with potential prey items for their young, for example, aphids or ERM eggs (Thistlewood 1986). There is speculation (e.g. Edwards 1998) that the lack of prey items during late summer and fall

may discourage oviposition in the orchard and thus cause false positive results. However, since many of the blocks had high populations of secondary pests, especially ERM eggs, this did not explain false positives in any of the orchards. Alternatively, spring applications of chlorpyrifos (which is toxic to *C. verbasci* nymphs) may have caused false positive results in the risk assessments. Reding (2000) proposed higher thresholds for commercial orchards than for untreated orchards. Therefore, the conservative use of thresholds derived from untreated orchards would cause false positive errors in our data.

***Apple cultivar susceptibility:*** In both 2002 and 2003, none of the 'Braeburn' fruit in the trial sustained any damage, including the fruit in the check (empty) cages. The percentage of 'Golden Delicious' fruit damaged was higher than that of 'Delicious' in both years (Fig. 3). These data confirm other research and provisional thresholds that indicate 'Golden Delicious' is much more susceptible than 'Delicious' (Beers et al. 1993). The percentage of fruit damaged in trials of 'Gala', 'Fuji', and 'Granny Smith' appeared to be intermediate between the percentage damaged in trials of 'Golden Delicious' and 'Delicious'. However, in both years, for these three cultivars, damage was significantly lower than that of 'Golden Delicious' but was not significantly different than that of 'Delicious' ($2\times2$ $\chi^2$, $\alpha=0.05$). Thus, the economic injury level for 'Gala', 'Fuji', and 'Granny Smith' appears to be somewhat lower than, but close to, that of 'Delicious'. Based on this assay, a provisional conservative estimate of 2 nymphs/tap could be used for these three cultivars (see next section for refinement of 'Gala' threshold). Results for 'Cameo' were not consistent between the two years. In 2002, the percentage 'Cameo' fruit damaged was significantly lower than that of 'Golden Delicious' but was not significantly different from that of 'Delicious'. In 2003 the results were opposite ($2\times2$ $\chi^2$, $\alpha=0.05$). Pending further investigation, a conservative estimate of 1 nymph/tap could be used for 'Cameo'.

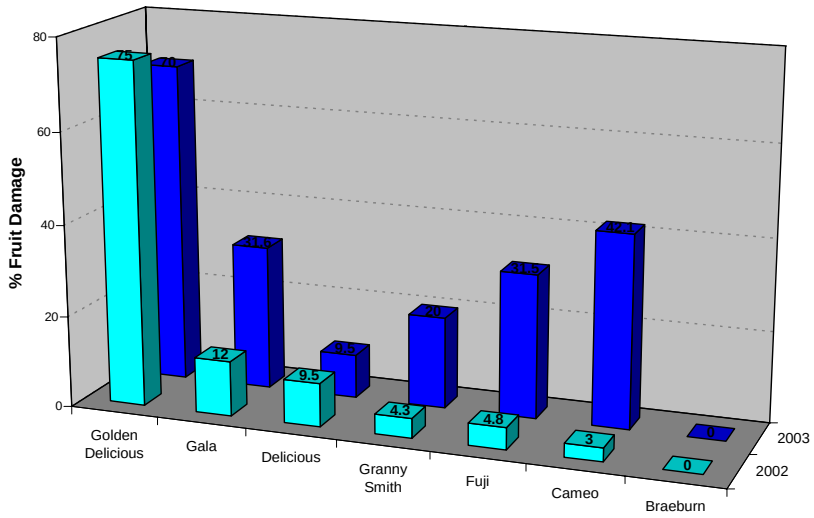



**Fig. 3.** Percentage of fruit damaged by *C. verbasci* in seven apple cultivars, 2002 and 2003.

***Economic threshold for 'Gala':*** Tap samples taken at petal fall, when cages were removed, ranged from 3 to 36 nymphs per tap and recovered an average of 42% of the nymphs introduced in the cages. The branches had an average of 15 flowers along the length of 45 cm. Of these, an average of 9 per branch had formed fruit on 29 May, and 5.8 per branch remained on the tree by 7 July. About the same number, 5.5 per branch, remained on the trees by harvest on 20 Aug. Most of the injuries caused by nymphs were single, raised growths or rough areas smaller than 0.5 mm diameter. By July, many

of these were barely visible, less than 1 mm, and resembled an abnormal lenticel (Plates 2a, 2b). A few injuries were larger than 0.5 mm initially and expanded to a wider area by harvest. Significant injuries sometimes resembled russet (Plates 3a, 3b) or large bumps. Many were surrounded with a dent in the apple surface or caused extreme growth reduction around the feeding site. Some fruit had multiple injuries, resulting in fruit distortion (Plates 4a, 4b). Injury resembling russet has not been described in the literature but may be common in orchards.

Plate 2a. Single injury site; not counted as damaged ('Gala', 20 Aug 2003).

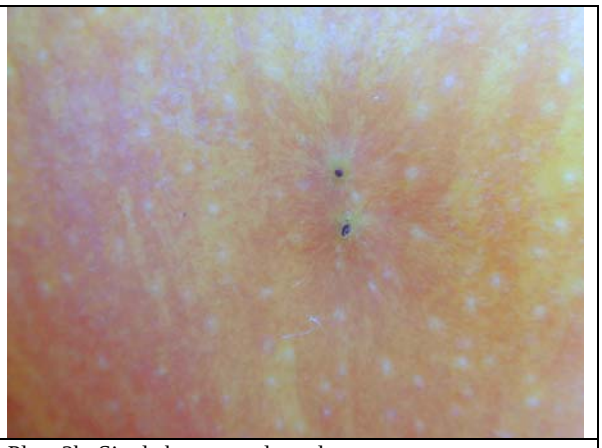


Plate 2b. Single bumps, enlarged.

Plate 3a. Damage causing fruit skin russet ('Gala', 20 Aug 2003).

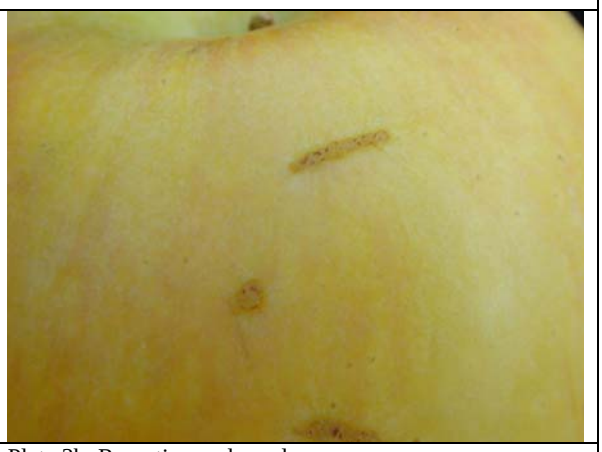


Plate 3b. Russeting, enlarged.

Plate 4a. Multiple injuries causing fruit distortion ('Gala', 20 Aug 2003).

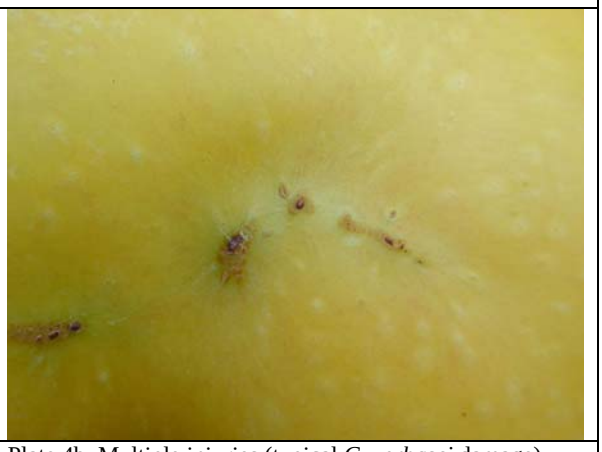


Plate 4b. Multiple injuries (typical *C. verbasci* damage) ('Gala', 20 Aug 2003).

After examining the dataset, low numbers of fruit in the formerly caged areas appeared problematic when calculating the proportion of fruit damage, and therefore all replicates with <3 fruits were deleted. In the first evaluation (27 May), the relationship between nymph population and fruit damage was excellent ($F$=38.5, $P$=0.0001, Adj. $R^2$=0.73) (Fig. 4A). At this time, most injury sites were small, and all were counted as damage. The relationship deteriorated by 7 July, when single injury points with no other associated distortion or enlargement were no longer counted as damaged ($F$=16.5, $P$=0.001, Adj. $R^2$=0.52) (Fig. 4B). There was little change from the 7 July to the 20 August evaluation because fruit numbers and damage classification had stabilized; however, the line was slightly flatter by the harvest evaluation ($F$=15.1, $P$=0.002, Adj. $R^2$=0.50) (Fig. 5).

The flattening of the lines over time is an indication of both shedding of damaged fruits and relegating some technically damaged fruits into a non-economic (undamaged) category as the fruit enlarges. The latter phenomenon is shown best by the data points that lie directly on the x-axis (up to 17 average nymphs/tap but no resulting injury) (Fig. 5). Had the fruit in this study been hand-thinned (a normal commercial practice where damaged fruit is selectively removed), the relationship would be even more obscure.

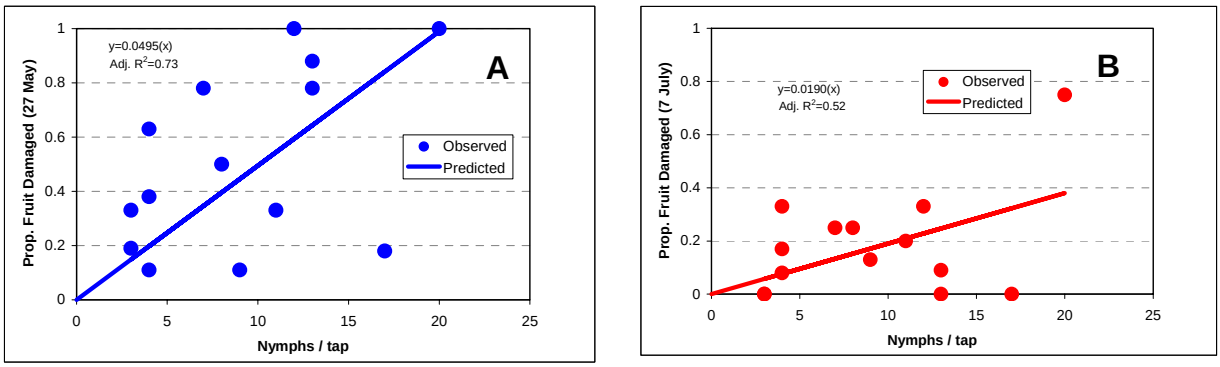
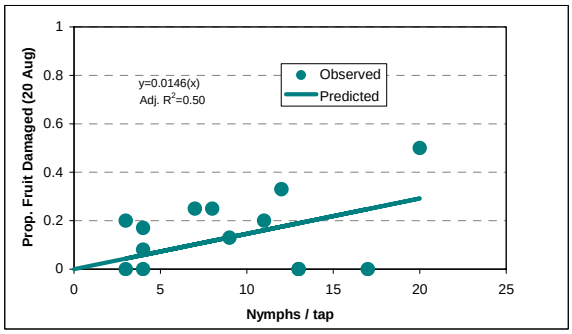



**Fig. 4** Regression of nymphs / tap and the proportion of 'Gala' fruit damaged on 27 May (A) and 7 July (B) 2003.




In 2004, the first evaluation (3 May) occurred soon after petal fall, before most symptoms developed ($F$=18.4, $P$=0.0008, Adj. $R^2$=0.54) (Fig. 6A). By 8 June, the relationship was strengthened ($F$=43.3, $P$=0.0001, Adj. $R^2$=0.74) (Fig. 6B). When fruit damage could be better classified (6 July) the relationship was less pronounced (F=26.2, P=0.0002, Adj. $R^2$=0.63) (Fig. 7A), becoming more obscure at harvest ($F$=17.8, $P$=0.0009, Adj. $R^2$=0.53) (Fig. 7B).

**Fig. 5.** Regression of nymphs/tap and the proportion of 'Gala' fruit damaged on 20 Aug 2003.

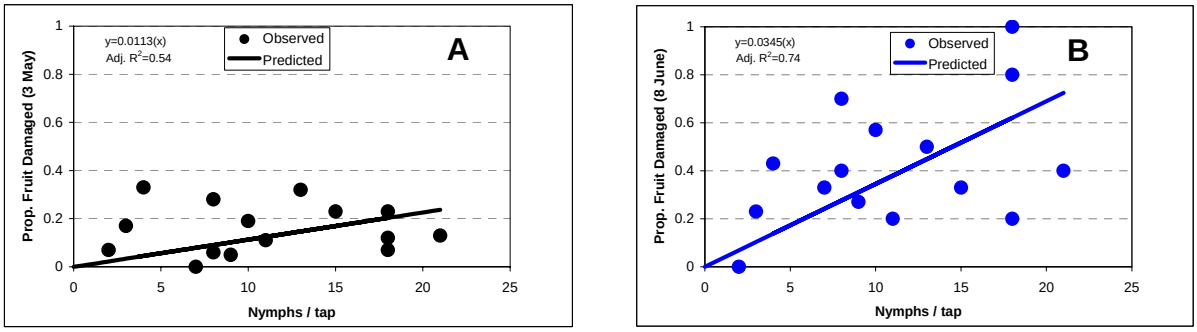



**Fig. 6.** Regression of nymphs / tap and the proportion of 'Gala' fruit damaged on 3 May (A) and 8 June (B) 2004.

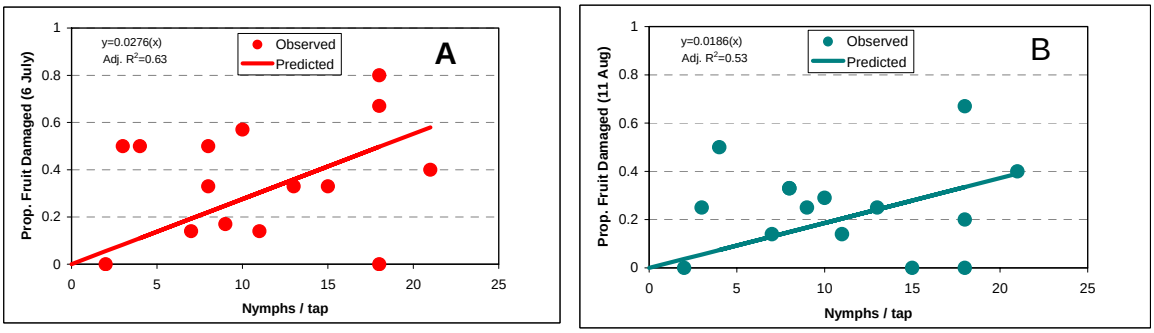



**Fig. 7.** Regression of nymphs/tap and the proportion of 'Gala' fruit damaged on 6 July (A) and 11 Aug (B) 2004.

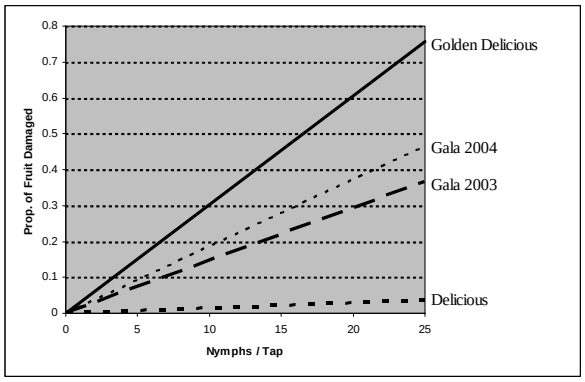



**Fig. 8.** Regression equations for nymphs / tap and proportion of 'Gala' fruit damaged compared with similar equations for 'Delicious' and 'Golden Delicious' (Thistlewood et al. 1989).

Using the regression developed from the harvest data, an average of 1.5 (2003) and 1.9 (2004) nymphs/tap would produce 1% fruit injury at harvest. A provisional (conservative) threshold of 1 nymph/tap can be used. Although data were collected in different manners, the results can be compared with equations reported for 'Delicious' and 'Golden Delicious' (Thistlewood et al. 1989) (Fig. 8). As in the field assay, 'Gala' appears intermediate in susceptibility to *C. verbasci*.

Microscopic photography of the surface of fruit during flowering revealed great differences in the density of trichomes. 'Golden Delicious', the cultivar most susceptible to *C. verbasci* feeding injury, had the most clearly visible surface (Plate 5a). Other cultivars such as 'Gala' and 'Delicious' had surfaces obscured by dense trichomes (Plates 5b, 5c). The sparse trichomes of 'Golden Delicious' may allow greater access by the mouthparts of *C. verbasci* nymphs and so would allow more frequent feeding. Conversely, cultivars such as 'Gala' and 'Delicious' may be less accessible to nymphs and thus less susceptible to injury.

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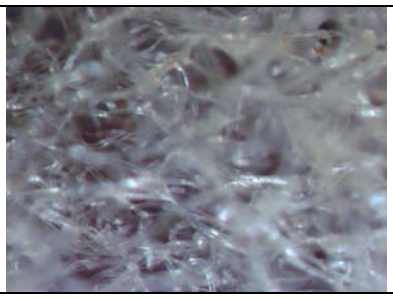
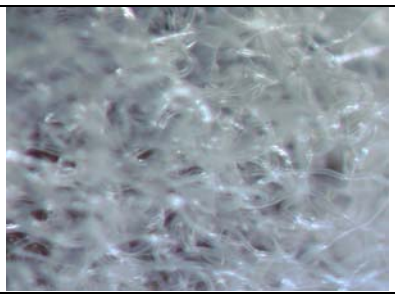
|  |  |  |
| Plate 5a. Surface of 'Golden Delicious' fruit at flowering. | Plate 5b. Surface of 'Gala' fruit at flowering. | Plate 5. Surface of 'Delicious' fruit at flowering. |

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**Thistlewood, H. M. A. 1986.** The bionomics and monitoring of *Campylomma verbasci* (Meyer) on apple in the Okanagan valley, British Columbia. Ph. D. Simon Fraser University, Burnaby, B.C.

**Thistlewood, H. M. A., R. D. McMullen, and J. H. Borden. 1989.** Damage and economic injury levels of the mullein bug, *Campylomma verbasci* (Meyer) (Heteroptera: Miridae), on apple in the Okanagan valley. Can. Ent. 121: 1-9.

**Budget**:

**Project title**: Feeding behavior, thresholds, and pheromone trapping of *Campylomma verbasci*
**PI**: Elizabeth H. Beers
**Project duration**: 2002 through 2004
**Current year**: 2004
**Project total**: $78,132

| | **Year 1** | **Year 2** | **Year 3** |
|---|---|---|---|
| **Total** | **$27,385** | **$24,156** | **$26,591** |

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

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

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

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

**OBJECTIVES:**

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

**SIGNIFICANT FINDINGS- *LYGUS***

**1. *Lygus* Economic Thresholds.** Branch cage studies were conducted to determine the period of time at which Lygus feeding resulted in the most damage. These studies helped quantify proportional *Lygus* abundance to fruit damage. From these studies we determined that Lygus feeding can result in sub-surface superficial feeding injury at any point between fruit set and harvest, but that early to mid-spring feeding is cosmetically more damaging then summer feeding.

**2. Cover Crops/ Indigenous Plants.** Replicated plots of 14 cover crop blends were established on the Roza unit at WSU IAREC in May 2003. We have documented significant differences among cover crop blends in their potential to build populations of *Lygus*.

**3. Biological Control/ Orchard Surveys for Lygus Parasitoids.** A parasite *Peristenus* spp. attacks the nymph stages of *Lygus* and keeps individuals from reaching sexual maturity by emerging in the late instar nymph or early adult stage. Extensive surveys conducted by Walsh in 2002, 2003 and 2004 determined the presence of Lygus parasitism by *Persitenus* spp. in most of the orchards surveyed in Washington State.

**4. Inoculative Releases of *Persitenus* into refugia.** Populations of *Persitenus* are inconsistent over the sites we surveyed annually and innoculative releases of paraitized will not be effective at establishing populations of *Peristenus*.

**5. Orchard Floor Treatments.** In 2004 we compared the efficacy of Carzol, Round-up, Asana and mowing as orchard floor treatments. *Lygus* were not detected before the treatments were applied. A week following the treatments, Lygus began to inhabit the orchard. The sweep net samples showed that *Lygus* were significantly less abundant in the mowed plots when compared to the other treatments. The following week showed a reduction in Lygus abundance across all treatments, but the Asana and Roundup treatments hosted fewer *Lygus*.

**RESULTS/ DISCUSSION LYGUS-**

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

Figure 1. April 2001 Feeding Injury

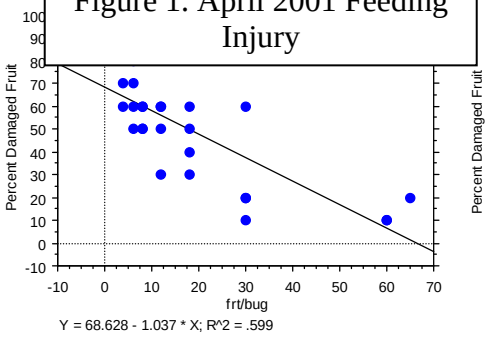



Figure 2. July 2001 Feeding

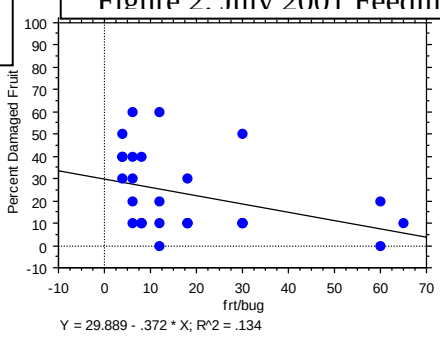



Figure 3. May 2002 Feeding

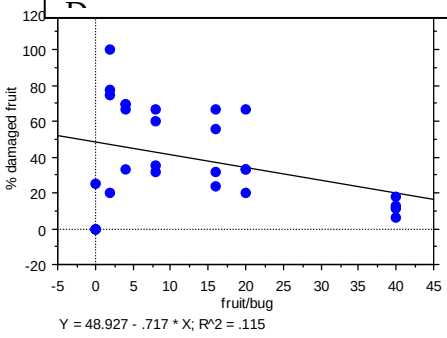



Figure 4. Sequential feeding injury 2002

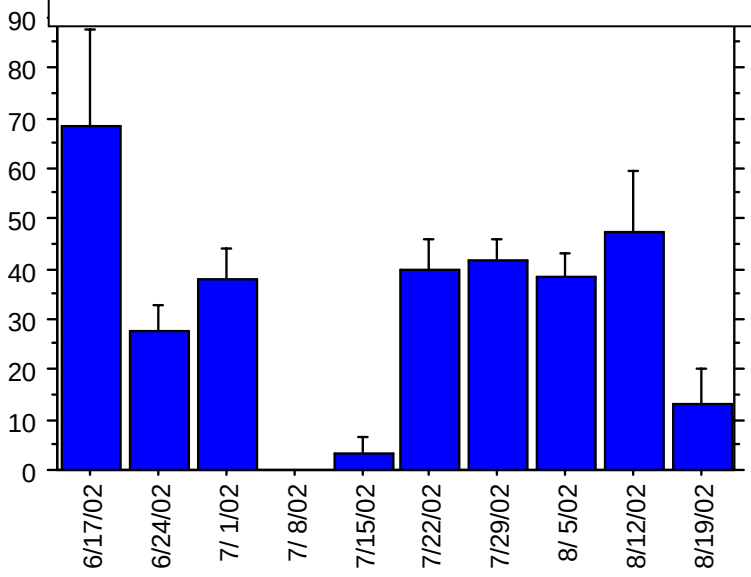
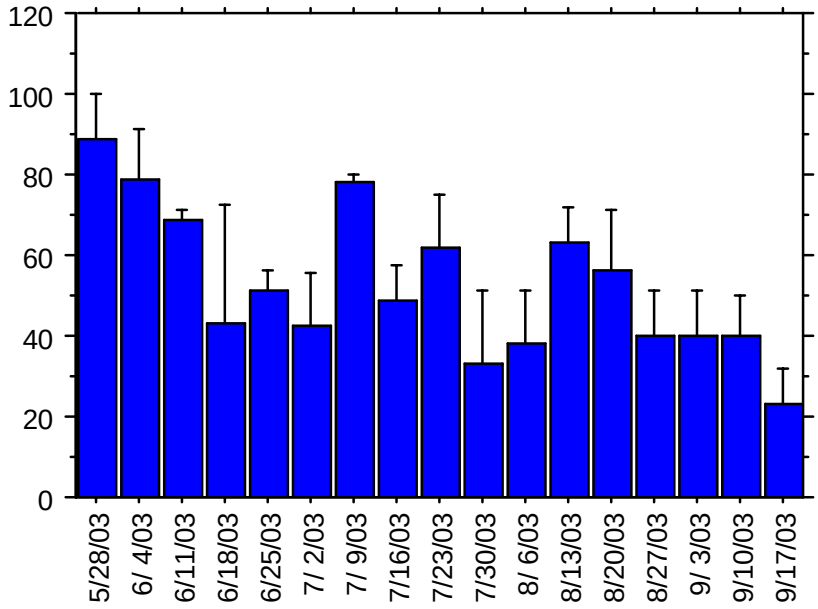
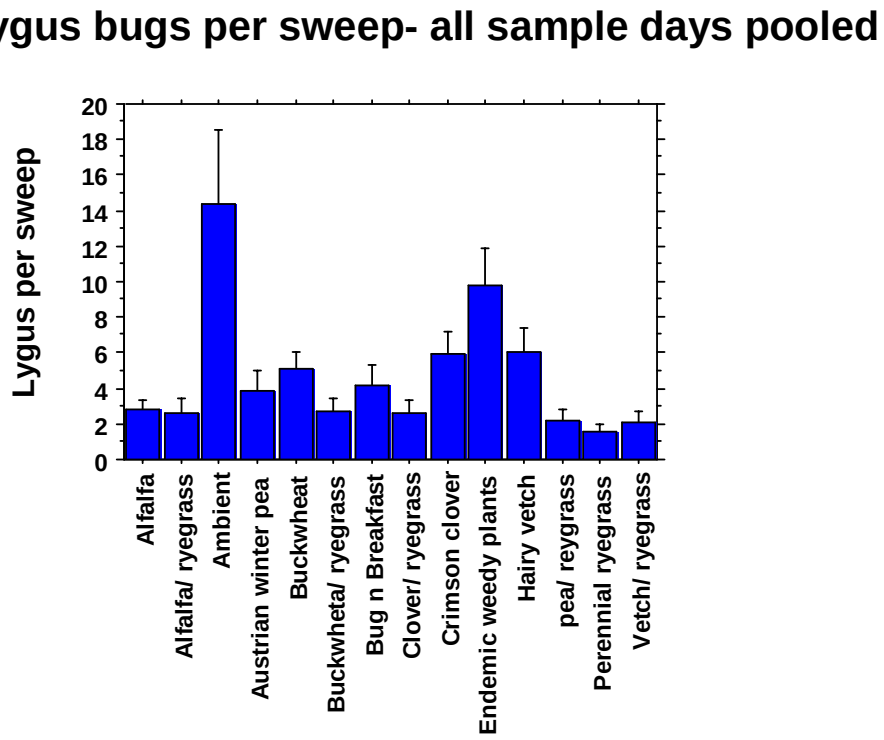


Figure 5. Sequential feeding injury 2003

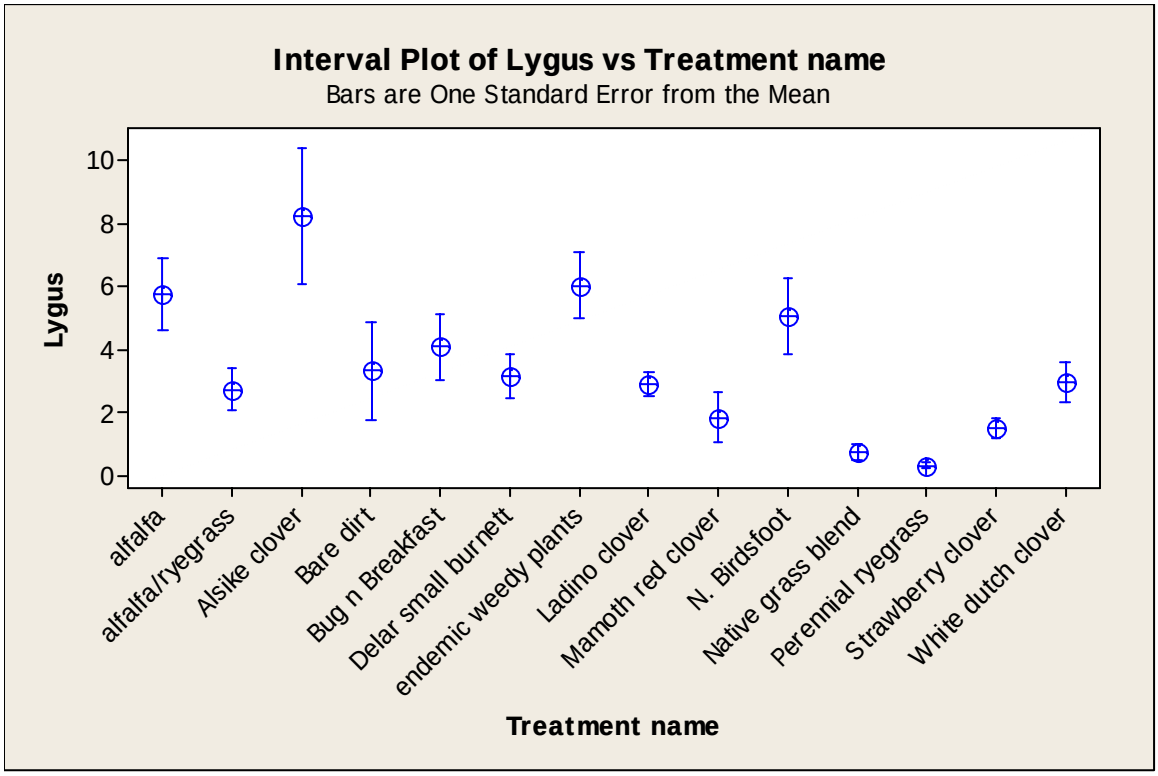


**2. Orchard floor cover crops.** Replicated plots of 14 cover crop blends were established on the Roza unit at WSU IAREC in May 2003. Cover crop blends included perennial rygrass, buckwheat, buckwheat/ryegrass, alfalfa, crimson clover, hairy vetch, alfalfa/ryegrass, clover/ryegrass, vetch/ryegrass,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




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

In 2004 Lygus abundance was greatest in the alfalfa, alsike clover, endemic weedy plants, and birdsfoot trefoil cover crops. Lygus numbers were low in the native grass blend, perennial ryegrass, and strawberry clover treatments. The bare dirt and native grass blends hosted significantly fewer leafhoppers than did the other treatments. This data indicates that the native grass blend and perennial ryegrass were the treatments least likely to host the Lygus.



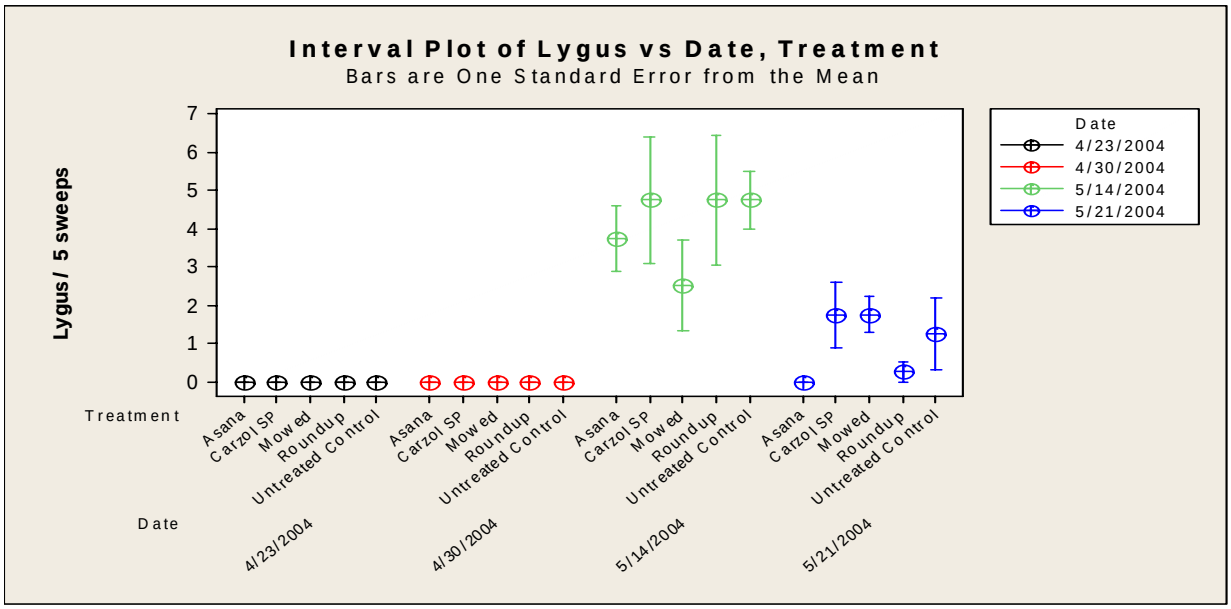

**3 & 4. Biolgical control**. A parasite attacking Lygus spp. was discovered in 1995 in Washington State and subsequent collections in Parma, Idaho in 1996 and 1997 showed that the parasite was present (Mayer unpublished data). The parasite has been described as *Peristenus howardi* Shaw (Hymenoptera: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

Collections made in 2000 (Mayer, unpublished data) did not document the parasite's presence beyond the Touchet, Washington and Parma, ID regions. Extensive surveys conducted by Walsh (AE News 2003) in 2002, 2003 and 2004 determined the presence of *Lygus* parasitism by *Persitenus* spp. in several important fruit production regions in Washington State. However, the results of the survey were disappointing in that levels of parasitism were low or not detected in several important fruit growing areas Extensive surveys in 2002 and 2003 determined the presence of Lygus parasitism by *Persitenus* spp. In total over 150 sites were surveyed over 3 years and over 11,000 Lygus were dissected to determine if *Peristenus* spp were present. In 2002, 38 sites were surveyed and 4,724 nymphs collected of which 190 nymphs were parasitized. In 2003, 67 sites were surveyed and 4,468 nymphs were collected of which 48 nymphs were parasitized. In 2004, 79 sites were surveyed and 3,450 nymphs were collected of which 173 were parasitized. Parasitism never exceeded 30%. And unfortunately was not detected in the majority of orchards that were surveyed over 3 years. Parasitism of Lygus by *Peristenus* was greatest in areas that were less disturbed by human activity.

**5. Orchard Floor Treatments:**
Replicated 5,000 square foot blocks were established in a commercial c.v. 'Granny Smith' orchard on the Roza Unit near WSU Prosser. Carzol, Asana, and Roundup were applied at recommended field rates with an ATV mounted boom sprayer. Controls were left unaltered while in the mowing treatment vegetation was cut to four to five inches. Pesticide treatments and mowing were conducted on April 26, 2004 in an attempt to assess how thrips and Lygus would respond. For each sample date, four yellow sticky cards were placed on the orchard floor and canopy in each plot to assess thrips abundance. Lygus were surveyed by sweeping the orchard floor with a sweep net five times per plot.

Lygus were not detected before the treatments were applied. A week following the treatments, Lygus began to inhabit the orchard. The sweep net samples showed that Lygus were significantly less abundant in the mowed plots when compared to the other treatments. The following week showed a reduction in Lygus abundance across all treatments, but the Asana and Roundup treatments hosted fewer Lygus. By this time, the vegetation in the Roundup treatments was dead and subsequently unlikely to host Lygus. This data indicates that frequent mowing of the orchard floor may help to reduce Lygus abundance.

Orchard Floor treatments 2004.



**FINAL REPORT**

**Project Title**: Management of Codling Moth
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**Objectives**:

1. Optimizing the use of microencapsulated sex pheromone for codling moth.
2. Refine and field-test the A.K.I.S.S. (Attractive Killing Interception Sensory Station) baited with the DA lure (pear ester) for control of codling moth.
3. Monitoring the mating success of codling moth under different sex pheromone-based control programs.

**Significant findings:**

- **Apple orchard plots treated with an ultra low volume application of a microencapsulated sprayable sex pheromone formulation for codling moth had significantly lower levels of fruit injury than similar plots treated with a standard air blast application and untreated check plots.**
- **The ultra low volume application deposited nearly seven times more capsules per leaf than the air blast application. The lower leaf surface in the upper canopy had the highest mean capsule density.**
- **Apple, pear, and walnut leaves treated with 40 capsules per leaf remained attractive for at least 4 weeks under field conditions. The influence of rain was to primarily remove capsules from the top of leaves.**
- **The addition of Asana to the sprayable sex pheromone killed moths for two weeks and mortality was dependent on the density of capsules deposited per leaf.**
- **The addition of Asana with the sprayable sex pheromone further reduced moth catch 80% for two weeks in replicated orchards.**
- **A new formulation of the microencapsulated material significantly extended the disruption of sex pheromone-baited traps with commercial formulation from 2 to 5 weeks.**
- **A killing station (AKISS) was developed and field-tested in 28 apple plots during 2004.**
- **Data from 16 orchards were summarized at mid-season and fruit injury was reduced ca. 50% versus the untreated orchard plots.**
- **Several problems were encountered with the killing station including, breakage, loss of residue from foliage, repellency, lack of clarity with the grease, and poor positioning of the lure.**
- **A new improved killing station has been designed and tested in the laboratory.**
- **Other fruit volatiles tested were less effective in catching female codling moth than the pear ester.**

- **Greater than 85% of female codling moths caught in traps baited with pear ester were mated in orchards treated with Pheromone Mops, microencapsulated sex pheromone, hand-applied dispensers, and left untreated.**

Methods:

**Microencapsulated sex pheromone.** Studies were conducted in replicated (n = 5) 1-2 ha apple blocks to compare the efficacy of applications of Checkmate® CM-F (Suterra Inc., Bend, OR) with either an air blast (926 liters per ha) or an ultra low volume sprayer (12 liters per ha). Codlemone was applied in all plots at a rate of 49.0 g A.I. per ha. Six applications were made 2-4 weeks apart during the season. Spray intervals of less than 4 weeks were used in some cases due to the occurrence of rainfall and indications that the air blast application was failing. Untreated blocks were included in the study. All plots received three insecticide applications during the season. Blocks were monitored with traps and fruit injury was sampled at mid-season and prior to harvest. Transformed injury data were analyzed with ANOVA.

The density and distribution of microcapsules deposited with the ultra low volume and air blast sprayers were estimated by spraying a similar formulation with 0.50% fluorescent dye added. The density of fluorescent microcapsules per leaf were counted on ten leaves from twenty shoots collected from the lower and upper canopy. Capsule density as a function of canopy height and between the top and bottom of leaves were analyzed with a paired t-test for each spray method separately. Apple, pear, and walnut leaves were treated with 40 microcapsules per leaf surface. Codling moth males (n = 3) were flown in a flight tunnel to detached leaves (n = 5) weekly for five weeks. The apple test was repeated due to the occurrence of rainfall during the first test.

The impact of adding an insecticide to the ultra low volume application of microencapsulated sex pheromone was evaluated in two types of tests. In the first test, the maximum rate of Asana XL (14.6 fl oz) was added to the microencapsulated sex pheromone plus the fluorescent dye and sprayed on potted apple trees. Leaves with 0 – 150 microcapsules per leaf were identified and labeled. Every 7 days leaves with a range of microcapsule densities were selected. Moths were touched to the upper leaf surface for 3 seconds and mortality was assessed after 24 hours. Asana XL (14.6 fl o z) was added to the microencapsulated sprayable material and applied with the ultra low volume sprayer to four apple orchards. Each block was split in half and treated with sex pheromone plus Asana on half and only sex pheromone on the other half. Two sex pheromone-baited traps were placed in each block and monitored for two weeks.

A new formulation of Suterra's microencapsulated sex pheromone was tested during August and September. Five apple orchards were split into halves. Each half was sprayed either with the commercial formulation or the new formulation. Two sex pheromone-baited delta traps were placed in each orchard half and monitored weekly for 6 weeks.

**Development of a killing station**. The AKISS design developed during 2003-04 was a clear 0.1 $m^2$ square plastic sheet coated with a clear FDA-approved food service grease (Royal Purple) mixed with 6.0% esfenvalerate. Eight apple plots were established (0.5 – 1.2 ha) in Orondo and 20 plots in Moxee during 2004. All plots were treated with 60 killing stations per ha. Treated plots were paired with similar untreated plots. Plots in Orondo were treated with 2 insecticide applications and plots in Moxee were treated with two applications of microencapsulated sex pheromone. Fruit injury from the Orondo plots and 8 Moxee plots was assessed in early July and transformed data were analyzed with ANOVA.

A new AKISS design was developed during 2004 that uses an open plastic grid design coated with a clear water-white gel mixed with 6.0% esfenvalerate. The lure was placed in the center of the trap. A series of male codling moths were flown to either the 2004 design or the new design in a flight tunnel. Killing stations were hung from an artificial apple branch with foliage.

Studies were conducted to evaluate the attractiveness of several reported fruit volatile kairomones for codling moth: pear ester, Dimethyl nonatriene, Z-3 Hexenyl Acetate, Beta Farnesene, and Farnesol. Compounds were loaded into gray halobutyl elastomer septa and placed in delta-shaped

traps in a heavily infested orchard on 14 July. Sex pheromone-baited traps and traps baited with a solvent control were included in the study. Traps were checked and rotated every few days until 26 July.

**Mating success of codling moth**. The mating success of female codling moths was assessed with 100 delta-shaped traps placed within 20 apple orchards treated with Pheromone Mops in the Brewster area and in 10 orchard plots treated with microencapsulated sex pheromone in the Moxee area. Similar data were also collected from two orchards treated with Isomate-C PLUS dispensers, and five orchards left untreated. Data are presented for the first and second moth flight (> 5 July).

**Results and discussion:**

**Microencapsulated sex pheromone**. Moth counts were reduced 68% and 92% in the air blast and ULV-treated plots versus the untreated plots (Fig. 1). Significant differences occurred in the levels of fruit injury among treatments with the ULV treatment having significantly less injury than the untreated plots (Table 1). The mean density of microcapsules in the air blast treatment was 2.9 per leaf, and the highest density was 14 microcapsules per leaf. The ULV application deposited an average of 19.7 microcapsules per leaf and a maximum of 157 per leaf (Table 2). Significantly more capsules were deposited in the upper than lower canopy with the ULV application. Both application methods deposited nearly twice the number of microcapsules on the underside than on the top of leaves in the upper canopy. Apple, pear, and walnut eaves treated with 40 microcapsules loss their attraction gradually over time and some leaves were still attractive after 5 weeks (Table 3). Natural rainfall reduced the attractiveness of apple leaves treated on their upper surface but not with leaves treated on their lower surface. Significant levels of adult mortality occurred following a 3-s contact with leaves sprayed with the microencapsulated sex pheromone and Asana (Fig. 2). A strong linear dose response was found and the toxicity of treated leaves declined over time. The occurrence of rainfall reduced the toxicity of treated leaves. The addition of Asana to the microencapsulated sex pheromone further reduced moth catch 80% versus the sex pheromone-alone treatment over the two-week test period (Table 4). The new microencapsulated formulation significantly reduced moth catch for 5 weeks while the commercial formulation was effective for only two weeks in this test (Fig. 3).

**The use of microencapsulated sprayable formulations for codling moth has been widely field tested for more than 5 years in the United States but have not been adopted by growers. Despite their promise of a flexible tactic that can be applied with standard equipment and is compatible with other pesticide applications they have not performed well. This poor performance has been blamed on their short residual activity in the field due to instability of their formulations. Alternatively, my studies have focused on improving their efficacy by increasing the deposition and retention of capsules within the orchard canopy. The preliminary studies reported here suggest that the use of ultra low volume applications can dramatically improve their performance. The effectiveness of the ultra low volume applications was first demonstrated in 2000 and last year I began to work with these materials with the idea of creating hundreds of attractive point sources on trees. My research has suggested that the current hand-applied dispenses are not effective in disrupting mating and that sex pheromones reduce populations through a combination of disruption and a delay in mating. Similar results have been found for oriental fruit moth and European corn borer. Studies are needed to expand the evaluation of the ULV approach to grower orchard trials and to compare this approach with the standard use of hand-applied dispensers. Furthermore, a number of factors associated with the use of microencapsulated sex pheromones need to be explored to further optimize this technology, such as spray pressure, amount of water, addition of stickers, angle of nozzle and nozzle type.**

Development of a killing station. **Significant and near significant differences in fruit injury occurred with the addition of killing stations (Fig. 4). Plots treated with killing stations averaged 9.4% versus 16.6% fruit injury in the untreated plots in Orondo ($P = 0.06$) and 0.8% versus 2.1% in Moxee ($P = 0.02$), respectively. A number of problems were noted with this**

**approach during the season and the test was terminated at midseason. These included a loss in the toxicity of the panes due to removal of grease from abrasion by foliage and the cumulative effects of rain and wind; and the breakage of stations due to wind. Fruit injury data from 12 of the Moxee plots were not collected due to the loss of most of the stations. Repellency of the killing stations and placement of the lure above the station were later found to have reduced moth contact with the treated surface of the station.**

**Moth contact with the killing station was affected by several factors. The insecticide mixture was found to be repellant after 24 h and not at 120 h of field aging. During June the contact toxicity of the killing stations was reduced after 4 weeks (15% alive) and after 7 weeks (25% alive). In contrast, stations hung free of foliage last season and again this year both caused 100% mortality for 10 weeks. Similarly, the new open grid design was 100% effective during an 8-week trial this fall. The loss in toxicity observed in early summer this year was thought to be due to contact with foliage and weathering from rainfall and wind. During the season we noticed that the grease was not clear and stations did not get covered with scales or other insects that we typically see with the use of STP-coated traps. Flight tunnel tests found that the grease was somewhat repellant (Table 5). The new open grid design with the lure placed in the center had a significantly higher proportion of moths contacting the killing station than the solid pane design with the lure clipped at the top.**

**In studies with several apple volatiles traps baited with the pear ester were the only ones to catch female codling moths. The sex pheromone-baited traps averaged 70 moths per trap and the pear ester traps averaged 5 males and 4 females during this trial. Traps baited with the other volatiles averaged < 1 moth per trap.**

**The potent attraction of the pear ester for female codling moth has offered a new approach to manage codling moth. Killing stations could become an important addition to sex pheromones especially along the borders of orchards and near bin piles and other extra-orchard sources of codling moth. This ongoing research is focused on developing an effective tactic that is also cost effective and user friendly. Future studies will continue to focus on an improved design and to better understand the link between moth behavior and trap design.**

Mating status of codling moth**. Very high levels of mating by female codling moths were found in all orchards regardless of sex pheromone treatment (Table 6). The percentage of mated females was somewhat lower in the ULV microencapsulated-treated orchards. Pear ester lures are known to be somewhat biased for mated versus virgin female codling moths. However, this year's data are the highest percentage of mated females trapped in orchards treated with sex pheromones than any other in the past six years. This extended data set supports the idea that reductions in populations of codling moth in orchards treated with sex pheromones are likely due to a combination of mating disruption, delay of mating, and enhanced biological control.**

**Budget:**

| Item | Year 1 (2004) |
|---|---|
| Salaries[1] | 22,388 |
| Benefits (34%) | 7,612 |
| Equipment | 0 |
| Supplies | 0 |
| **Total** | **30,000** |

[1] Six months salary for a GS-7 term .

**Table 1. Mean percent fruit injury in plots (n = 5) treated with two application methods of microencapsulated sex pheromone and an untreated control. All plots were sprayed with three applications of insecticides.**

| | Mean % ± SE fruit injury | |
|---|---|---|
| Treatment | Mid-season 8 July | Pre-harvest 1 Sept. |
| Untreated | 9.2 ± 2.3a | 27.8 ± 8.7a |
| Air blast | 4.9 ± 2.2ab | 8.7 ± 2.3b |
| ULV | 1.2 ± 0.2b | 3.3 ± 1.1b |
| ANOVA df = 2, 12 | $F = 4.78$, $P < 0.05$ | $F = 6.04$, $P < 0.05$ |

**Table 2. Mean deposition of microcapsules on the bottom and top of leaves within apple canopies following either an air blast or an ULV spray application.**

| | Mean ± SE microcapsules | | | | | | |
|---|---|---|---|---|---|---|---|
| | Lower canopy | | | Upper canopy | | | Tree |
| Treatment | Bottom | Top | Average | Bottom | Top | Average | Average |
| Air blast | 2.2 ± 0.3 | 0.6 ± 0.1 | 2.8 ± 0.3 | 2.0 ± 0.2 | 0.9 ± 0.1 | 2.9 ± 0.3 | 2.9 ± 0.2 |
| ULV | 7.5 ± 2.3 | 9.8 ± 1.0 | 17.3 ± 2.7 | 15.8 ± 2.8 | 6.3 ± 0.8 | 22.1 ± 3.3 | 19.7 ± 2.1 |
| P-value | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

A fluorescent dye was added to the microencapsulated formulation to facilitate counting of capsules.

**Table 3. Mean proportion of apple, pear, and walnut leaves treated with 40 microcapsules per leaf surface and aged in the field for 1 – 35 d that were contacted by male codling moths flown in a flight tunnel.**

| | | Mean ± SE percent male moths contacting leaf | | | | | |
|---|---|---|---|---|---|---|---|
| Leaf surface | Start of test date | 1 d | 7 d | 14 d | 21 d | 28 d | 35 d |
| Apple top | 4 June | 0.9 ± 0.1 | 0.2 ± 0.2[a] | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.2 ± 0.2 | 0.2 ± 0.2 |
| Apple bottom | | 0.8 ± 0.1 | 0.7 ± 0.2[a] | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.6 ± 0.2 | 0.5 ± 0.2 |
| Walnut top | 9 June | 0.8 ± 0.1 | 0.6 ± 0.2 | 0.5 ± 0.2 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 |
| Walnut bottom | | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.3 ± 0.2 | 0.6 ± 0.2 | 0.4 ± 0.2 | 0.4 ± 0.2 |
| Pear top | 17 June | 0.7 ± 0.1 | 0.5 ± 0.2 | 0.4 ± 0.2 | 0.3 ± 0.2 | 0.1 ± 0.1 | - |
| Pear bottom | | 0.9 ± 0.1 | 0.7 ± 0.2 | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.3 ± 0.2 | - |
| Apple top | 21 July | 0.7 ± 0.1 | 0.7 ± 0.2 | 0.5 ± 0.2 | 0.2 ± 0.1[a] | - | - |
| Apple bottom | | 1.0 ± 0.0 | 0.9 ± 0.1 | 0.6 ± 0.2 | 0.6 ± 0.2[a] | - | - |

[a] Leaves were exposed to 0.5" rainfall prior to this date.

**Table 4. Reductions in trap catch of male codling moths in sex pheromone-baited traps in apple orchards (n = 4) sprayed with microencapsulated sex pheromone with and without Asana added or untreated.**

| | Mean ± SE moth catch per trap | | |
|---|---|---|---|
| Weeks after spray | Untreated | Sex pheromone | Sex pheromone + Asana |
| 1 | 26.8 ± 4.6 | 2.5 ± 1.1 | 0.6 ± 0.5 |
| 2 | 26.8 ± 6.2 | 1.1 ± 0.7 | 0.1 ± 0.1 |

**Table 5. Flight tunnel tests of new open grid AKISS design. The sex pheromone lure was placed in the center of the open grid and at the top of the solid pane in these tests.**

| | Proportion moths contacting: | | | | | |
|---|---|---|---|---|---|---|
| | No foliage | | Foliage added | | | |
| | Station | | Station | | Foliage | |
| Test | Solid pane | Open grid | Solid pane | Open grid | Solid pane | Open grid |
| Station only | 0.86a | 0.79a | - | 1.00a | - | 0.13a |
| Station w'grease | 0.60b | 0.50b | 0.40A | 0.84bB | 0.84B | 0.28bA |
| Station'w grease + Asana | 0.60b | 0.60ab | 0.36A | 0.76bB | 0.92B | 0.36bA |

Column (small letters) and row means (cap letters for tests with foliage for either station or foliage contact) followed by different letters were significantly different, $P < 0.05$.

**Table 6. Mating status of female codling moth in apple orchards treated with Pheromone Mops, microencapsulated formulations, hand-applied dispensers, and no sex pheromone during 2004.**

| | Percentage females mated | |
|---|---|---|
| **Sex pheromone treatment** | 1st flight | 2nd flight |
| Pheromone Mops (n = 20) | 97.2 | 94.7 |
| Microencapsulated (n = 10) | 87.9 | 92.5 |
| Hand-applied (n = 2) | 100.0 | 100.0 |
| Untreated (n = 5) | 96.4 | 90.8 |

**Figure 1. Weekly moth catch in apple plots treated with microencapsulated capsules either sprayed with an air blast sprayer or an ULV sprayer and in plots left untreated.**

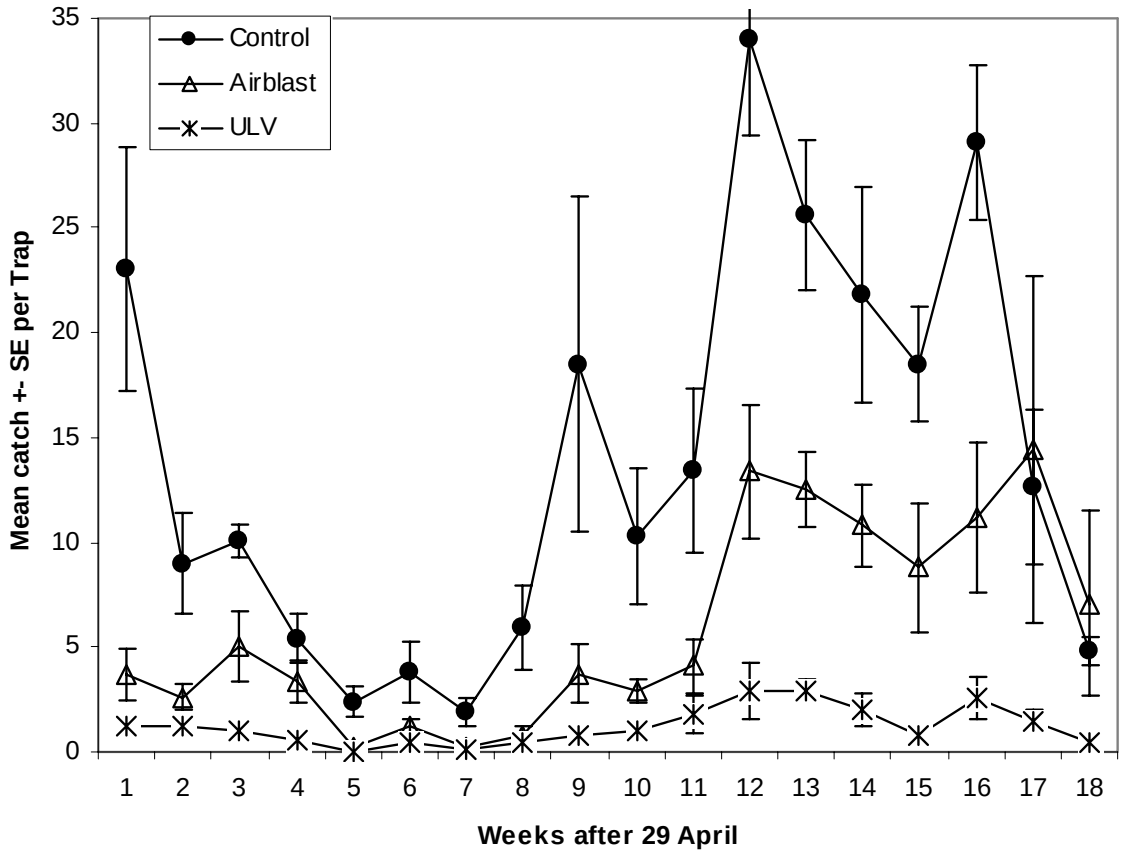
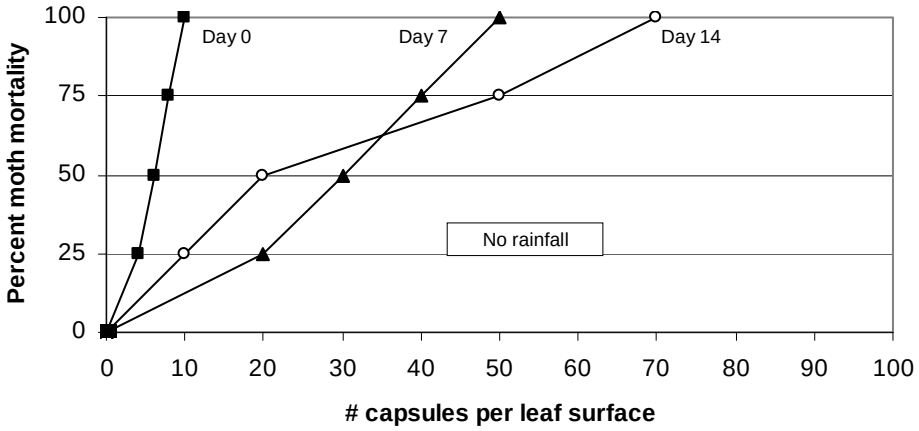


**Figure 2. Mortality of adult codling moths exposed to leaf surfaces for 3 s treated with variable numbers of microcapsules plus Asana. No rainfall occurred during test 1 but nearly 0.5" occurred on day 3 after the start of the second test.**



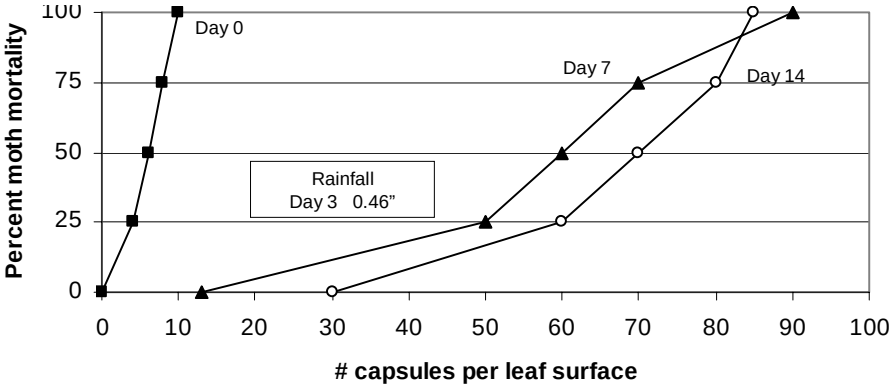



**Fig 3a. Comparison of moth catches in four apple orchards sprayed with a new formulation of Suterra's Checkmate CM-F. Moth catches were significantly reduced in the treated blocks for five weeks.**

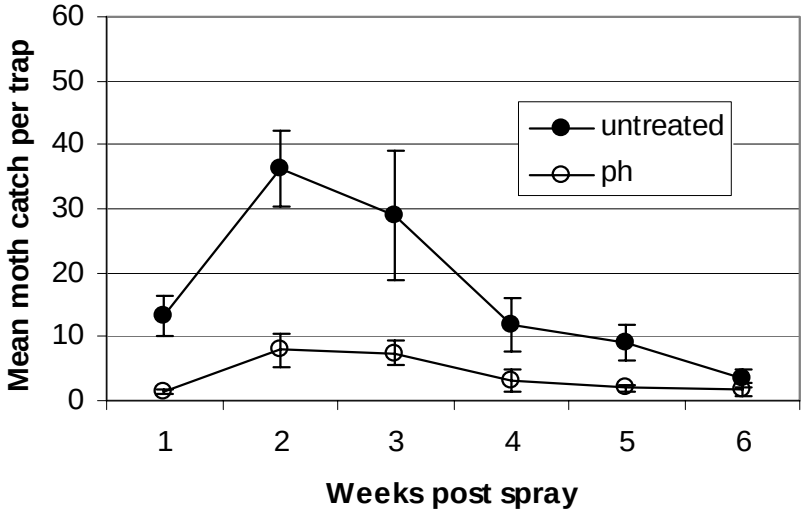



**Fig 3b. Comparison of moth catches in four apple orchards sprayed with the standard formulation of Suterra's Checkmate CM-F. Moth catches were significantly reduced in the treated blocks for two weeks.**

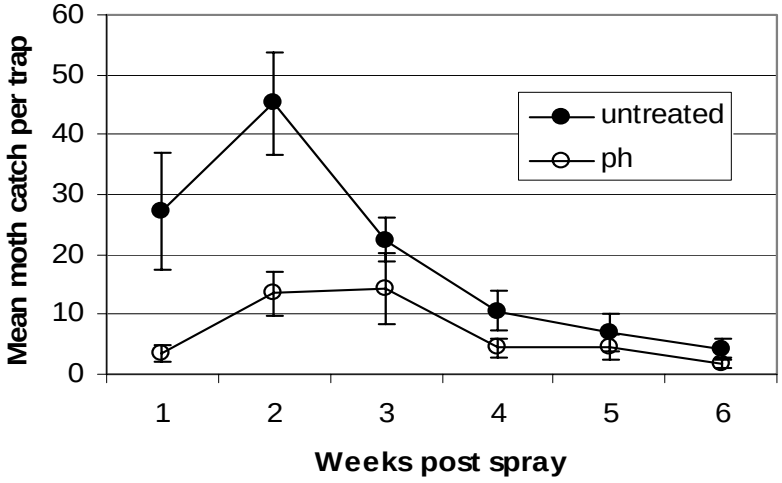
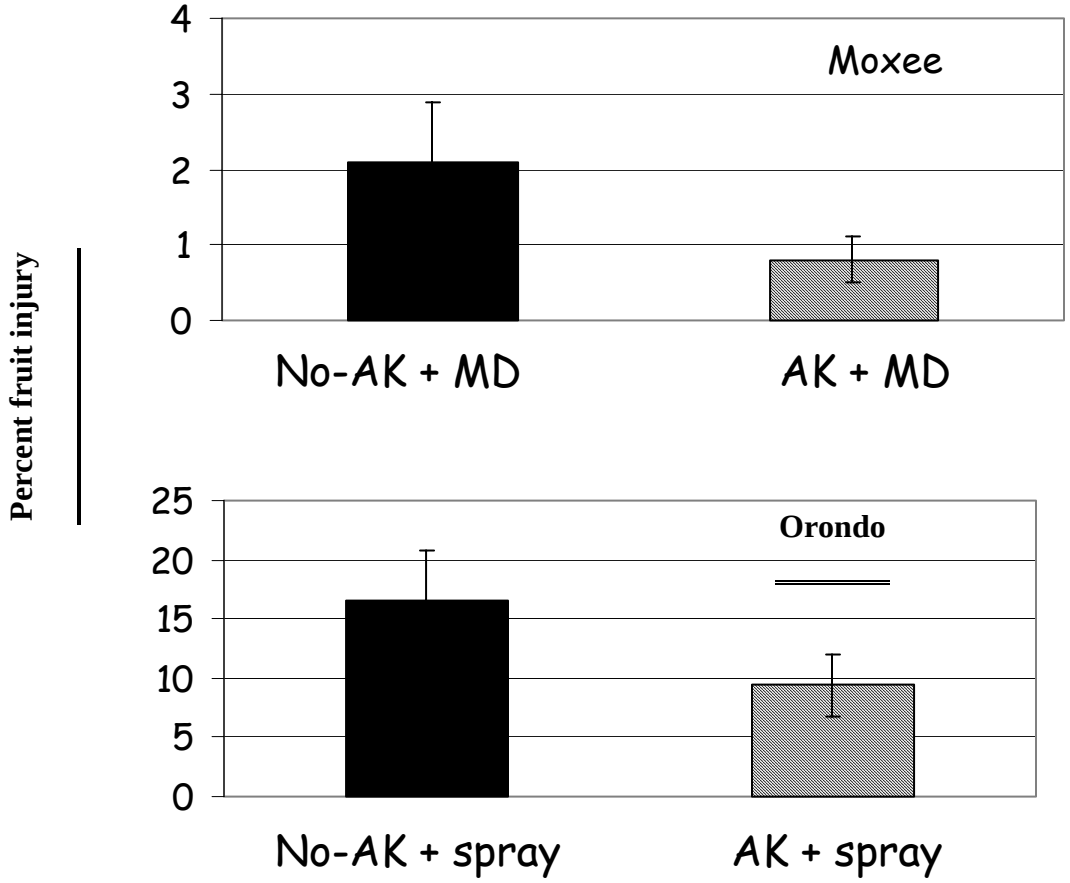



**Figure 4. Percent fruit injury in replicated apple blocks (n = 8) treated with and without pear ester and sex pheromone-baited killing stations (AK). Moxee orchards were also treated with microencapsulated sex pheromone and Orondo orchards**

**were sprayed with insecticides.**



**FINAL PROJECT REPORT**

**Project Title**: Codling moth selection of pupation and hibernation sites: enhancement of tree banding.

**PI**: Peter J. Landolt, Research Entomologist and Research Leader.
**Organization**: USDA, ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951.
**Co-PI(s) and affiliations**: Alan Knight, USDA, ARS

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

**Objectives**:

Project objectives:

1. Determine larval codling moth dispersal behavior from fruit and identify physical parameters of cocooning sites.
2. Determine roles of chemistry and of visual stimuli in codling moth larval attraction to and cocooning at weathered versus freshly cut wood.
3. Document larval responses to conspecific larvae and cocoons.
4. Isolate and identify attractants and arrestants in weathered wood, if experiments confirm a chemical role in the behavior.
5. Explore improvements in tree banding and other interception techniques, using information on larval dispersal behavior and responses to visual and chemical stimuli.

2005 Objectives/goals:

1. Demonstrate activity of extract and extract isolates from weathered woods.
2. Repeat quantitation of the percent of mature larvae that drop from fruit, versus spin up on the tree trunk and develop laboratory assay for drop or walk behavior.
3. Determine specificity of larval attraction to cocooning larvae.
4. Document anemotaxis of male and female larvae to synthetic aggregation pheromone.
5. Characterize possible larval footprint pheromone.

Significant Findings:

1. Field experiments showed a significant percentage of larvae drop from the tree, rather than crawl down the trunk.
2. Cocooning larvae orient towards dark objects and spin up preferentially but not exclusively under dark vs light pieces of wood.
3. Cocooning larvae exhibited preferential attraction responses to weathered wood versus “new” wood, but not consistently.
4. Cocooning larvae exhibited anemotactic attraction in an olfactometer to cocooned larvae.
5. Codling moth larvae leave a trail that influences the behavior of other codling moth larvae.

**Methods for 2005**:

1. Isolate attractant from weathered wood. We have conducted an assessment of volatiles from weathered wood using solid phase microextraction (SPME), which revealed the presence of a set of chemicals. In the coming season, we will conduct replicated analyses using extracts and volatile collections to compare odorants from weathered wood that is attractive in bioassays, weathered wood that is not attractive in bioassays, and non-weathered wood that is not attractive in bioassays. This will involve steeping pieces of wood in methylene chloride to obtain an extract, and also volatile collections by passing air through a chamber possessing the odor source, filtering that air through an adsorptive trap, and then solvent-extracted the trap for GC-MS analysis. This will provide 1) the means for a subtractive analysis, effectively

reducing the numbers of peaks to consider as potential larval attractants, and 2) samples of volatiles that can be tested in assays for responses by larvae. A second study will be to fractionate the behaviorally active chemical samples, and test the fractions again for behavioral activity, using arena and olfactometer assays. Fraction testing will involve comparisons of a solvent blank, each fraction alone, the starting material, and a combination of the fractions. Also, progress may be made using a molecular approach to be developed under a proposal by Dr. Barcenas. This approach involves the cloning of odor and taste receptor genes of codling moth into cells and determining the specificity of the receptors expressed in those cells using fluorescence assays of cell depolarization. This technique will provide information on which compounds in an active extract or volatile collection are reacting with receptor proteins and may then be detected by larvae. This would be a valuable technique to use with larvae, because we do not yet know where all larval sensory receptors are located and how to set that up with neurophysiological detectors. This work (testing of fractions and possibly the molecular technique) should provide us with a short list of compounds to test for attractiveness to larvae.

2. Quantify larval drop. In 2005 we will follow up on the 2004 tests in two ways, but largely using the same methods (comparing banding with interception traps for dropping larvae). First, the test of 2004will be repeated to provide more replicates to improve the scientific rigor. In addition, this test will be expanded to compare the two generations of larvae. This will involve then 3 sites, each with 20 trees banded and 20 interception traps. These plots will be monitored through both generations, to sample larvae looking for spin up sites. Bands and traps will be collected at the end of the larval emergence period, and numbers tallied and compared to further quantify the ratio of larvae that drop compared to moving down the trunk, and to determine if this pattern is similar for the first generation of non-diapausing larvae, and second generation largely diapausing larvae.

3. Compare diapausing/non diapausing behavior. In addition to the potential results of the experiment to compare first and second generation spin ups in bands versus interception traps, laboratory experiments will be developed to the dispersal and search behavior of diapausing and non-diapausing larvae. Larvae will be released in the center of a plexiglass box, with cardboard tubes providing access up, down, and in the 4 cardinal directions. The larvae will be monitored to determine in which direction they move, and the cardboard tubes will be discarded following each assay replicate to remove larval footprint effects. This test will be conducted with 50 lab reared non-diapausing larvae, and 50 diapausing larvae. The hypothesis tested is that diapausing larvae tend to move and spin up high up, and non-diapausing larvae tend to move and spin up low. This hypothesis comes from observations of escaped larvae in the insectary. This assay will be useful also in later experimentation with wild larvae in field collected fruit.

4. Determine specificity of larval response to cocoons. Olfactometer assays will be conducted to determine if observed anemotactic responses of larvae to larvae in cocoons varies with larval sex and diapausing state. Assays will compare responses of male and female larvae to male and female larvae in cocoons, and will compare codling moth larval responses to codling moth that are non-diapausing versus diapausing.

5. Larval codling moth attraction to synthetic pheromone. Using the parallel tube olfactometer to look at anemotactic attraction responses, we will initially make a comparison of larval upwind movement in response to a) a system blank, b) a pheromone blend, and c) 6 larvae in cocoons. This will be a non-choice assay, comparing the forward movement of larvae in response to airflow from these treatments. If results are positive with the synthetic blend,

additional tests will be conducted to evaluate the responses of males and females, as well as diapausing and non-diapausing larvae.

6. Characterize larval footprint pheromone. Solid phase microextraction (SPME) and solvent extracts will be used to characterize the chemical residue deposited by traveling mature larvae. Mature larvae will be placed in a glass petri dish for a measured period of time (probably one hour) and then removed. The bottom of the dish will be wiped with an SPME fiber and then rinsed with methylene chloride, for GC-MS analysis. This procedure will be performed for male larvae, for female larvae, and for blank petri dishes. This work will provide a tentative set of compounds to use in bioassays and as a basis of comparison with the aggregation pheromone produced by cocooned larvae.

**Results and Discussion**:

1. Larval movement in the field. The comparison of numbers of larvae per tree band versus per one square meter catch basin under tree foliage showed first that a significant number of larvae were captured in the catch basins. This supports the hypothesis that some larvae drop from the fruit or foliage to the ground while seeking spin up sites. When the data was standardized for the area of a tree canopy, it was estimated that about ½ as many larvae dropped from the tree as crawled down the trunk (assuming 100% capture with both methods). These findings are important for interpreting banding results, and also for designing experiments to attract larvae that are seeking spin up sites. This test was conducted in one orchard and for one generation, and should be repeated to look for variance between generations, years, and varieties.

2. Orientation of cocooning larvae to light/dark. Field tests in 2004 confirmed 2003 results showing greater numbers of larvae spinning up in dark bands compared to light colored bands. Ten trees were banded with bands painted black while ten trees were banded with bands painted black. Although statistically significant, these number differences are not dramatically large, but indicate that banding may be more efficient with very dark bands, and also indicate that bands may not be 100% efficient in capturing larvae moving down the trunk to seek spin up sites.

3. Larval response to weathered wood. Additional olfactometer assays were conducted to evaluate codling moth larval responses to weathered versus fresh wood, and to also evaluate codling moth larval responses to white versus black painted woods. Larvae did again exhibit strong preference for small pieces of some weathered boards, but not to others. When pieces of wood were painted, larvae in choice arena tests chose black over white a significant percentage of the time. Preliminary analyses were conducted on the odor chemistry of weathered wood. Solid phase microextraction was used, coupled to GC-MS. This technique showed the presence of a number of compounds consistently from weathered boards, but does not provide information on which of these compounds may be capable of eliciting behavioral responses from codling moth larvae.

4. Larval attraction response to cocooning larvae. Mature codling moth larvae in a parallel tube olfactometer moved twice as far upwind when exposed to the odors from other larvae in cocoons, compared to other larvae no exposed to the odors from cocoons. When a second larva was tested immediately after the first larva, the second larva moved farther and faster through the olfactometer, compared to the first. This indicates the possibility of a chemical residue left by the first larva that stimulated the second larva. This situation occurred with larval responses to the control, and not to the treatment (cocooned larvae). These results

show that larvae looking for spin up sites are attracted to the odors from other larvae in their cocoons, and that those same larvae may leave a trail pheromone on the surface that stimulates other larvae.

5. Cardboard bands placed on trees received twice as many cocooning codling moth larvae when they were artificially infested with codling moth larvae before they were put on the tree trunk. This finding suggests a practical application of the pheromone produced by cocooning larvae that attracts other larvae.

**Budget**:

**Project Title**: Codling moth selection of pupation and hibernation sites: enhancement of tree banding.
**PI**: Peter J. Landolt
**Project Duration**: 2003-2005
**Current Year**: 2005
**Project Total (3 years)**: $53,200
**Current Year Request**: $34,000

| Year | | | 2003 |
|---|---|---|---|
| | | 2004 | 2005 |
| Total | | | $33,000 |
| | | $34,000 | $34,000 |

| Current year Breakdown | | Year 1 | |
|---|---|---|---|
| | | | Year 2 |
| | | | Year 3 |
| Item | | | |
| Salaries | | | $29,000 |
| | | $30,000 | $31,000 |
| Benefits | | | |
| Wages | | | |
| Benefits | | | |
| Equipment | | | |
| Supplies | | $ 4,000 | $ |
| | 4,000 | $ 3,000 | |
| Travel | | | |
| Miscellaneous | | | |
| Total | | | $33,000 |
| | | $34,000 | $34,000 |

Salary request is for partial support for a biological technician (½) time, and partial support for a chemist (1/4 time)

Supplies include glassware, gases, and solvents for maintenance of olfactometers, materials for field traps, and solvents, SPME fibers, and gases for chemical analysis.

**FINAL REPORT**
**WTFRC Project #AE-01-222** **WSU Project #13C-3643-4093**


| | |
|---|---|
| **Project title**: | Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm. |
| **PI**: | Jay F. Brunner |
| **Organization**: | WSU Tree Fruit Research and Extension Center, 1100 North Western Avenue, Wenatchee, WA. phone: 509-663-8181; FAX: 509-662-8714; jfb@wsu.edu |
| **Co-PIs and affiliations**: | Betsy Stutzman, Associate in Research, Tree Fruit Research and Extension Center, Wenatchee, WA<br>Peter Landolt, USDA-ARS Yakima |
| **Contract administrator**: | Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691 |


**Objectives:**

1. Evaluate sprayable pheromone systems for behavioral control of codling moth.
2. Evaluate fiber pheromone formulations for behavioral control and attract & kill possibilities for leafrollers and codling moth.
3. Evaluate an attract & kill technology LastCall formulation for leafrollers in large field trials for efficacy and rate effect.
4. Develop a bait & kill system for control of lacanobia fruitworm and assess the impact on other noctuids in orchards. **(Not addressed due to lack of pest importance)**
5. Evaluate the potential of a new flake pheromone formulation for CM control.

Significant findings:

***2002***

1. Sprayable pheromone for CM control did not perform well in orchards with moderate to high pressure. This technology may 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.

***2003***

1. Suterra CM-F sprayable pheromone contributed to suppression of codling moth (CM) activity when combined with supplemental insecticides in low-pressure orchards. Higher rates of CM-F sprayable pheromone performed as well in low-pressure orchards when

compared with hand-applied dispensers at half rates. CM-F sprayable does not perform as well as the full rate of hand-applied dispensers in moderate- to high-pressure orchards.

2. The Scentry fiber pheromone showed promise in large field trials for suppression of CM and leafroller adult flight activity when applied with the modified ground applicator. An "attract & kill" product, when combining fibers with permethrin, looked promising in field trials this year, particularly for OBLR.
3. The IPM Technologies' OBLR and PLR LastCall attract & kill formulation reduced moth activity significantly in large field studies in both generations. The reduction of adult moth activity could not be directly correlated with larval populations.
4. The female OBLR traps used in the Scentry fiber and LastCall trials in the first generation showed a low percentage of female recovery, either due to predation or escape. The modification in the second generation allowed for a high recovery rate of females, but percentage of females mated and males captured was very low overall. A system for assessing female leafroller mating status remains an issue.

***2004***

1. The Scentry fibers continue to look promising for codling moth and OBLR when applied with the modified ground applicator to large field trials. The 100-gram rate of fibers seems to be comparable to Isomate C-Plus hand-applied at 200 dispensers per acre in low-pressure situations. Air applications of fibers made by helicopter also looked promising, but efficiency of the application is in question because the "pods" used under the helicopter tend to swing, allowing for variable consistency in distribution of fibers. Fruit injury at harvest in the majority of blocks was minimal. There was no difference between treatment rates but significant difference between treatments and untreated (no pheromone) control blocks. All codling moth and leafroller sites had at least one cover spray per generation targeted at the respective key pest.
2. Hercon CM Disrupt "flake" pheromone technology remained attractive through the duration of a CM generation. Retention on foliage was good. The application systems tested provided a good distribution of flakes in trees. In small plots and single-tree experiments the Hercon CM Disrupt flakes significantly reduced male moths' ability to locate females or female mimics in traps. The Flake formulation performed well in second generation compared to hand-applied and untreated control. The modified leaf blower application has the potential to be more efficient and easier for commercial applications by the grower. More experience with this technology is needed.
3. Hercon CM Disrupt "fringe" hand-applied dispensers did not perform as well as Isomate C-Plus or Isomate CTT in the trials that were conducted. In low-pressure situations the Hercon Disrupt dispensers were unable to effectively reduce pheromone trap catch compared to Isomate hand-applied treatments, even at "half rates."
4. IPM Technologies, Inc. LastCall OBLR formulation continued to perform well at half or full rates. There was no significant difference between treatments, but all treatments performed better than the untreated control. The PLR formulation was used in a low-pressure situation and did reduce trap catch compared to the control but was not significant. Application of the product may be the only restricting aspect of its commercial use in the future.

**Methods:**

**See details of methods in previous years proposals and progress reports.**

Evaluations of different pheromone formulations (sprayable, hand-applied, fibers, attract & kill) were conducted in commercial orchards with cooperating growers. Treatments were

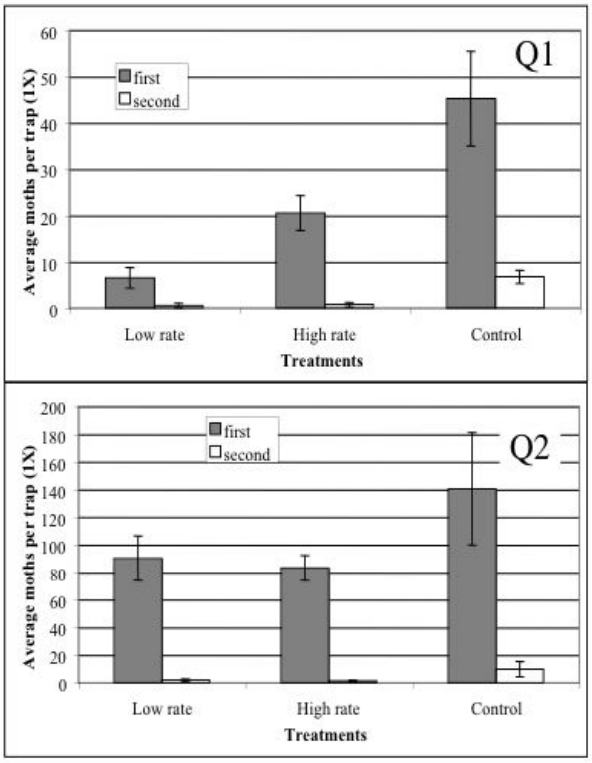
applied to large plots (2 to 10 acres each) replicated (three times) at different locations. Plots were monitored for moth activity with pheromone and non-pheromone lures one or two times per week. Sampling foliage or fruit from each plot at appropriate times of the year was used to assess pest density or fruit injury.

The duration and retention of new pheromone delivery technologies (fibers, flakes, attract-and-kill) was determined through replicated studies. Treatments were applied to different tree parts and retention followed over time to determine retention times on surfaces. Fibers, flakes or attract-and-kill technologies were placed in pheromone traps to determine their attractancy over time. Moth captures in traps were recorded and compared to a standard attractant source (lure).

**Results and discussion:**

**CM sprayable pheromones:**
Sprayable pheromone formulations (Suterra and 3M) were evaluated in 2002 and 2003 in replicated large plots in commercial orchards. The Suterra formulation provided some suppression of CM using 5 applications per season similar to a half-rate of Isomate C-Plus. Sprayable formulations in general are not robust pheromone technologies for CM management when applied through a speed sprayer or highly concentrated specialized device.

**CM Scentry fibers - ground application:**
***Codling moth*** **-** Treatments of 33 and 66 grams of Scentry codling moth (CM) fibers were mixed with BioTac 25/100 weight (50:50) for first generation application to three sites in Quincy (Q1 and Q2) and Malaga areas. The targeted application rate was for 50 and 100 grams of fibers per acre, but product mislabeling caused reduced rates in first generation. Each treatment was 5 acres in size, with either hand-applied dispensers or untreated (zero mating disruption) as comparison blocks. The first generation application was targeted for 14 days after petal fall with the cooperator/grower applying Intrepid at 12 oz/acre 7 days after petal fall. This timing was to accomplish two things: 1) optimal foliage/surface area for fibers to adhere to; 2) Intrepid application of 12 oz/acre would allow efficacy of codling moth eggs already laid and any future eggs deposited on top of Intrepid. The two Quincy trial blocks were moderate to high pressure with a total trap reduction of 55-86% compared to the untreated controls. The other blocks in Malaga had very low pressure and showed no difference in total trap catch between treated and hand-applied dispenser blocks.




At the start of the second flight, application of fibers increased to 50 and 100 grams mixed with BioTac 100/300 weight (50:50) at the Quincy sites and 100 grams at Malaga. Results in the second generation at the Quincy sites showed a statistically significant reduction in trap catch ranging from 82-93% compared to the untreated comparison blocks. The Malaga sites showed no difference between treatments. There was no difference in mating status in fiber treated or untreated blocks in

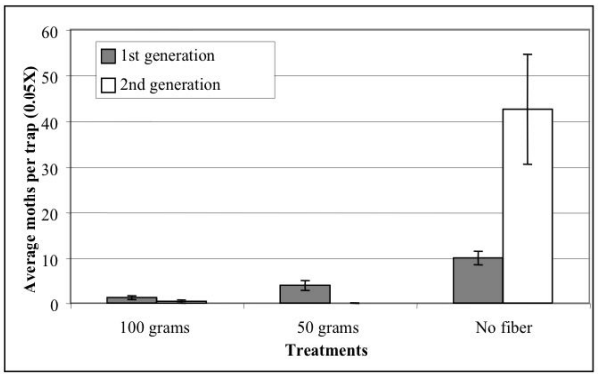
either locations. Fruit injury remained very low at all sites in both first and second generations, with the Quincy sites averaging <1% damage and Malaga showed a reduction in damage compared to the hand-applied blocks but it was not significant.

## CM Scentry fibers - air application:

***Codling moth*** **-** Two sites in Chelan and Malaga received commercial applications of Scentry CM fibers by helicopter at the discretion of the growers and their consultants. Monitoring was done to gain more experience with methods of application that are available to the grower. There was no untreated block to use as comparison at either site. At the Chelan site, average moth catch per trap was 1.7, and Malaga catch ranged from 6.6 to 36.5 per trap. Traps were placed at a rate of 1 trap/lure per acre. Fruit damage at the end of first generation was 0% at Chelan and ranged from 0%-3.8% at the Malaga site.

In the second generation the Chelan site was discontinued because the grower and consultant did not treat again with fibers due to very low pressure. A new site with high pressure was added at Bray's Landing and had an untreated control for comparison. In the fiber treatment, 1x lure baited traps averaged 34 moths per trap compared to the untreated control at 37 moths per trap. Fruit injury was significant with 7.7% and 9.3% in the fiber treated area and 4.4% and 2.5% in the untreated control area.

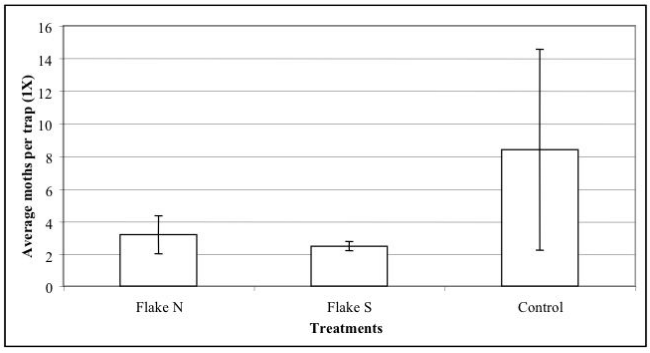
## OBLR Scentry fibers:




Scentry OBLR fibers were applied at 50 and 100 grams mixed with BioTac 25/100 weight first generation and 100/300 weight in second generation to three locations, Desert Aire (figure shown), Quincy and Monse. Both treatments showed a significant reduction in trap catch at Desert Aire in both generations compared to the untreated with reductions of 60-80% in first generation and 99-100% in second generation. Due to application problems the Quincy site received only 25 and 50 grams respectively in the first generation. Even at this reduced rate there was a reduction of 83-100% in trap catch compared to the untreated control. In the second generation the full rate was achieved and had near trap shut-down at reductions of 88-97%. The Monse site was very low pressure with no difference in trap catch first generation but in the second generation trap catch was significantly different with 85% reduction in treated blocks compared to the untreated comparison.

This year the live female traps that were developed by Dr. Vince Jones in 2002 were replaced with acetic acid food-based baited traps developed by Dr. Peter Landolt. These traps were used to assess mating status in all OBLR trials in both generations.

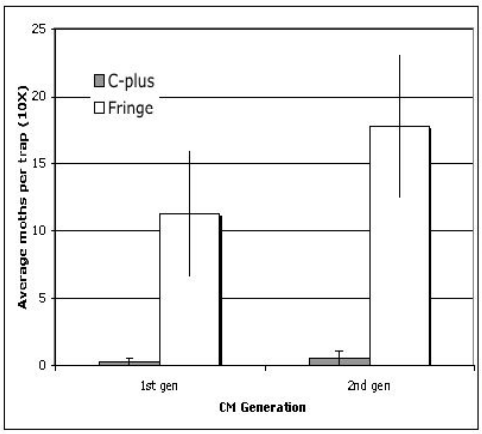
## Hercon CM Disrupt "flakes":



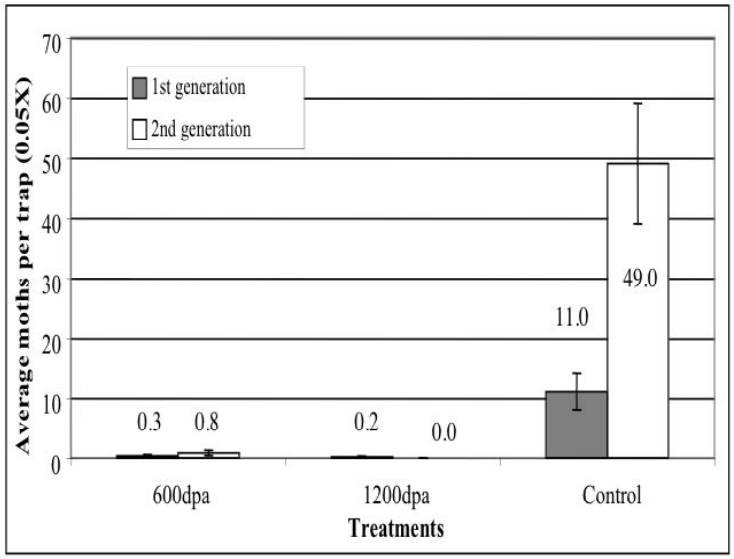

***Codling moth*** **-** This was one large site located in Quincy that we monitored in the second generation only. Cooperating with the Hercon technical consultant and commercial grower, this site was set up to compare the new technologies of pheromone "flakes" with the new hand-applied Hercon CM Disrupt "fringe" and an untreated control. Reductions

in pheromone trap catch in the "flake" treatments were 43-70% compared to the untreated control. There was no difference in trap catch between the hand-applied and the untreated control. The flake treatments mating status was 43-75% mated females compared to hand-applied at 88% mated and untreated control at 75%. Fruit injury assessment prior to harvest showed 88-95% reduction in the "flake" treatments and 17% reduction in the hand-applied compared to the untreated control at 10.8%.

**Hercon CM Disrupt "fringe" hand-applied dispensers vs. Isomate C-Plus/CTT:** Two 10-acre sites in the Wenatchee valley were treated with Hercon CM Disrupt "fringe" hand-applied dispensers at 120 dispensers/acre compared with either Isomate C-Plus at 200 dispensers per acre or Isomate CTT at 200 dispensers/acre. The Isomate C-Plus and Isomate CTT performed better in both generations with 10x red septum pheromone traps showing 67-100% reduction compared to the Hercon hand-applied technology. DA lure trap catch in first generation was very low but in second generation increased, and mating status from all hand-applied treatments ranged from 80-100% mated. Fruit injury was very low in all treatments with no significant difference.

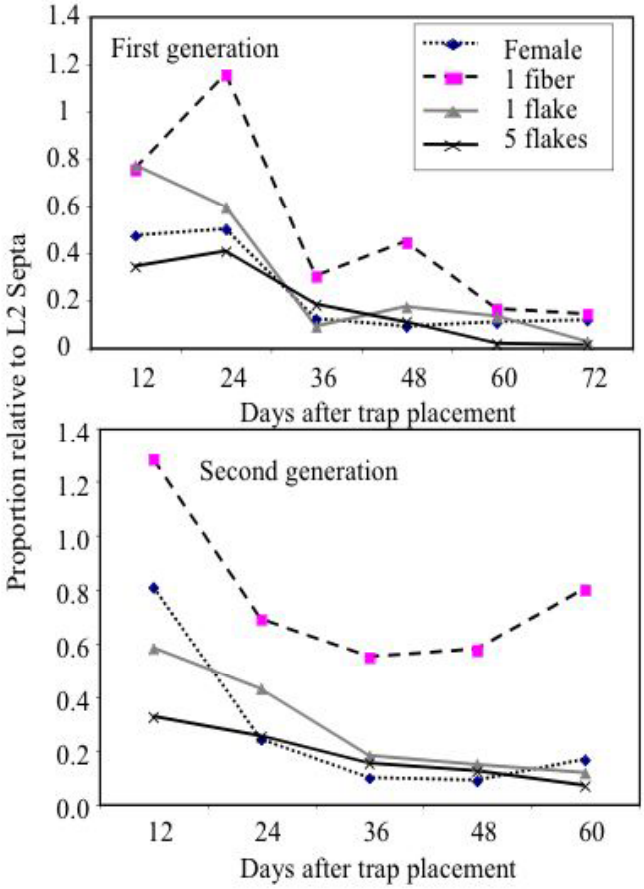



**OBLR LastCall:** Attract & kill formulations for OBLR were used in large 5-acre plots to determine efficacy on obliquebanded leafroller. Rates of 600 and 1200 drops per acre were used. Sites were located in Quincy (figure shown) and Pateros. There was no effect on trap catch between rates of LastCall with 97-100% reduction at all sites compared to the control. Two of the three sites had supplemental control for leafroller.

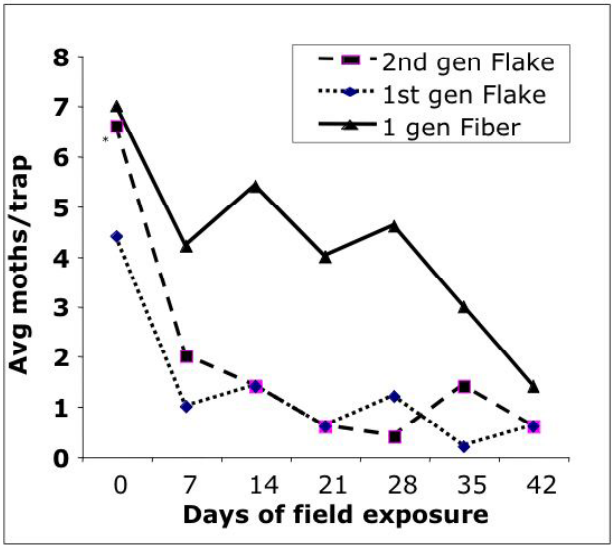



**PLR LastCall**: This was a site just south of Wenatchee on Stemilt Hill, which received two supplemental sprays for control. The site received only one treatment of 1200 drops per acre compared to an untreated control. Pressure was very low in both generations with 90-100% reduction in 0.1x baited pheromone traps compared to the control.

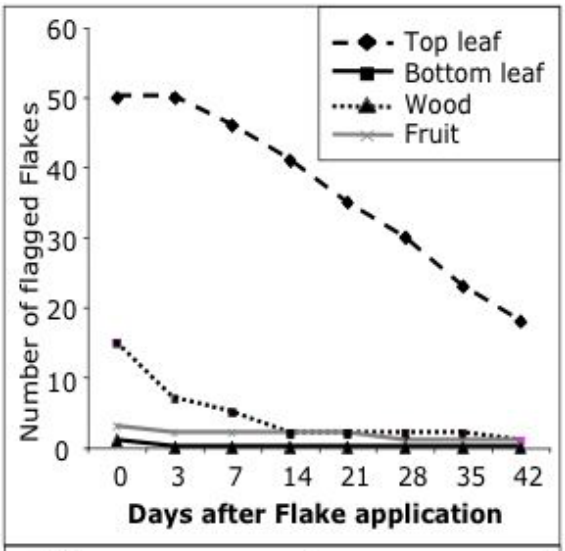
**NoMate CM fiber and Disrupt CM flake attractiveness:** NoMate CM fibers and Disrupt CM flakes were attractive to CM males over the entire first and second generation flights (figure to right). The L2 lure was the most attractive lure used during both generations. During the first generation test, the fiber lure attracted as many moths as the L2 lure through 24 days but was significantly less attractive for the rest of the flight period. The flake lure attracted as many CM males as the fiber lure through 36 days, but appeared to lose relative attractiveness for the rest of flight. The flake lure was close in attractiveness to CM males as a virgin female lure. During the second flight, both the fiber and flake lures were statistically equivalent to the L2 lure through 24 days. From day 24-60 the fiber lure maintained its relative attractiveness to the L2 lure, attracting 60-80% as many CM males. The fiber lure was statistically equivalent to the L2 lure after each rotation, except 48 days. The fiber lure attracted more CM males than the flake lure at 48 and 60 days. Again, the flake lure appeared to be as attractive as a CM female over the entire flight.

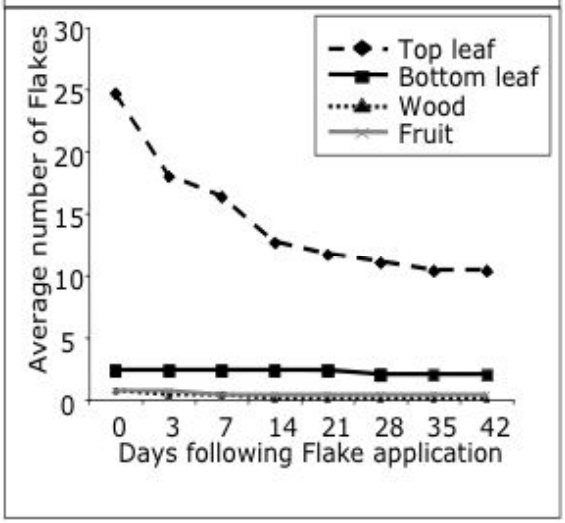



**Effect of field-aged exposure on NoMate CM fibers and Disrupt CM flakes on attractiveness and longevity:** The effect of field-aging Disrupt CM flakes resulted in a decrease in relative attractiveness after 7 days of exposure (figure at right). However, in only during the second generation test was the decrease in attractiveness statistically significant (F ratio 2.86, df 6, Prob>F 0.03). These data suggest that additional exposure from 7-42 days did not affect relative attractiveness during the first or second generation. NoMate CM fibers appeared to lose relative attractiveness later in the first generation; however, at no time was the loss in attractiveness relative to day 0 fibers statistically significant. Fibers attracted numerically more CM males over the entire first generation than the flakes; however, only at 7 days (*t* 2.67, df 8, Prob>*t* 0.03) and 28 days (*t* 2.29, df 8, Prob>*t* 0.05) was this difference statistically significant.



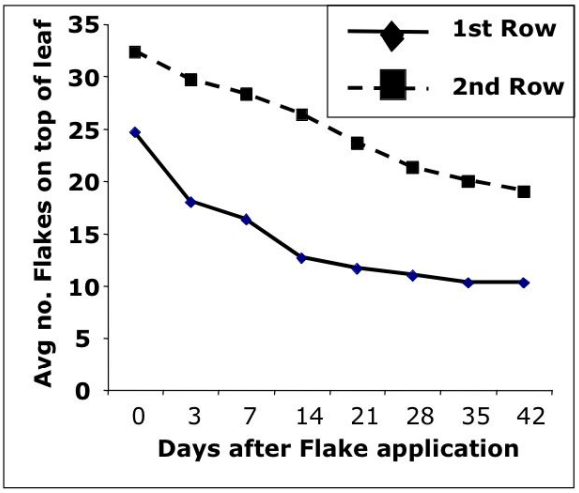
**Disrupt CM flake (photo) application efficiency and retention:** Statistical analysis was difficult with the hydroseed applicator trial due to the unreplicated experimental design, but clear trends were still apparent in this study. The recommended rate of Gelva was 48 fl oz/5 gal, and at this rate the hydroseed applicator was able to stick many more Disrupt CM flakes on the tops of the leaves than on the bottom of leaves, wood or fruit (right top). It was apparent that this rate of Gelva was insufficient to stick flakes on these other surfaces. Flake retention on the tops of leaves was a fairly linear decline with approximately 40% of the flakes remaining after 42 days. The adhesive study indicated that 48 fl oz rate of Gelva may have outperformed the 32 fl oz rate with more flakes being retained at 3 and 7 days. From that point on, the loss rate was slightly more in the 48 fl oz treatment, and at 42 days both treatments had the same number of flakes remaining on the upper surface of the leaves. It is clear that adding Guar Gum to Gelva significantly increased flake adhesion to the tops of leaves (72% after 42 days) but also to the bottom of leaves (48% at 42 days), wood (44% at 42 days) and fruit (28% at 42 days). However, at the high volume used in the hydroseeder applications, the addition of Guar Gum resulted in stickiness on the trees and fruit that probably would not be horticulturally acceptable.

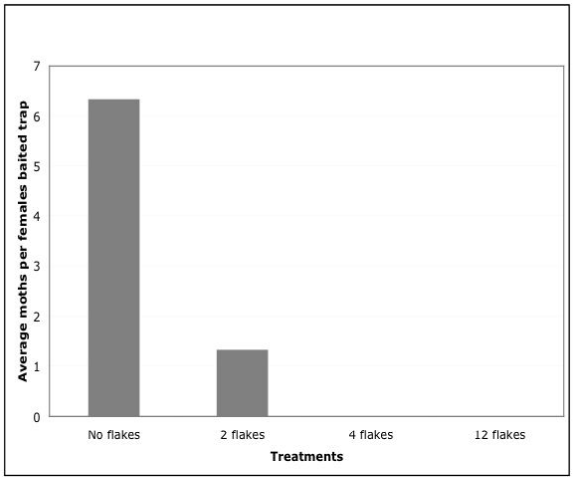


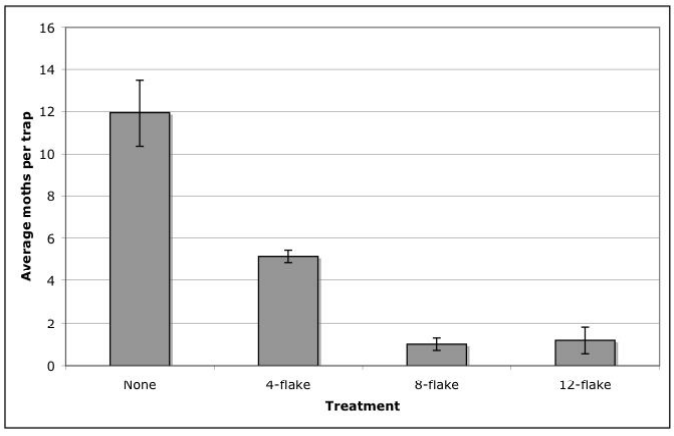

The blower applicator was also more efficient at applying flakes to the upper surface of leaves, as very few were stuck on the bottom of leaves, wood or fruit (right bottom). An average of 25 flakes per tree were found within 24 hr of application. Flake retention declined linearly through 14 days, but from that point on very few flakes were lost and after 42 days 40% remained. Although Guar Gum was necessary to adhere flakes using the blower applicator, the blower operated at a much lower volume and the same sticky residue on fruit and leaves was not noted. Interestingly, more flakes were deposited on trees in the second row from the blower application than on the first row adjacent to the application (figure on next page). The difference in flake deposition between the rows was not statistically significant, but these data suggest that the blower was operating efficiently over two rows. Blower applications traveling at 8 mph should be able to efficiently cover a large acreage in a relatively short time.

**Point source density experiments:** In Experiment #1 an average of 6.3 moths were captured in the trees without flakes. Where there were 2 flakes per tree an average of 1.3 moths were captured. No moths were captured in female baited traps in trees with 4 or 12 flakes per tree (see bar graph below).




In Experiment #2 an average of 6.0 moths were captured in the 0-flake (control) treatment. We had another control in trees in the same orchard but at a longer distance from the treated trees (see bar graph below). In this control an average of 8.6 moths were captured, slightly more than the treatment control (no flakes) indicating that there may have been some interference due to proximity of the flake treatments. All of the flake treatments significantly reduced moth capture in fiber-baited traps. We know that fibers used as lures are more attractive than virgin CM females. This is because they either release more pheromone or because they are always calling while females call for only a short period each night. There seemed to be little difference associated with the location of flakes, though when only 2 flakes were used and placed to the side of the trap vs. above and below fewer moths were captured. Could this indicate something about how competition between different sources occurs?




In Experiment #3, which was very similar to Experiment #1, an average of 11.9 moths were captured in the trees without flakes. Where there were 4 flakes per tree, an average of 5.1 moths were captured. The number of moths captured in trees with 8 or 12 flakes per tree was not different from each other but significantly less than the 4 flake treatment (figure at left).

In Experiment #4 an average of 2.0 moths were captured in the trees without flakes and monitored with the fiber-baited trap. The number of moths capture in the 2 flakes per tree treatment was only slightly lower (1.6 moths per trap) than the untreated control, but the 4 flake treatment had significantly lower catch, 0.8 moths per trap. When the L2 lure was used as a monitoring tool fewer moths were captured in all treatments but the same trend in moth capture was evident but not statistically different.

**Budget:**

**Project title:** Developing behavioral-based control tactics for codling moth, leafrollers and lacanobia fruitworm.
**PI:** Jay F. Brunner
**Project duration**: 2002-2004 (3 years)
**Project total (3 years)**: $104,267

| **Year** | Year 1 (2002) | Year 2 (2003) | Year 3 (2004) |
|---|---|---|---|
| **Total** | 37,280 | 32,365 | 34,622 |

| Item | Year 1 (2002) | Year 2 (2003) | Year 3 (2004) |
|---|---|---|---|
| Salaries[1] (0.5 Betsy Stutzman, formerly Valdez) | 15,000 | 15,450 | 18,694 |
| Benefits (30%) | 4,500 | 4,635 | 5,608 |
| Wages[1] | 8,000 | 8,000 | 7,000 |
| Benefits (16%) | 1,280 | 1,280 | 1,120 |
| Equipment[4] | 5,000 | 0 | 0 |
| Supplies[2] | 1,000 | 1,000 | 700 |
| Travel[3] | 2,500 | 2,000 | 1,500 |
| Miscellaneous | 0 | 0 | 0 |
| Total | 37,280 | 32,365 | 34,622 |

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

Additional funding supporting this project in the amount of $40,000 per year was provided.

**FINAL REPORT**
**WTFRC Project # AE-03-328** **Organization Project # 5352 22000 015 13T**

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

**PI:** Eugene Miliczky
**Organization:** USDA-ARS, Wapato, WA

**CO-PI:** David Horton, USDA-ARS, Wapato, WA

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

**Objectives:**

1. Assess the arthropod inhabitants of alder (*Alnus* spp.) leafrolls, especially with regard to beneficial insects that also occur in Washington orchards or may be of value in orchard biological control. Evaluate the potential of *Clubiona* spiders, common occupants of alder leafrolls, as predators of orchard pest leafrollers: PLR and OBLR.
2. Conduct a survey of native plant species for leafrolling caterpillars that may serve as alternate hosts for *Colpoclypeus florus*, an important parasite of orchard pest leafrollers; determine if non-pest leafrollers on native host plants harbor parasitoids of potential value in biological control of orchard pest leafrollers; test suitability of non-pest leafrollers from native host plants as hosts for *C. florus*.
3. Conclude western flower thrips/extra-orchard host plant survey by sampling a series of important WFT host plants that spans the season; assess numbers and occurrence of WFT and its predators on the plants.
4. Determine if the density of western flower thrips in apple blossoms varies as distance from adjacent extra-orchard habitat increases; does the level of WFT damage (pansy spot) vary as distance from extra-orchard habitat increases; assess the efficacy of field sampling for WFT by the blossom flick method to determine thrips densities in apple bloom.
5. Determine if psyllid species found on extra-orchard host plants are attacked by *Trechnites insidiosus*, a parasitoid of the pear psylla, or other parasitoid species.

**Significant findings:**

- Alders are heavily infested with leafrolling caterpillars and the leafrolls are occupied by a variety of beneficial arthropods. These include species of known importance in orchard biological control and species that may be of potential value to orchard biological control. Sac spiders in the genus *Clubiona* are frequently found in alder leafrolls. In laboratory trials *C. pacifica* was an effective predator of PLR and OBLR larvae on apple seedlings. Pupae and adults were also attacked and the spiders may occasionally feed on eggs. The alder leafrollers are parasitized by several kinds of wasps and flies.
- Western Flower Thrips density in apple blossoms showed a significant decline as distance from adjacent extra-orchard habitat increased. The decline was most marked between the orchard border and 30 feet into the orchard from the border. Incidence of thrips damage also showed a significant decline as distance into the orchard from the orchard border increased. Flick sampling for WFT was less than 100% effective at removing all thrips from the blossoms. Efficiency was poorest with pink stage blossoms but improved at each subsequent stage of bloom development – open king bloom, full bloom, and petal fall.

- Extra-orchard habitats rich in flowering plant species may allow the western flower thrips to pass through a continuous series of generations on native plant species as they successively come into flower. Thrips populations can in turn support important predators such as the minute pirate bug, *Orius*, and the small immatures of several kinds of spider.
- Many species of native plants found in extra-orchard habitats are hosts for leafrolling type caterpillars. Many of these caterpillars are attacked by parasitoid wasps and flies, some of which may be of potential value against orchard pest leafrollers. *Colpoclypeus florus* successfully parasitized 2 tortricid leafrollers from alder and one from dogwood in preliminary laboratory trials.
- Several species of psyllid found on extra-orchard host plants were found to be hosts for parasitoid wasps, although none yielded *Trechnites*.

**Results and Discussion:**

Determine how the density of western flower thrips changes within the orchard as distance from adjacent native habitat increases, especially within 100' of the orchard border: This study was conducted in collaboration with Steve Cockfield and Elizabeth Beers who sampled the 4 Wenatchee area orchards. The Western Flower Thrips, *Frankliniella occidentalis*, was the most abundant species by far in our samples, based on adult specimens. For example, other species comprised less than 2% of the 1,754 adult thrips taken in the 4 Yakima Valley orchards. WFT abundance varied widely among the 8 orchards with a 60-fold difference between the least and most densely populated blocks (Table 1). Immature thrips were detected in all orchards and tended to be more abundant in samples taken later in the bloom period. While not certain, it is likely that most immature thrips were *F. occidentalis*.

Table 1. Numbers of adult WFT taken at 4 Yakima and 4 Wenatchee area orchards in flower clusters at 6 distances from adjacent, non-orchard habitat. Thrips numbers at each distance are the totals for the collections made at pink, king bloom, full bloom, and 100% petal fall.

| | **Yak 1** | **Yak 2** | **Yak 3** | **Yak 4** | **Wen 1** | **Wen 2** | **Wen 3** | **Wen 4** | **Totals** |
|---|---|---|---|---|---|---|---|---|---|
| **Border** | 30 | 92 | 116 | 164 | 10 | 59 | 77 | 2 | **550** |
| **30'** | 13 | 79 | 92 | 141 | 5 | 43 | 34 | 8 | **415** |
| **60'** | 3 | 55 | 88 | 160 | 8 | 44 | 21 | 0 | **379** |
| **100'** | 2 | 31 | 66 | 178 | 5 | 29 | 19 | 2 | **332** |
| **200'** | 7 | 12 | 59 | 117 | 9 | 41 | 9 | 2 | **256** |
| **300'** | 4 | 16 | 58 | 146 | 11 | 15 | 30 | 1 | **281** |
| **Totals** | **59** | **285** | **479** | **906** | **48** | **231** | **190** | **15** | **2213** |

Western flower thrips density declined significantly as distance from non-orchard habitat increased for the full bloom samples (repeated measures ANOVA; P = 0.007) and the overall summed samples (P = 0.007). Furthermore, the density decline was most marked near the orchard border. Profile contrast analysis showed that a significant decline in thrips density occurred between the orchard border and 30' from the border for the full bloom sample (P = 0.035) and the overall summed sample (P = 0.013). A significant decline was also noted in the full bloom sample for the 60' vs. 100' comparison (P = 0.05).

Thrips damage due to pansy-spotting (Table 2) showed a significant decline with distance effect (repeated measures ANOVA: P = 0.013). The decline was most marked near the edge of the orchard (profile contrast analysis: border vs. 30'; P = 0.018). There was not always, however, a close correspondence between thrips density during bloom and the level of thrips damage observed in an orchard (Tables 3a and 3b). Damage in orchards with high thrips densities did not always exceed damage in orchards with considerably lower thrips densities, while, on the other hand, the orchard

with the lowest thrips density had the 5th highest level of damage. Thrips density in an orchard during bloom appears to be a less than optimal predictor of damage. A possible contributing factor is that thrips activity in the orchard is not restricted to the immediate bloom period. Thrips are active outside the orchard throughout the season and they pass through numerous generations. Within the orchard, they are found on many of the flowering plants that occur as weeds or deliberately planted species such as dandelions, clovers, and mustards. Thrips are present in and around an orchard well after bloom and may continue to visit apples and perhaps cause damage by continued egg laying in the young fruit. Beers et al. (1993: Orchard pest management: a resource book for the Pacific Northwest) noted the poor correlation between thrips density and oviposition damage, as well.

Table 2. Number of apples showing thrips damage (pansy spot) in 4 Yakima and 4 Wenatchee area orchards at 6 distances from adjacent, non-orchard habitat. Sample size: 250 apples @ each distance.

| | **Yak 1** | **Yak 2** | **Yak 3** | **Yak 4** | **Wen 1** | **Wen 2** | **Wen 3** | **Wen 4** | **Totals** |
|---|---|---|---|---|---|---|---|---|---|
| **Border** | 15 | 12 | 34 | 22 | 12 | 10 | 17 | 16 | **138** |
| **30'** | 7 | 7 | 40 | 17 | 5 | 3 | 11 | 8 | **98** |
| **60'** | 7 | 11 | 38 | 10 | 10 | 0 | 7 | 11 | **94** |
| **100'** | 6 | 3 | 35 | 13 | 5 | 4 | 11 | 6 | **83** |
| **200'** | 4 | 4 | 42 | 20 | 12 | 0 | 12 | 7 | **101** |
| **300'** | 4 | 2 | 40 | 8 | 12 | 3 | 8 | 7 | **84** |
| **Totals** | **43** | **39** | **229** | **90** | **56** | **20** | **66** | **55** | **598** |

Table 3a. Total number of WFT collected per orchard ranked from highest to lowest with corresponding number of pansy spot damaged fruit.

| **Orchard** | **Yak 4** | **Yak 3** | **Yak 2** | **Wen 2** | **Wen 3** | **Yak 1** | **Wen 1** | **Wen 4** |
|---|---|---|---|---|---|---|---|---|
| **No. of Thrips** | 906 | 479 | 285 | 231 | 188 | 59 | 48 | 15 |
| **No. of damaged fruit** | 90 | 229 | 39 | 20 | 66 | 43 | 56 | 55 |

Table 3b. Orchards ranked from highest to lowest thrips density and corresponding ranking with regard to number of pansy spot damaged fruit.

| **Orchard** | **Yak 4** | **Yak 3** | **Yak 2** | **Wen 2** | **Wen 3** | **Yak 1** | **Wen 1** | **Wen 4** |
|---|---|---|---|---|---|---|---|---|
| **Thrips ranking** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| **Damage ranking** | 2 | 1 | 7 | 8 | 3 | 6 | 4 | 5 |

Efficacy of the "blossom flick" method of field sampling for Western Flower Thrips: Flick sampling to determine thrips densities in apple flower clusters in the field was less than 100% effective in dislodging thrips from the bloom in our trials (Table 4). Efficiency of thrips dislodgement from flower clusters was lowest for clusters in the pink stage and a mean of only 43% (N = 4) of the thrips present in pink stage clusters was dislodged by flicking. However, flicking efficiency improved at each subsequent stage of flower cluster development and a mean of 91% (N = 6) of the thrips present in 100% petal fall clusters was dislodged by flicking.

Table 4. Number (%) of Western Flower Thrips removed from flower clusters (N = 25) in the field by the flick sampling technique and the number (%) undetected by flick sampling but removed from the same flower clusters by detergent sampling in the laboratory. One set of samples was taken along the orchard border and a second set was taken in the orchard interior, about 300' from the border.

| | | BORDER | | | | INTERIOR | | |
|---|---|---|---|---|---|---|---|---|
| **Orchard –stage** | | **Flick** | **Detergent** | **Total** | | **Flick** | **Detergent** | **Total** |
| **Yak 2 – king** | | 12 (60%) | 8 (40%) | 20 | | 3 (60%) | 2 (40%) | 5 |
| **Yak 2 – full** | | 7 (70%) | 3 (30%) | 10 | | 1 (25%) | 3 (75%) | 4 |
| **Yak 2 – petal** | | 29 (94%) | 2 (6%) | 31 | | 21 (95%) | 1 (5%) | 22 |
| | | | | | | | | |
| **Yak 3 – pink** | | 1 (20%) | 4 (80%) | 5 | | 3 (43%) | 4 (57%) | 7 |
| **Yak 3 – king** | | 4 (50%) | 4 (50%) | 8 | | 0 (0%) | 3 (100%) | 3 |
| **Yak 3 – full** | | 80 (77%) | 24 (23%) | 104 | | 21 (72%) | 8 (28%) | 29 |
| **Yak 3 – petal** | | 52 (95%) | 3 (5%) | 55 | | 19 (90%) | 2 (10%) | 21 |
| | | | | | | | | |
| **Yak 4 – pink** | | 7 (50%) | 7 (50%) | 14 | | 3 (50%) | 3 (50%) | 6 |
| **Yak 4 – king** | | 13 (37%) | 22 (63%) | 35 | | 5 (36%) | 9 (64%) | 14 |
| **Yak 4 – full** | | 89 (86%) | 14 (14%) | 103 | | 48 (84%) | 9 (16%) | 57 |
| **Yak 4 - petal** | | 78 (87%) | 12 (13%) | 90 | | 42 (93%) | 3 (7%) | 45 |

Arthropods associated with rolled leaves on alders (*Alnus* spp.): Thirty-two samples of rolled alder leaves were collected at 16 sites between 23 June and 9 September, 2004. Thirteen sites were in Yakima Co. along Highway 410 or subsidiary roads; 1 site, Cascade Park, was near the border between Yakima and Kittitas Counties; and 2 sites were in Chelan Co. along Highway 20 near Rainy Pass. Nine sites had not been sampled in previous years. Individual sites were sampled 1 to 5 times and the number of rolled leaves examined per sample ranged from 23 to 135.

Several types of predatory, parasitic, and plant-feeding arthropods were found at all or nearly all sample sites as shown in Table 5. Many of the gaps in the table could probably be filled in with additional sampling.

Table 5. Presence (+) or absence of plant feeding (upper half of table) and predatory and parasitic (lower half of table) arthropods in rolled alder leaves at 16 sites sampled in 2004.

| **Sample site[1]** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gelechiid LR** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Tortricid LR** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | + |
| **Plant-feeding mites** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| **Thrips** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Aphids** | + | + | + | + | | + | + | + | + | + | | + | | | + | + |
| **Psyllid[2]** | + | + | + | + | + | + | + | + | + | + | | + | + | + | | |
| **Springtail** | | + | + | + | + | | + | + | + | + | + | + | + | + | | |
| **Rust? mite** | | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| | | | | | | | | | | | | | | | | |
| **Gelechiid parasitoids** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **Tortricid parasitoids** | | + | + | | | | + | | | | | | | | | |
| ***Clubiona* spiders** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **other spiders** | + | + | + | + | + | + | + | + | | + | + | + | | + | | |
| **Brown lacewing** | + | + | + | + | | | + | + | + | + | | + | + | + | | |
| **Green lacewing** | | + | + | | | + | | | | + | | | | + | | + |
| ***Anthocoris* bugs** | + | + | + | + | + | | + | + | | + | + | + | + | | | |
| ***Deraeocoris* bugs** | + | + | + | | | | + | + | | | | | + | | | |
| **Predatory mites** | | + | + | | + | + | + | + | | + | | | + | + | | + |

[1] Site 1 – Cascade Park; Sites 2 through 14 located along Highway 410 and subsidiary roads all of which paralleled rivers or streams; Sites 15 and 16 along Highway 20 near Rainy Pass.

[2] By the time most samples were collected this single generation psyllid was evidenced primarily by cast skins and waxy deposits.

Alders were heavily infested with a number of species of leafrolling caterpillars at all sites. Two species in the family Gelechiidae were recognized, 1 of which was found on all 3 species of alder that were sampled – *Alnus incana, A. rubra*, and *A. sinuata*. The second gelechiid was largely restricted to *A. sinuata*, a species that we found most abundantly at our higher elevation sites. In addition, several species of leafrollers in the family Tortricidae occurred on the alder, one of which was quite common. This species was tested as a host for *Colpoclypeus florus* (see below) and is the subject of a new research proposal.

Plant-feeding mites were found on the vast majority of rolled leaves at most sites and more than 1 species was probably represented. Rust(?) mites were found on many rolled leaves especially later in the season when their numbers seemed to build up.

Thrips were found on nearly as many rolled leaves as phytophagous mites early in the season, but while the percentage of mite infested leaves remained high through August, thrips infestation declined in that month. More than one species of thrips was present, including probable predatory types, and both suborders of thrips were represented although the terebrantia (the group that includes the western flower thrips) was far more common.

Aphids were found on a low percentage of rolled leaves at most sites. Springtails were seen occasionally in the early part of the season but the number of infested leafrolls increased late in the season. Perhaps the senescing leaves were more attractive to these insects, many of which feed on decaying plant material.

Alder is of particular interest because of the large number of beneficial arthropods that colonize the plants some of which occurred in high numbers in some of our samples. Included in this group are a number of generalist predators that are also found in orchards where they prey on pest insects.

Brown and green lacewings were found in a small percentage of leafrolls at most sites. The former included *Hemerobius neadelphus* and the latter included *Chrysoperla plorabunda* (*carnea*) and *Chrysopa coloradensis*, all 3 of which occur in orchards as generalist predators of soft-bodied insects.

Predatory bugs found on the alders that also colonize orchards, where they prey on pest insects, included *Deraeocoris brevis* and *Anthocoris antevolens*. *D. brevis* occurred in low abundance at several of the sites but *A. antevolens* was more abundant and in several of our samples individuals were found in 5% to 10% of the leafrolls examined (19 of 45 leafrolls in 1 sample). We assume that an important prey item for both predators is the native psyllid found on the alders as both bugs are important predators of pear psylla in orchards.

Spiders were well represented in alder leafrolls with sac spiders in the genus *Clubiona* the most numerous. *Clubiona* have potential as leafroller predators (see below) and 2 species have been identified on alder. *C. pacifica* was the most commonly encountered but *C. moesta* was also found, and it has been taken in Washington orchards. Other spiders found on alder that also occur in Washington orchards include the jumping spiders *Pelegrina aeneola* (a dominant member of the spider fauna in some organic orchards) and *Eris militaris*, *Misumena vatia* (a crab spider), the cobweb weaver, *Enoplognatha ovata*, the line weaver, *Spirembolus mundus*, and the philodromid, *Philodromus rufus*.

The predatory mites included as the last item in Table 5 belong to the family Anystidae. They are distinctive rather large, long-legged, red mites that run swiftly and were observed preying on plant-feeding mites and their eggs. Other predatory mites of more typical appearance were probably present in the leafrolls but I did not distinguish them from the similar appearing phytophagous species.

Both the gelechiid and tortricid leafrollers served as hosts for a number of species of insect parasitoids and parasitism rates exceeded 10% in some samples. Ichneumonid, braconid, and chalcidoid wasps and tachinid flies were reared from the leafrollers and various stages of the leafrollers were attacked by different parasitoids. Some of the parasitoids, especially those that attack the alder tortricids, may potentially utilize orchard pest leafrollers as alternate hosts.

The importance of rolled leaves to a number of the plant-feeding and predatory arthropods was shown by collecting samples of rolled and unrolled leaves at 3 sites on 31 August. Similar numbers of both types of leaves were collected at the 3 sites and examined in the laboratory. Results of the comparison are shown in Table 6.

Table 6. Number of rolled and unrolled alder leaves on which various arthropod groups were found at 3 sites on 31 August 2004.

| **Site #** | **1** | **1** | | **2** | **2** | | **3** | **3** |
|---|---|---|---|---|---|---|---|---|
| **Type of leaf** | **rolled** | **unrolled** | | **rolled** | **unrolled** | | **rolled** | **Unrolled** |
| **# of leaves examined** | **43** | **42** | | **57** | **57** | | **63** | **50** |
| **# of leaves w/*Clubiona*** | 17 | 0 | | 4 | 0 | | 2 | 2 |
| **# w/other spiders** | 4 | 0 | | 1 | 2 | | 9 | 0 |
| **# w/spider spin-ups** | 14 | 0 | | 26 | 0 | | 13 | 0 |
| **# w/*Anthocoris*** | 2 | 0 | | 5 | 0 | | 0 | 0 |
| **# w/phytophagous mites** | 39 | 9 | | 55 | 9 | | 51 | 2 |
| **# w/thrips** | 4 | 1 | | 6 | 0 | | 9 | 3 |
| **# w/springtails** | 10 | 0 | | 18 | 2 | | 7 | 0 |
| **Rust (?) mites** | 11 | 18 | | 7 | 54 | | 45 | 42 |

Rolled alder leaves were colonized in greater numbers by most arthropod groups in Table 6 than were nearby unrolled leaves. Rolled leaves may offer certain advantages over unrolled leaves such as more constant and higher humidity, protection from direct sun, protection from wind and rain and perhaps protection from some predators (leafrolls are quite visible, however, and visually oriented predators may well cue in on rolled leaves). Rolled leaves are probably used by all these groups as protected sites for molting as evidenced by the large number of rolled leaves that contained cast spider skins. Shed *Anthocoris* skins were also frequently found in rolled leaves. Egg sacs of various spiders were found in rolled leaves, including *Clubiona*, and *Anthocoris* may used rolled leaves as a mating site as males and females were found in the same roll on a number of occasions. The rust (?) mite was one group that showed the opposite trend in 2 of the 3 samples and unrolled leaves colonized by the mites heavily outnumbered rolled leaves that were colonized.

Three species of alder were sampled in 2004: mountain or thin-leaf alder (*A. incana*), red alder (*A. rubra*), and sitka or wavy-leaved alder (*A. sinuata*). A fourth species, white alder (*A. rhombifolia*) also occurs in eastern Washington. The 3 sampled species occurred together at some sites but mountain alder was the most common species overall. Sitka alder was the dominant species at higher elevations according to our samples and red alder is the dominant species west of the Cascades. All three were infested with both tortricid and gelechiid leafrollers and most of the other arthropod groups listed in Table 5 were found on all 3 species as well. Sitka alder, however, was much less heavily infested with plant-feeding mites than the other 2 species.

Preliminary screening of leafrollers from native plant species as possible alternate hosts for *Colpoclypeus florus*: Seven species of leafrolling caterpillars from four native plants were exposed to *C. florus*, an important parasitoid of our pest leafrollers, in laboratory trials. The plants were among those known to usually be infested with leafrollers based on sampling in previous years. Results of the tests are summarized in Table 7

Table 7. Summary of acceptability tests of native leafrolling caterpillars as alternative hosts for the leafroller parasitoid, *Colpoclypeus florus*.

| Host plant | Leafroller family | # larvae exposed | # larvae parasitized | # *C. florus* adults/host |
|---|---|---|---|---|
| Lupine | Unknown | 7 | 0 | -- |
| Dogwood | Tortricidae sp.1 | 16 | 3 | 3-13 |
| Dogwood | Tortricidae sp. 2 | 2 | 0 | -- |
| Alder | Gelechiidae | 10 | 0 | -- |
| Alder | Tortricidae sp. 1 | 8 | 6 | 8-15 |
| Alder | Tortricidae sp. 2 | 1 | 1 | 8 |
| Strawberry | Tortricidae[1] | 25 | 20 | 1-11 |

[1]Tentatively identified as the strawberry leafroller, *Ancylis comptana*.

The alder gelechiid and the lupine leafroller, also not a tortricid, were not accepted as hosts by *C. florus* in these tests whereas four of the tortricid species were parasitized. In the case of the leafroller from strawberry this would not be surprising if the species is, as it appears to be, the strawberry leafroller.

One additional similar test was run with a small, unidentified parasitoid wasp that attacks a small leafroller on rose. The leafroller, also unidentified, has been found at a couple of sites, may be quite abundant, and rate of parasitism is fairly high. Multiple adult parasitoids develop from a single host, as in *C. florus*. Adult parasitoids reared from the small rose leafroller were tested against PLR and tortricid leafrollers from alder, cottonwood, and dogwood. However, none of the leafrollers was parasitized.

Tests of *Clubiona* spiders as predators of oblique-banded and pandemis leafroller larvae: Four tests were conducted to evaluate the efficacy of *Clubiona* spiders as predators of pandemis and oblique-banded leafroller larvae. All adult spiders used in these tests were *C. pacifica* Banks, which is the species we have collected most frequently. Since conditions varied somewhat among the 4 tests each is described briefly and the results are summarized in the table below.

Test #1. This test utilized potted pear seedlings, 14" to 16" tall, inoculated with OBLR larvae and placed in cylindrical, clear plastic cages. There were 5 cages and one *Clubiona* spider was introduced into each cage. Two medium size immatures, 1 sub-male, and 2 females were used. A confounding factor in this test was that OBLR fared poorly on the pear plants. Some larvae appeared sickly, fed little, and died or became moribund before the end of the experiment. Also, the 3 immature *Clubiona* molted during the experiment and as a result would not have been actively hunting for at least a portion of the time during which the experiment was running. No definite predation could be attributed to these 3 spiders. One of the female spiders died during the experiment. The second female *Clubiona*, however, consumed all 4 OBLR larvae available to her. The only leafrollers alive at the end of the test were a large larva and a pupa, both in the cage occupied by the female spider that died.

Test #2. This test was run in a similar fashion to the first, except that apple seedlings inoculated with 5 pandemis larvae each, were used. There were 6 cages and each received 1 adult female *Clubiona*. All spiders had reached adulthood in the laboratory and were unmated. Only 2 PLR did not fall victim to spiders and they could not be accounted for at the end of the test. Two PLR reached the pupal stage but they also were located and consumed by spiders.

Test#3. For this test, a 3 ½ ' tall apple tree in a 5 gallon pot was inoculated with 24 OBLR larvae ($4^{th}$ and $5^{th}$ instar). Nineteen larvae established on the plant, which was then enclosed in a large, organdy screen cage and 3 medium to large immature *Clubiona* added. The test was run for 19 days after which the cage was checked for surviving and dead OBLR. Twelve pupae and 1 large larva survived. Five larvae were definitely killed by the spiders and a $6^{th}$ was a probable victim, thus accounting for all 19 OBLR. Only 1 *Clubiona* survived. This was a female which molted to the adult stage during the test. Two shed *Clubiona* skins were found as were several spider parts, the former

indicating 2 spider molting events and the latter probably indicating that the surviving spider cannibalized 1 or both of the other 2 spiders.

Test #4. Five branches of a small apple tree in a 5 gallon pot were inoculated with 5 or 6 medium to large OBLR larvae apiece. The next day each branch was covered with a small screen cage and a female *Clubiona* introduced. The test was run for 14 days and the cages checked for predation. One spider died during the test and one failed to kill any OBLR. The other 3 spiders killed 1 or 2 OBLR apiece.

Table summarizing the results of the 4 *Clubiona* predation on leafroller experiments in 2004.

| **Test** | **Host** | **# of Spiders** | **# of available LR's** | **# LR's predated (%)** | **# LR's surviving** |
|---|---|---|---|---|---|
| 1 | Pear | 5 | 18 | 4 (22%) | 2 |
| 2 | Apple | 6 | 27 | 25 (93%) | 0 |
| 3 | Apple | 3 | 19 | 5 or 6 (26% or 32%) | 13 |
| 4 | Apple | 5 | 17 | 5 (29%) | 12 |
| **Totals** | **--** | **19** | **81** | **39 or 40 (48% or 49%)** | **27** |

Eighteen *Clubiona*, 6 females and 12 immatures of various sizes, were exposed to PLR egg masses to determine if the spiders showed any propensity to feed on eggs. One female *C. pacifica* completely consumed an egg mass that had been deposited on wax paper and a second female fed on a mass that had been deposited on the side of a plastic vial and consumed about 10% of the eggs. None of the other 16 spiders fed on the egg masses although most fed on newly eclosed neonate larvae.

Host plant suitability studies:

1) Apple as a host for the alder tortricid leafroller. Six medium to large, field collected alder tortricid leafroller larvae were placed on foliage of a small potted apple tree in the greenhouse on 29 July 2004. Six similar alder tortricid larvae were placed on a small potted alder tree to serve as an alder control. Finally, 6 PLR larvae from a laboratory colony, also of similar size, were placed on a small potted apple tree to serve as an apple control. Leafroller survival was evaluated on 10 August. None of the 6 alder tortricid larvae placed on apple was recovered and there had been very little feeding or leafrolling activity by the alder leafrollers. In contrast, 6 leafrolls were found on the alder plant that had been infested with alder leafrollers and there was feeding damage, some quite extensive, associated with each leafroll. One large larva and one pupa were recovered from the plant. Three large PLR larvae and 2 pupae were recovered from rolled leaves on the apple tree and at least 13 leaves were found that had feeding damage. These results, while preliminary, indicate that apple is probably not a suitable host plant for the alder tortricid leafroller.

2) Pear as a host for the alder tortricid leafroller. Three, small, potted, pear plants were each inoculated with 2 field-collected alder tortricid larvae and 2 OBLR larvae of comparable size from a laboratory colony for comparison. Larvae were small to medium sized, the latter being somewhat less than half grown. Larvae were preferentially placed on young, developing leaves of the plants on 21 July. A check on leafroller establishment the following day indicated some leafrolling activity on the young, terminal leaves of each of the 3 plants. On 6 October each plant and the cage in which they were kept were carefully examined for leafrollers, feeding damage, leafrolling activity, and frass. The oldest developmental stage recovered in this test was a dead, abnormally developed OBLR pupa in the spun-together, heavily fed-upon, terminal leaves of the plant. Other than this, individual examination of all leaves on the 3 test plants showed only 6 of 84 leaves with feeding damage, the largest of which was a rectangular area of 8mm x 10mm. A few leaves had a small amount of webbing present. Three, dead, OBLR larvae were also found but they had developed little. No alder leafroller larvae were recovered. Again these results are preliminary but they indicate the unsuitability of pear as a host for the alder tortricid.

3) Alder as a host for PLR and OBLR. Fifteen small OBLR larvae (probable 2$^{nd}$ and 3$^{rd}$ instars) were placed on young, developing leaves of a potted mountain alder (*Alnus incana*) plant on

14 July 2004. Ten PLR of similar size were likewise placed on a second alder plant (fewer PLR were used because I had fewer available). Equal numbers of OBLR and PLR were placed on apple cuttings in vials of water as controls. The apple cuttings did not prove suitable for the leafrollers and none of them survived for more than a few days. The first check on leafroller survival on alder was made on 30 July. One OBLR pupa and 5 feeding larvae in leafrolls were found as well as extensive feeding damage and frass. No PLR larvae or pupae were found, however, and there was little evidence of feeding and very little frass. Final evaluation of leafroller survival on alder was made on 6 August when 4 normal OBLR pupae were found in leafrolls but no PLR of any stage were present. These results, while preliminary, indicate the probable suitability of *Alnus incana* as a host for OBLR. While PLR did poorly on alder in this test, *A. incana* and *A. rubra* have been listed as host plants for this species (Miller, J. C. 2003. Lepidoptera of the Pacific Northwest: caterpillars and adults. FHTET-2003-03, USDA-Forest Service).

Survey of extra-orchard host plants for leaf-rolling caterpillars: The leafroller on extra-orchard host plant survey was completed in 2004 when 145 samples of foliage apparently infested with leafrolling caterpillars were collected and reared. Collections were made from 29 plant species and moths were obtained from 24 species. Some of the moths are tortricids. Parasitoids were reared from the leafroller material from 8 host plants: lupine, willow, paintbrush, tall buckwheat, chokecherry, dogwood, cottonwood, and Ceanothus. Most parasitoids were wasps, but wasps and a tachinid fly were obtained from leafroller material found on tall buckwheat. Insects obtained from stinging nettle proved to be butterflies and a web-spinning sawfly was obtained from rose. Overall, 102 moths or their parasitoids were obtained from the 145 specimens that were collected in 2004, a rearing success rate of 70%. In addition to this material many specimens from the alder work were reared. Parasitoids of both the tortricid and gelechiid leafrollers were obtained and some hyperparasitoids as well. Several species, including wasps and tachinid flies, were found to attack the alder leafrollers.

**Budget**

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

**PI:** Eugene Miliczky

**Project Duration:** 2003-2004

**Project Total (2 years):** $66,700

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

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

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

Unruh-Effects of new insecticides-no report submitted

FINAL REPORT
**WTFRC PROJECT # TR-02-235**

**Project Title:** Reduction of Pesticide Inputs, Worker Exposure, and Drift Through Alternative Sprayer Technology
**PI:** Allan S. Felsot (Washington State University, Dept. of Entomology; afelsot@tricity.wsu.edu)
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**Cooperator(s):** Tom Auvil (Washington Tree Fruit Research Commission)
Jay Brunner, Mike Doerr, Keith Granger (WSU, Dept. of Entomology)
Linda Finch & Pauline Anderson (USDA-Wapato)
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**Objectives:**

The goal of our research has been to help growers reduce the cost of pesticide applications while simultaneously maintaining efficacy, reducing worker exposure, and off-target drift. This project was designed to help meet the goals of the 'technology roadmap' to reduce production costs while enhancing fruit quality and sustaining a quality environment. The following objectives were studied during a three year project that involved a combination of field and laboratory experiments.

1. Determine residue deposition from a reduced volume alternative sprayer (i.e., the Proptec tower) using reduced rates of active ingredient application.
2. Determine efficacy of reduced application rate residues deposited by a reduced volume sprayer and the conventional 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:**

- LC50/LC95 of azinphos-methyl (Guthion), acetamiprid (Assail), and methoxyfenozide (Intrepid) against neonate codling moth (CM) larvae were respectively 0.017/0.075 µg/cm$^2$, 0.007/0.166 µg/cm$^2$, and 0.077/1.130 µg/cm$^2$. The slopes of the dose-response curves for Intrepid and Assail were flatter than the curve for Guthion suggesting greater population variability in susceptibility to these new reduced risk insecticides.
- Neonate codling moths often died within 2-3 hours of exposure to leaf disks treated in the field or the lab with Guthion or Assail without any evidence of feeding. Thus, Assail seems to have good contact activity against codling moth larvae.
- During crop years 2002 and 2004 when five-rows were sprayed per plot, residues deposited by the Proptec sprayer were higher than by either a Rears airblast or Pak-blast sprayer. When spray plots consisted of only one row during crop year 2003, initially deposited residues were higher in the Pak-blast treatments.
- Fluorescent tracer dye photographs showed uneven coverage on apples from the second cover spray regardless of sprayer type. In July, the surfaces of treated apples often had large areas lacking spray residues. In contrast, apples sprayed during May or early June had more uniform coverage around the whole fruit.

- Cessation of feeding activity, rather than lethality should be used as the appropriate field-relevant toxicological endpoint for Intrepid.
- Bioactivity of Guthion, Assail, and Intrepid as determined by the leaf disk and apple assay was not significantly affected by sprayer type (Rears airblast vs. Proptec), did not differ between leaves collected from mid canopy and leaves collected from the top of the canopy, and were equally efficacious at half-rates and full rates.
- Bioactivity of Guthion persisted for about one month (i.e., bioactivity generally reached ~90% neonate CM mortality) on treated foliage but only persisted for about two weeks on treated apples. Similar persistence in bioactivity of foliage and apples was observed for Assail.
- Intrepid (methoxyfenozide) bioactivity was lower than Guthion and Assail. Persistence of bioactivity (as evidenced by <50% mortality within 24 hours) lasted for less than three weeks on foliage and was poor on apples.
- Persistence of Guthion and Assail bioactivity generally paralleled persistence of residues. As residues dropped below the LC50 after 30 days, 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. Overhead irrigation increased the rate of dissipation.
- Alternate-row middle and skip-row spray patterns deposited sufficient Guthion and Assail residues to cause 100% mortality of larvae regardless of sprayer type. Residues were still above the LC50 for each insecticide 28 days after application.
- Dislodgeable foliar residues of Guthion observed after 21 days were at least two times lower than residues used by the EPA to assess post-application worker exposure at both the 0.5 and 1 lb AI rate/acre.
- Drift of Guthion, Phosmet, and Assail were detected to a distance of 200 feet in several drift trials during experimental plot applications and commercial scale applications.
- When nozzles were turned off on one side of an airblast sprayer, significant levels of spray drift still occurred on this "upwind" side due to the rotation of the axial fan. However, the Proptec sprayer did not drift significantly to the upwind side of the fans. These observations suggest that airblast sprayers applying chemical to outside rows need to shield the unused side of the axial fan to avoid inadvertent contamination outside of the orchard.

**Methods:**

All experiments were conducted at commercial orchards in the Quincy area during crop years 2002-2004. At each location, cooperators allotted a block of 'gala' apples for delineation of treatment plots. Plots consisted of five tree rows by 10 trees long. All experiments involved comparing the deposition, persistence, and bioactivity of several insecticides after application by either an axial fan airblast sprayer or a Proptec single tower sprayer. Two cover sprays were made during each growing season—late May or early June and during the third week of July. During 2002 and 2004, all five rows in a plot were sprayed, but in 2003, only a single row was sprayed from both sides. During 2003, alternate-row-middle and skip-row spray patterns were also tested.

Insecticide formulations (active ingredient; lbs AI/acre) applied in the various experiments included Guthion Solupak (azinphos-methyl; 0.25, 0.5, 1.0 lbs AI/acre), Assail 70WP (acetamiprid; 0.075, 0.15 lbs AI/acre), Intrepid 4F (methoxyfenozide; 0.125, 0.250 lbs AI/acre), and Imidan 70W (phosmet; 3.5 lbs AI/acre). Fluorescent dye was added to some spray tank mixtures to visualize the pattern of spray deposition on foliage and apples.

Following each insecticide application and at different intervals thereafter, foliage and apples were collected for laboratory bioassays with neonate codling moths (all insecticides) and for analysis of insecticide residues (Guthion and Assail treatments only). During 2002 and 2004, the tree canopy was divided into mid (waist high to extended arm length) and top (at least 2 ft above extended arm length) segments. Samples were kept separate by canopy location. Dose-response relationships (LD50/LD95) were also determined for each insecticide by exposing neonate CM to laboratory treated leaf disks.

For data analysis, the independent variables were sprayer type, canopy location, and insecticide rate. During 2003, row spraying pattern (i.e., alternate row middle or skip row) were also independent variables. Over all years, dependent variables were percent mortality of neonate CM exposed for at least 24 hours to leaf disks punched from field-treated foliage, percent reduction in entry holes on field-treated apples, and residues on leaves (expressed as $\mu g/cm^2$) and residues on apples (2002 and 2004 only [analyses of 2004 apple samples still in progress]).

Downwind drift and cross row movement of sprays during application were monitored during all years of the project. Downwind out-of-orchard drift was monitored by collecting residues on silica gel (SG) cards (20 cm x 10 cm) laid on the ground at different distances from the outside tree row (i.e., row one). To measure cross row movement SG cards were also laid at different distances from row one several rows inside the orchard. Cards were placed both in the tree rows and in the alleys between rows. During 2002, single row applications were made using either an airblast or Proptec sprayer traveling between rows one and two and between rows two and three. Only one side of the row was treated (toward the downwind side). During 2003 and 2004, both commercial applications (Phosmet, Guthion) and multiple row experimental applications (Assail, Phosmet) were monitored for drift. Data were used to determine the validity of the model AgDRIFT.

**Results & Discussion:**

Objective 1. *Determine residue deposition from a reduced volume sprayer (i.e., the Proptec tower) using reduced rates of active ingredient application.*

During crop year 2002, the Proptec sprayer was calibrated to deliver the insecticides at a volume rate of 100 gallons/acre and speed of ~4 mph. The airblast sprayer delivered insecticide at a volume rate of 100 gallons and speed of 1-2 mph. Initial deposition of Guthion residues was nominally higher in the Proptec treatments at both canopy levels except at mid canopy level after the second cover spray (Figure 1).

During crop year 2004, the Proptec was calibrated to deliver spray at a volume rate of 35 gal/acre and all five rows in a treatment plot were sprayed. Deposition of both Assail and Guthion residues applied at their nominal 1X rates (0.15 lb AI/acre or 1 lb AI/acre, respectively) was again higher in the Proptec treatment except for the mid canopy level of the first cover spray (Figure 2). However, during crop year 2003, when only one row was treated and no segmentation of the canopy used in sampling, initially deposited residues from the Proptec application were lower than residues from the airblast (Pak-blast) application (Figure 3, 4). Nevertheless, bioactivity (as measured by mortality) against neonate CM larvae was over 90% for all treatments.

Objective 2. *Determine efficacy of reduced application rate residues deposited by a reduced volume sprayer and the conventional airblast sprayers.*

Based on larval exposure to laboratory-treated leaf disks, LC50 and LC95 were estimated for Guthion, Assail, and Intrepid (Figure 5). Without any other food source, larvae would eat the leaves and would remain healthy through 3 or 4 instars. Furthermore, larvae would "weave" cocoons of frass and undigested leaf waxes around themselves. Contact toxicity as opposed to feeding activity was evident for all insecticides because larvae would die without any observable feeding on the leaf surface.

When exposed to leaves treated in the field with Guthion and Assail, especially after initial deposition and for at least two weeks thereafter, larvae would die or appear moribund within about four hours of exposure. On leaves treated with Intrepid, larvae would become lethargic and stopped feeding. Only after initial Intrepid deposition and one week later did larvae die within 24 hours, but they always became sick even months after application.

Initially deposited residues were significantly above the LC95 for Guthion at both the reduced and full application rates (Figure 1, 2, 4) but near the LC95 for Assail (first cover spray only) (Figure 2, 3). Nevertheless, after 24 hours of exposure to initially deposited residues off all insecticides, 80-100% of larvae generally died regardless of rate of application, type of sprayer, and row spray pattern.

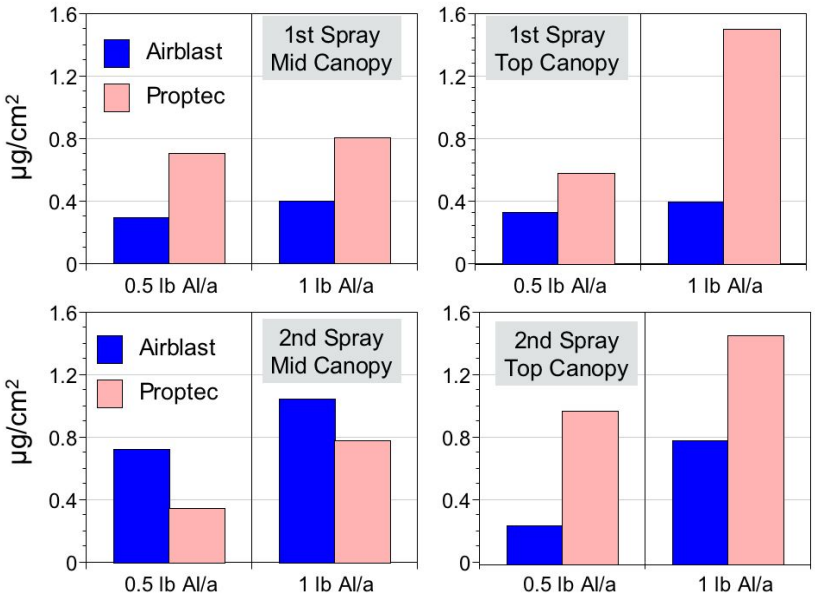


Figure 1. Deposition of Guthion residues (µg/cm$^2$) on foliage at mid canopy and top canopy levels after the first and second cover spray during crop year 2002.

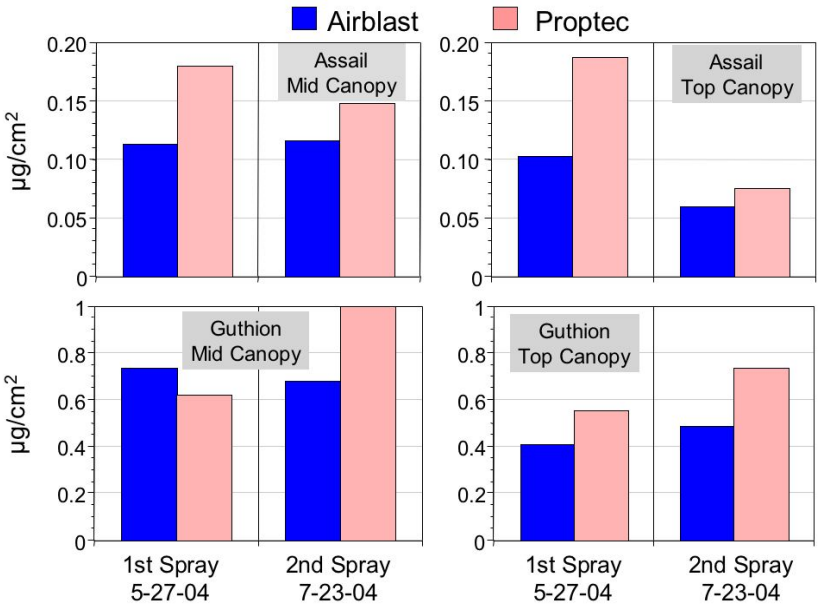


Figure 2. Deposition of Guthion (bottom) and Assail (top) in the mid and top canopy segments after spraying during crop year 2004. Both insecticides were applied at the rate of 1 lb AI/acre.

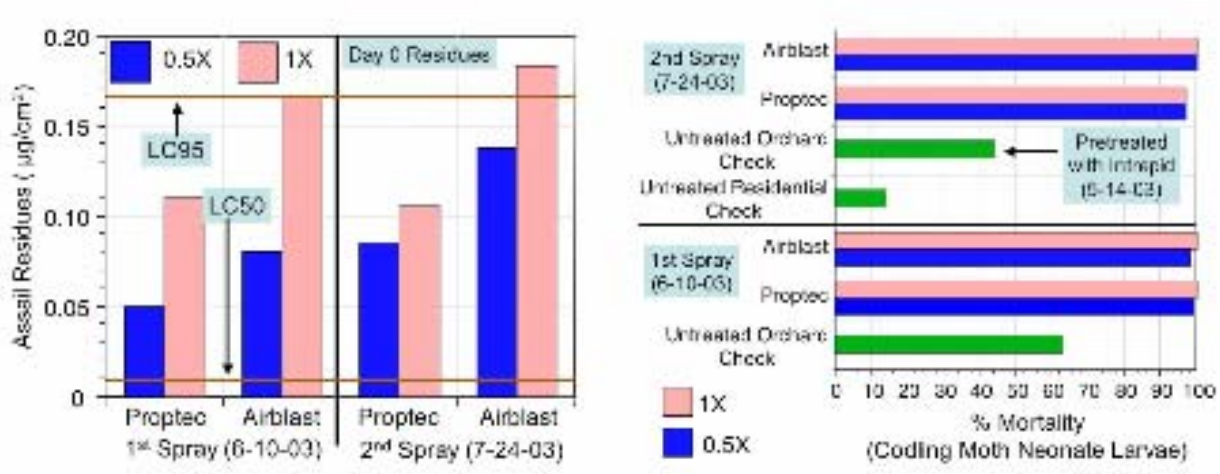


Figure 3. Initial deposition (µg/cm$^2$; left side) on foliage and bioactivity (% neonate CM mortality; right side) of Assail residues applied during June and July 2003 by a Proptec and airblast (Pak-blast) sprayer. Both sides of a single row were sprayed. Note that foliage collected from the orchard had received a cover spray of Intrepid during May, and larval mortality was subsequently very high, even after two months. Therefore, leaves from an untreated residential apple tree were used to gauge larval mortality in the absence of insecticide. Percentage mortality was uncorrected for untreated control mortality.

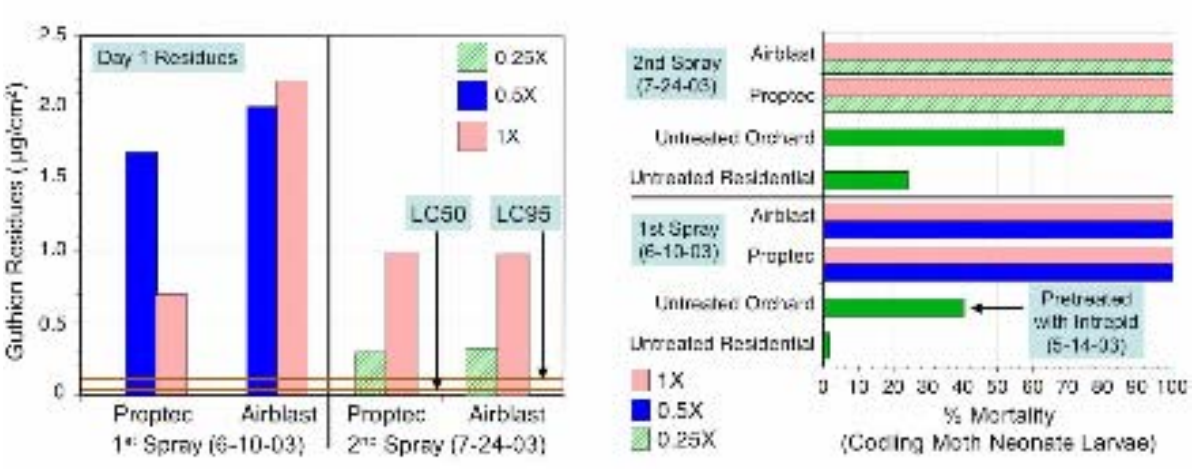


Figure 4. Initial deposition (µg/cm$^2$; left side) on foliage and bioactivity (% neonate CM mortality; right side) of Guthion residues applied during June and May 2003 by a Proptec and airblast (Pak-blast) sprayer. Single rows were sprayed from both sides.

Objective 3. *Determine the residue decline rate of reduced application rates using chemical and biological assays.*

The dissipation of residues and change in bioactivity against neonate CM were monitored following both the first and second cover sprays during crop years 2002 and 2003 and during the first cover spray of crop year 2004. During crop year 2002 and 2003, Guthion residues generally remained above the neonate foliar LC95 for about one month following application (Figure 6, 8). During crop year 2002, foliar Guthion residues and bioactivity tended to be higher in the Proptec treatment plots than in the airblast sprayer plots. For both sprayer types, bioactivity dropped significantly when residues had declined below the LC95 (compare Figure 7 with the Proptec treatments in Figure 6). Patterns in dissipation of residues and bioactivity were the same in both the 0.5X (0.5 lb AI/acre) and 1X treatments (1 lb AI/acre) (Figure 6, 7).

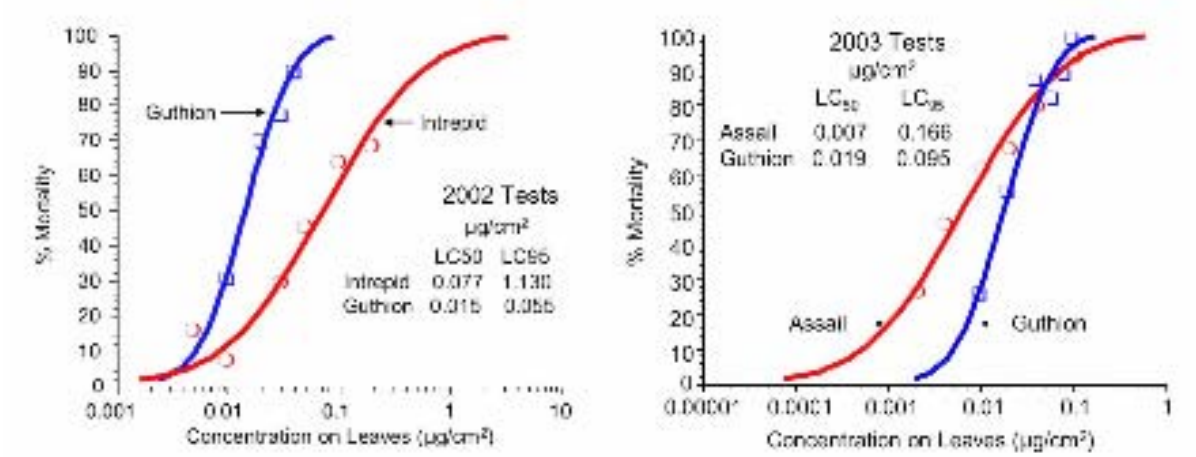


Figure 5. Dose-response relationships for neonate CM larvae exposed to laboratory-treated leaf disks for 24 h. One hundred microliters of insecticide formulated in a 2:1 acetone water solution was pipetted over the upper surface of individual leaf disks.

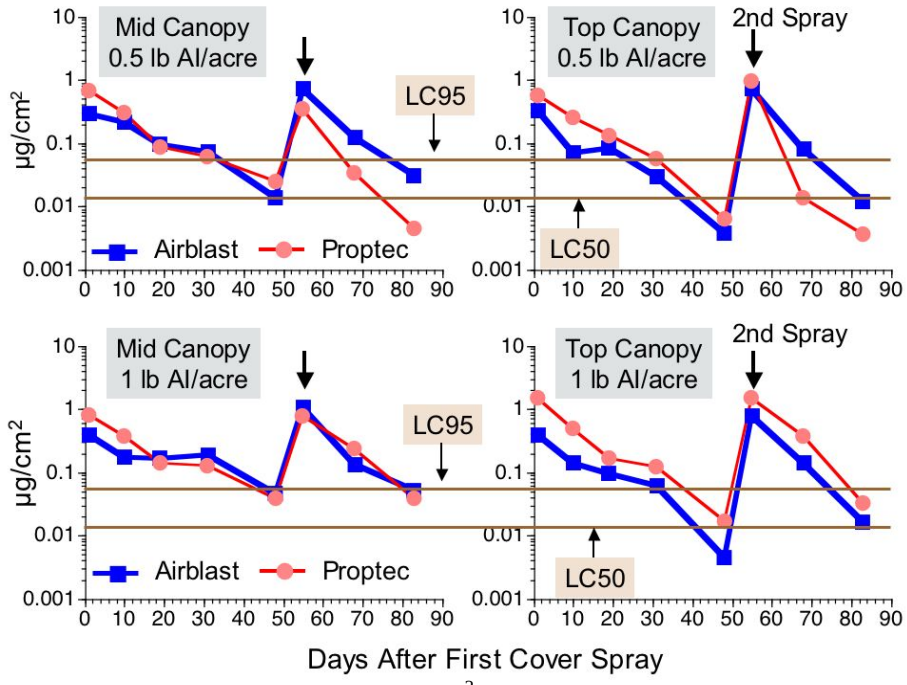


Figure 6. Persistence of Guthion residues (µg/cm$^2$) after two cover sprays in relation to the foliar LC50/LC95 against neonate CM. Experiments were conducted during crop year 2002, and five rows within each plot were sprayed at a volume rate of 100 gal/acre. This orchard used overhead sprinkler irrigation, which was turned on more frequently following the second cover spray than following the first spray.

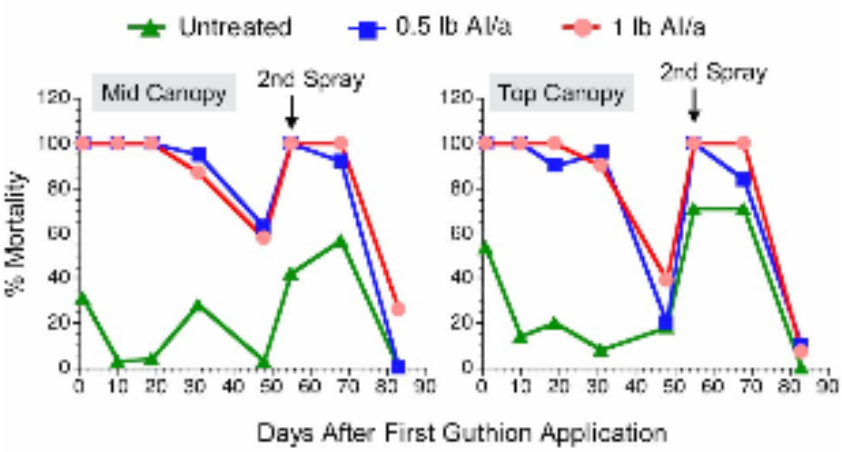


Figure 7. Persistence of bioactivity (% mortality) of Guthion residues against neonate CM larvae on field-treated foliage (crop year 2002). Application was made with a Proptec sprayer. Note that bioactivity dropped below 90% and 50% when residues had declined below the LC95 and LC50, respectively (see Figure 6).

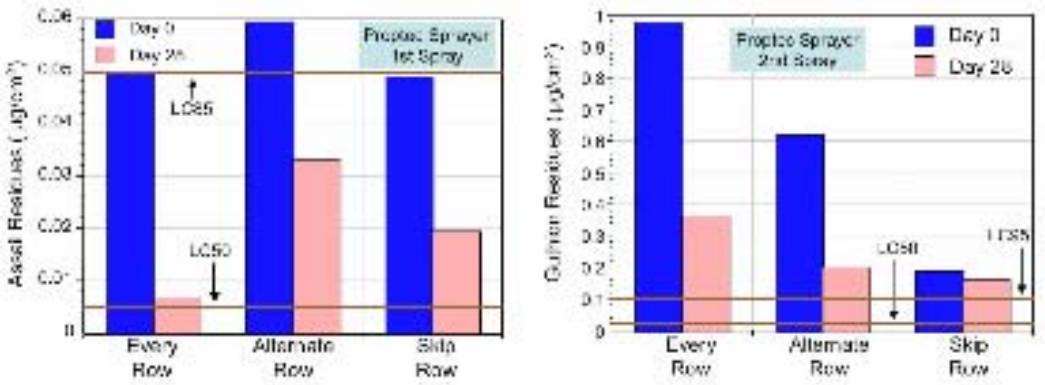


Figure 8. One-month persistence of Assail (left side) and Guthion (right side) residues on foliage in relationship to the LC50/LC95. Applications were made during crop year 2003 to single rows with the Proptec (35 gal/acre; ~4 mph) using conventional (every row) and alternative row spraying practices.

Assail residues following the Proptec sprayer treatment during crop year 2003 were lower than residues from the Pak-blast sprayer following the first and second cover spray throughout the monitoring period (Figure 9). Although the Assail residues from the Pak-blast treatment were significantly below the lab determined LC95, foliar bioactivity was generally around 90% or greater throughout the monitoring period, although lower than for the Pak-blast sprayer treatment.

Residues of Guthion following alternative row spraying practices with the Proptec sprayer remained above the LC95, even on foliage collected from a completely unsprayed row situated between two rows sprayed from the outer side only (Figure 8). Similar results (not shown) were noted for the airblast sprayer. For both sprayers, the skip-row treatments resulted in residues comparable to the 0.5X and 0.25X (0.25 lb AI/acre) treatment rates. Guthion residues from those reduced rate treatments were also above the LC95 after 28 days (data not shown). In contrast to Guthion, Assail residues from all row-spraying practices made with the Proptec during 2003 were below the foliar LC95 but remained above the LC50, even after 28 days (Figure 8).

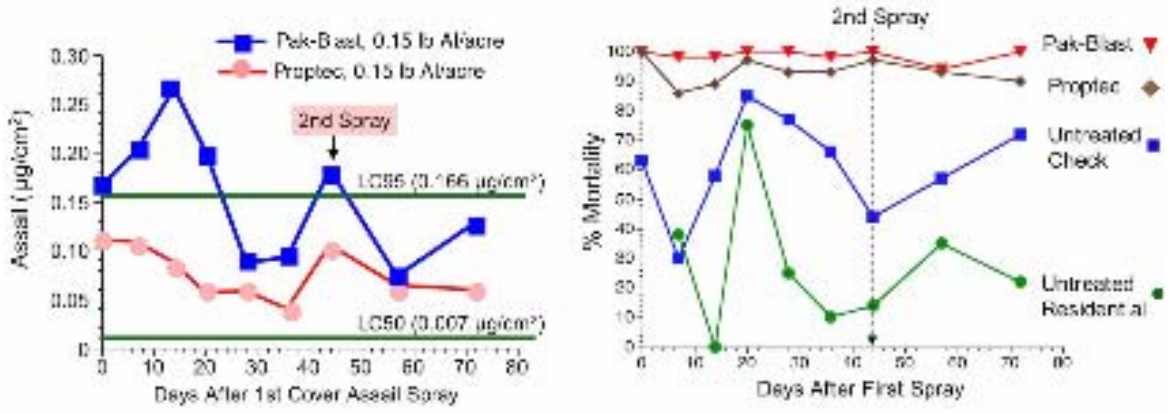


Figure 9. Persistence (left side) and bioactivity (right side) of Assail residues at the 1X rate (0.15 lb AI/acre) applied during crop year 2003 to single rows.

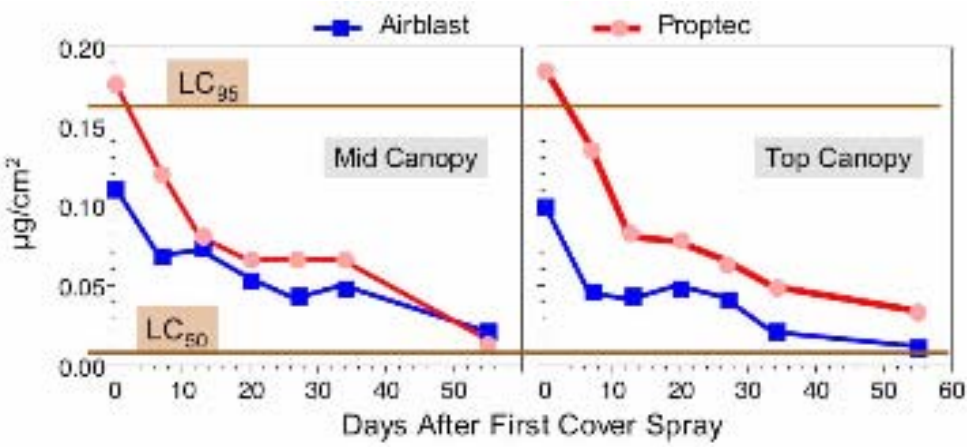


Figure 10. Persistence of Assail residues in relationship to the foliar LC95/LC50 after the first cover spray by a Proptec and Pak-blast sprayer during crop year 2004. Application was made to five rows within a treatment plot at a rate of 0.15 lb AI/acre and volume rate of 35 gal/acre or 100 gal/acre for the Proptec and Pak-blast sprayers, respectively.

During crop year 2004, Assail residues were above the foliar LC95 only immediately after application (Figure 10). Residues were higher in the Proptec treatments, especially at the top of the canopy. Residues remained above the LC50 for at least 35 days following application.

Biological activity against CM larvae on apples was measured using a larval entry hole assay. Persistence of biological activity (as measured by prevention of an entry hole) of deposited Guthion residues on apples was very short in comparison to persistence of bioactivity on leaves. Bioactivity dropped significantly after two weeks and paralleled a significant loss of pesticide residues (Figure 11). However, for apples collected from the top canopy level of the Proptec 1X treatment, over 80% reduction of entry holes was observed after two weeks. When apple residues dropped below 0.2 µg/cm$^2$, percent reduction of entry holes dropped to <60% (Figure 11). Trends were similar between 0.5X (data not shown) and 1X rate treatments. However, percent reduction in entry holes was initially lower in the 0.5X treatment than in the 1X treatment, and significantly lower after two weeks. The trend in loss of bioactivity on apples after two weeks was also observed in crop year 2004 (Figure 12).

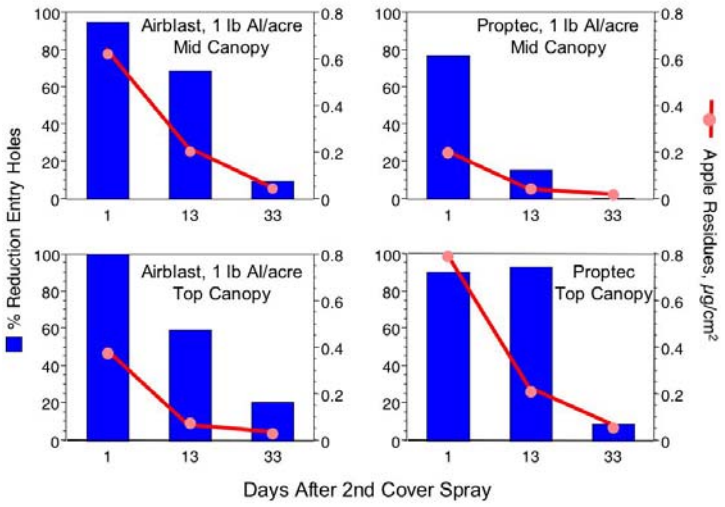


Figure 11. Percent reduction of neonate CM entry holes on apples in relationship to recovered surface residues of Guthion during crop year 2002. Multiple replicates of single apples were analyzed to derive an average surface residue.

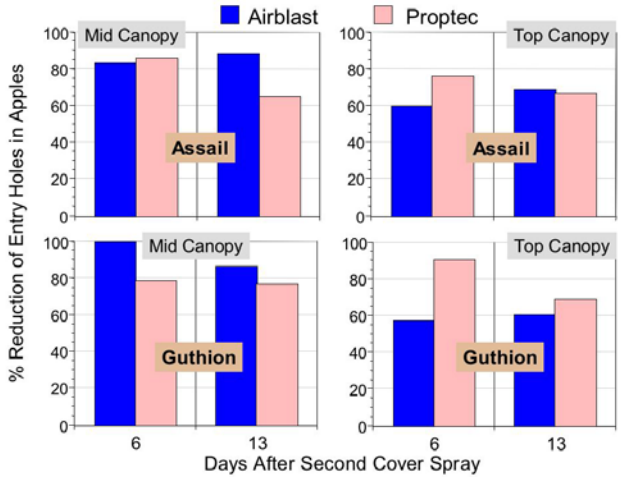


Figure 12. Percent reduction in neonate CM entry holes during crop year 2004 following 1X cover sprays of Assail and Guthion. No significant differences between sprayers were observed by the second week following application.

Objective 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.*

In the Guthion Registration Eligibility Decision documents (REDs), EPA used DFR (dislodgeable foliar residue) values from 0.5 lb (0.5X) and 1 lb AI (1X) per acre rates to determine whether risk was acceptable to post-application workers. The agency used 21-day post application residue values of 0.35 and 0.7 µg/cm$^2$, respectively. Using a stronger extraction solvent than typically employed for agency mandated DFR studies (acetone:water compared to soap solution), we observed in all experiments that Guthion residues at ~21 days were at least two times lower than the EPA estimates. Our observations suggested that the EPA database should be updated with more DFR studies of Guthion to increase the accuracy of post-application worker exposure. We noted no significant differences in residues recovered three weeks post application due to the airblast and Proptec sprayers when multiple rows (compared to single rows) were sprayed (see Figure 6).

Objective 5. *Determine the drift reduction potential of alternative sprayers.*

The drift potential from the Proptec sprayer could be compared to the airblast sprayer only at the orchard used during crop year 2002 owing to the lack of an open field next to the orchard used during 2003 and 2004. However, further characterization of drift from airblast sprayers was studied at an orchard in the Yakima Valley with an adjacent open field (data not shown). During 2002, single rows were sprayed with Guthion with the tractors first going though rows one and two (Spray A) only and then through rows two and three (Spray B) only (Figure 13). Rows were sprayed only from one side, inside to outside. The airblast sprayer nozzles on the leeward side were shut off during spraying.

Downwind out-of-orchard drift was significantly greater for the Proptec sprayer than the airblast, but the Proptec was oriented to spray from the inside to the outside of the rows rather than the inside to the outside. Pertinently, however, the airblast sprayer, but not the Proptec, deposited significant residues on the ground of untreated rows within the orchard (Figure 13) even though the inner nozzles were shut off, but the Proptec did not. We

hypothesized that the rotation of the axial fan on the airblast sprayer picked up spray and emitted it to the leeward unsprayed side. Thus, the data suggest that applicators should shield the unused side of the airblast sprayer when spraying the first two outside rows from only one side (i.e., the outside to inside direction). The Proptec sprayer should be operated on the two outside rows by spraying from outside to inside.

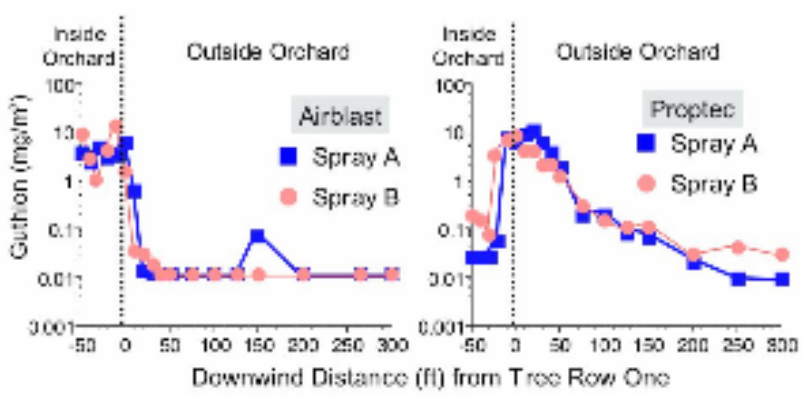


Figure 13. Downwind drift and cross row movement of Guthion applied by two sprayers during 2002.

**Budget**
**Project Title:** Reduction of Pesticide Inputs, Worker Exposure, and Drift
**PI:** Allan Felsot
**Project Duration:** 2002–2004 (3 years); **Project Total:** ($161,807)

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

**CONTINUING PROJECT**

**Project Title:** Sensor Webs
**PI:** Jay Brunner

Project referred to technology committee. Oral report presented only.

**CONTINUING (Final) REPORT** **YEAR 3/3**

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

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

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

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

**OBJECTIVES (2005):**

- Complete development and validation of molecular protocol to discriminate between apple maggot (*Rhagoletis pomonella*) and snowberry maggot (*R. zephyria*) captured in traps for accurate monitoring and quarantine actions.
- Develop DNA extraction method suitable for flies captured in traps (conservation of specimens far from optimum: the fly dries out and it is immersed in a sticky fluid).
- Complete characterization of putative new *Grapholita* species in roses (rose-hip worm).

**Significant Findings:**

- A 90% diagnostic system based on PCR-RFLP of a mitochondrial and a nuclear gene has been developed to discriminate *Rhagoletis pomonella* and *R. zephyria*.
- A pseudogene of mitochondrial COI that seems to be exclusive of *R. pomonella* has been discovered.
- Molecular diagnosis between *Rhagolettis basiola* (rose-hip maggot) and *R. pomonella* could be easily performed by amplification of mitochondrial COI or COII genes by real time PCR, or PCR-RFLP with conventional PCR.

Methods for 2005:

1. The simple and inexpensive method of Chelex will be tested to obtain DNA from legs and or heads of flies from sticky traps. If this does not work, we will try other methods that have been successful for poorly conserved DNA.
2. Single Stranded Conformational Polymorphism (SSCP) will be tested as an alternative cheaper and faster method to detect polymorphism in the diagnostic genes (obviating restrictions).
3. Characterize and optimize detection of the newly discovered pseudogene of mitochondrial COI.

**Results and Discussion (**Based on 2004 objectives in bold):

1. **Complete discovery and development of molecular protocol to discriminate between apple maggot (*Rhagoletis pomonella*) and snowberry maggot (*R. zephyria*) captured in traps for accurate monitoring and quarantine actions**.

   Backgroud: The different species of flies that are captured in traps can be classified by the wing pattern. However, *R. pomonella* and *R. zephyria* are identical and need to be taken to the lab for microscopic analysis. Adult female flies are distinguished by the size of the ovipositor, *R. zephyria* 0.9 mm or less, *R. pomonella* 1.0 mm or more. Flies with ovipositors that fall between 0.9 and 1.0 are considered to fall in a "gray area". A definitive ID is attempted by measuring wing band ratio and wing length, but these characters may also fall in a gray area. Adult males are separated by genital structure; *R. pomonella* has a parallel surstyli with broad surfaces facing directly lateral, *R zephyria* has divergent surstyli witn broad surfaces arranged obliquely. Similarly, this character has a continuous distribution and there are specimens that fall in a "gray area" as well (Westcott, 1982; and Mike Klaus, (pers. comm.). Depending in the collection area, specimens in the gray area could represent 0.1 to 11% of the flies. A DNA diagnostic protocol that could discriminate between the species in the "gray area" is being developed.

   **Follow up on the sequence data presented last year:**

   1) McPheron and Han (1997) reported one base-pair difference between *R. pomonella* and *R. zephyria* in a 460bp long fragment of the mitochondrial 16s rDNA gene. We found that we can use RFLP-PCR with primers LR-J-12887 and LR-N-13398 and Alu1 to distinguish between the two forms. However, further sequencing by us proved that the polymorphism is interspecific and hence can not be used for diagnosis.

   2) We found one base-pair difference between *R. pomonella* and *R. zephyria* in a 780 bp fragment of mitochondrial CytB, however this is not enough to design reliable species specific primers and there is no restriction enzyme that specifically cuts in this region. Therefore, the gene was discarded as potential for diagnosis.

   3) We found 3 base-pair differences in a 526bp mitochondrial COI gene, one of which can be resolved by PCR-RFLP with primers C1-J-1718 and C1-N-2191 and AluI. Analysis of specimens from Skamania, Cowlitz, Pierce, Thurston and Clark counties (*R. pomonella*) and from Pierce, Chelan, Kittitas and Yakima counties (*R. zephiria*) showed three haplotypes, only two of which are diagnostic (Table 1).

| COI Haplotype | A | B | C |
|---|---|---|---|
| R. pomonella N=24 | 0.75 | 0.25 | 0.00 |
| R. zephyria N=20 | 0.00 | 0.90 | 0.10 |

From this work, it became obvious that the two species are extremely similar and we needed to sequence more diverse genes, so we changed our target to introns of nuclear genes.

   4) We sequenced 350bp of the intron of Tubulin-3 and found only one difference, which can not be resolved by any known restriction enzyme. Not useful for diagnosis.

   5) We sequenced 180bp of nuclear intron EF-1A and found one difference, which can be resolved by the restriction enzyme SCrF1. We screened the same individuals as above and found that *R. pomonella* is monomorphic for genotype 1 but share the character with

10% of *R. zephyria*. Genotype 2 is exclusive of *R. zephyria* and can be used for diagnosis (Table 2).

| Intron EF1A genotype | 1 | 2 |
|---|---|---|
| *R. pomonella* N=24 | 1.00 | 0.00 |
| *R. zephyria* N=20 | 0.10 | 0.90 |

If we consider that diagnosis is economically critical in areas where the frequency of *R. pomonella* is 10% or less of the trap catches, this means that with the combination of the two genes we can determine accurately 88.5% to 90% of the flies in the gray area, while the rest remains as unknown, with a probability of 78-100% of being *R. zephyria*. The test would not allow any *R. pomonella* to go undetected .

We recently discovered a pseudo gene of mitochondrial COI that seems to be exclusive of *Rhagoletis pomonella*. Pseudo genes are non-functional copies of genes that because of their lack of function allow a more rapid evolution and diversification of gene sequences. When *R. pomonella* DNA is exposed to primers C1-J-1718 and C1-N-2191 two products become evident, the expected 525bp fragment of mitochondrial COI, and an extra 360bp fragment. DNA sequencing of the latter aligned with COI but showed 2 deletions 134 and 31 bp long and 25 individual base-pair differences. We obtained PCR products for almost 300 snowberry maggots and none showed evidence of the pseudo gene. In contrast, most of the PCR products of 60 apple maggots show the pseudo gene. We developed pseudo gene specific primers and validated its presence but have not been able to amplify it in all the *R. pomonella*. Further work needs to be done to assess the utility of this character.

The lack of a fully diagnostic method could be the result of genetic introgression between the two species, in which case, it is impossible to have a 100% diagnostic method. This hypothesis will be tested by genetic analysis of population of *R. zephyria* in areas free of *R. pomonella* versus areas where the two species occur in sympatry.

To validate this protocol and to estimate the frequencies of the mitochondrial and nuclear genes in different geographic areas throughout Washington, we made extensive collections of both species (Table 3). We will wait until the emergence of adult flies in late winter (February 2005) in order to correlate host plant, adult morphology and molecular data. This will allow us to validate our protocols with flies from the "gray area" and look for evidence of hybridization.

Table 3. Individuals of Rhagoletis to be characterized molecularly from a larger collection from Washington and Oregon.

| Species | Region | County | Quarantine? | 2003 | 2004 n |
|---|---|---|---|---|---|
| R. zephyria | West | Whatcom | YES | 0 | 8 |
| | West | Skagit | YES | 0 | 13 |
| | West | Snohomish | YES | 0 | 20 |
| | West | King | YES | 0 | 6 |
| | West | Pierce | YES | 4 | 0 |
| | Central | Chelan | NO | 2 | 17 |
| | Central | Kittitas | YES | 3 | 20 |
| | Central | Yakima | NO | 11 | 80 |
| | East | Stevens | NO | 0 | 20 |
| | East | Spokane | YES | 0 | 10 |
| | East | Columbia | NO | 0 | 9 |
| Total | | | | 20 | 203 |

| | | | | | |
|---|---|---|---|---|---|
| R. pomonella | West | Whatcom | YES | 0 | 10 |
| | West | Pierce | YES | 5 | 0 |
| | West | Thurston | YES | 2 | 0 |
| | West | Cowlitz | YES | 5 | 0 |
| | West | Clark | YES | 4 | 20 |
| | West | Skamania | YES | 8 | 0 |
| | Central | Kittitas | YES | 0 | 20 |
| | Central | Yakima | NO | 0 | 40 |
| | East | Spokane | YES | 0 | 20 |
| | Oregon | Pendelton | | | 6 |
| Total | | | | 24 | 86 |

***Rhagoletis pomonella*** versus ***R. basiola*.** A molecular method was developed to discriminate between the apple maggot and the rose-hip maggot. This work was performed to aid Dr. Wee Yee (USDA-ARS-YARL) in discriminating these flies as pupae without having to wait several months for emergence and positive identification. Given that these flies are phylogenetically well separated, the diagnosis is easy and straightforward. Melting profiles of PCR products of COI (primers C1-J-1718 and C1-N-2191) and COII (primers C1-J-2792 and C1-N-3287) genes are distinct; with *T. basiola* showing lower Tm´s (the temperature at which 50% of the PCR product melts). The method was validated with rose-hip maggots collected in six Washington counties (Okanagan, Chelan, Kittitas, Yakima, Stevens and Whitman). If conventional PCR it is necessary to do PCR-RFLP, for which we suggest Alu1.

2. **Continue collections of spider mites from different locations, design primers and test reliability and robustness of molecular protocols to discriminate among mites of quarantine importance, mainly Pacific spider mite (*Tetranychus pacificus*) and McDaniel spider mite (*T. mcdanieli*).**

We found a “hot spot” in mitochondrial gene COI suitable to design species-specific primers but were unable to continue the project due to the lack of specimens from different geographic populations to validate the methodology. We have recently been made aware of a source

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

No further work was done due to lack of specimens from exotic species. However, the possible discovery of a new species of *Grapholita* in Washington was pursued as follows:

2003 Background: A moth from Indiana classified by taxonomists as cherry fruit worm (CFW) had a distinct sequence from two other CFW moths from Michigan and Washington (28 out of 420 base pair differences as opposed to 2/420 between WA and MI), suggesting a different race or even a different species. This “CFW” proved identical in DNA sequences to two moths collected in rose-hips in ecological studies in Washington using rose as a host habitat for leafroller parasitoids. The rose-hip form looks like LAW as a larva (but has 33/420 base pair differences from LAW) and the adult resembles CFW (but has 28/420 differences from CFW). We think that this group represents an undescribed species of “rose-hip-worm”. We will make more collections and do more sequencing

next year to clarify this issue. We find no evidence that this rose form is a pest but it may be mistaken for CFW in traps.

Collections of specimens of the putative new species of *Grapholita* (rose-hip worm) in Washington were made during 2004. A total of 488 specimens from five counties (Okanagan, Douglas, Kittitas, Yakima and Whitman) were collected in three species of roses (*Rosa woodsii* 96.5%, *R. nutkana* 2.3%, and *R. canina* 1.2%). One third of the specimens were fixed in ethanol 70% as larvae, and the rest are being kept alive waiting for emergence in spring of 2005. Sample larvae and adults will be sent to taxonomist Dr. William Miller, University of Minnesota, for morphological characterization. Hopefully morphological characters will be discovered that could discriminate between this "rose-hip worm". If they can not be discriminated by morphological characters, it would be easy to design species specific primers if correct diagnosis becomes a quarantine issue, specifically if we find evidence of it in LAW or CFW traps.

Moths will also be compared to specimens of *Grapholita rosana*, a species that is known to attack roses in Europe but has not been previously reported in America. If the Washington specimens key out to this species, and molecular analysis confirm the identity, it would mean that *G. rosana* has been introduced and spread in America. If the moth turns out to be morphologically and or genetically separate from the European rose-hip moth, we can conclude that we discovered a new species of *Grapholita* in Washington based on molecular data. Fortunately, the new species does not seem to be of economic importance to the region.

**BUDGET**

**$ 0.0**

Current funding extends through July of 2005 and will be enough to complete the 2005 goals, so no further funding is being requested for this project. If there is no objection by the Commission, the poster and oral presentation will be given as a continuing project, and the final comprehensive report will be presented next year.

**CONTINUING PROJECT REPORT**

**YEAR 1/3**

**WTFRC Project #:** AE-04-426.doc

**Project title:** Alternate Hosts of Apple Maggot as a Threat to Apples
**PI:** Wee Yee
**Organization:** USDA-ARS, Wapato, WA
**Collaborator:** Mike Klaus, WSDA, Yakima, WA

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

**2004 Significant Findings:**
1) An established population of apple maggot exists in Yakima County in the Nile Valley, within 10 miles of apple orchards in Naches, with 22.5% of black hawthorns infested at low levels.
2) Apple maggots infested black hawthorn and apples in the Nile Valley.
3) No larval infestations were detected in any fruit in Ellensburg and Yakima, despite the presence of adult flies on traps in both areas.
4) In Vancouver and Puyallup in western Washington, apple maggots attacked pear, Asian pear, and plum, suggesting these are hosts used every year in areas with moderate to high fly populations.
5) Observations suggest early varieties of both apples and pear are attacked.

**Materials and Methods:**
1) *Determine apple maggot abundance and prevalence on alternate and normal host trees*. Studies to assess preferences for and abundance on different hosts were conducted in four regions in Washington: 1) Vancouver, 2) Puyallup, 3) Ellensburg, 4) Nile, and 4) Yakima. Each of these has its own characteristic flora and habitats and thus may influence apple maggot use of hosts. Traps were placed in apple maggot hosts beginning in mid-June: hawthorn, crabapple, early and late-maturing varieties of apples, rose, Asian pear, common pear, plum, and ornamental shrubs (cotoneaster, barberry, firethorn). Fruit from different hosts were collected in mid June to late August and larval infestation levels were recorded. Larvae were reared and adults will be positively identified in 2005.
2) *Effects of fruit maturity and spatial relationships between alternate host trees and normal trees on maggot infestations*. The fruit of selected hosts were collected weekly and measures of ripeness determined using color, weight and sugar content. Flies were trapped throughout the season in 2004.

In 2005, alternate hosts in three spatial categories with respect to apples, crabapples, or hawthorns will be trapped: 1) isolated or in stands ($\geq$ 1 mile from nearest host), 2) < 0.25 mile from infested apples or hawthorns, and 3) surrounded by apples or hawthorns in contiguous orchards or abandoned lots. This will establish the relationship between proximity of known hosts, host phenology, and the likelihood of infestations.
3) *Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies*. Preliminary observations were made in 2004 in the Vancouver area. Infestations of 1) cotoneasters, 2) firethorn (pyracantha), 3) plum, and 4) pears were studied with respect to proximity to apples and infestations. In 2005, more detailed studies are planned in which clean shrubs and trees will be placed in the following conditions: 1) in the middle of

infested apple orchards, 2) on the edge, 3) 25 yds, and 4) 100 yards away. Additional shrubs will be placed under the following host conditions: 1) near infested apple trees, 2) near infested hawthorn trees, and 3) near Asian pear or other known non-apple host trees such as pear. Shrubs will be placed around the trees in mid June though September. Fruit from the shrubs will be sampled to determine when and if infestations occur at particular times in relation to fruit development of the trees.

To determine if the shrubs are acceptable to the flies, 10 female flies collected from apple or hawthorn trees will be released on each plant and observations made of fruit acceptance (stinging or egg laying) and larval infestations. Field and laboratory no-choice studies will be conducted inside cages in Vancouver.

**Results and Discussion:**

1) *Determine apple maggot abundance and prevalence on alternate and normal host trees*.
Apple maggots were trapped on many hosts, but not all trees positive for adults were positive for larvae. Studies in 2004 confirmed previous work showing that apple maggot infests at least 9 hosts in Washington, of which 5 had not been previously (before 2002) recorded (Table 1). In Vancouver, apple maggots infested black and ornamental hawthorn, apple, crabapple, pear, Asian pear, plum, and cotoneaster, and in Puyallup, apple maggots infested hawthorn, apple, and Asian pear (Table 2). Infestations were greatest in hawthorn and apple, but were moderately high in pears and plums. Surprisingly, no pupae were recovered in Ellensburg, even though Washington State Department of Agriculture (WSDA) reported 32 positive traps sites, for 39 flies. Perhaps the cool temperatures in the storage unit where fruit were maintained slowed larval development or caused high mortality, although all fruit were cut and no larvae were seen inside them. The data indicate that when adults are present, infestations can still be very difficult to detect. Of great significance was the confirmation that an established apple maggot population exists in Yakima County in the Nile Valley. Here, apple maggot larvae were found infesting nearly a quarter of the black hawthorn trees at low levels (Table 2). In addition to the larvae/pupae recovered from hawthorn in the Nile (Table 2), WSDA also found fruit from three of five black hawthorns with adult fly catches were positive, for 18 total pupae. Also of great significance, WSDA found that apple fruit from one of nine trees with adult fly catches were infested with 5 larvae. This shows that the population is composed of flies attacked apples and that it is not sole composed of a "hawthorn race". In the Nile Valley, WSDA reported 51 positive trap sites (hawthorn and apple trees), with 79 flies caught. In Yakima, no apple maggot larvae were recovered from any fruit (Table 2), even though WSDA caught 3 adults on crabapple. Western cherry fruit fly, rose maggot, and snowberry maggot pupae were recovered from various hosts (Table 2), but rearing to the adult stage will be needed to determine if some of these hosts were also infested with apple maggot.

2) *Effects of fruit maturity and spatial relationships between alternate host trees and normal trees on maggot infestations*. Adult flies were found in all hosts as the season progressed and fruit ripened (Table 3). Flies appeared in highest numbers on hosts when fruit ripened, although data are not completely gathered. Fly populations were high enough only around Vancouver to allow for sufficient data collection.

Observations suggest flies occurred in higher numbers on alternate hosts where there were infested apples, crabapples, and hawthorns. In isolated trees or in stands in Skamania County (St. Cloud Ranch), flies occurred in moderate numbers on pears, but only when they were surrounded by apples and hawthorns.

3) *Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies*. Preliminary work based on observations was made to document the spatial relationships between ornamental shrubs and hosts shown or suspected of being alternate apple maggot hosts in the Vancouver area in preparation for 2005 studies. Plums were infested only near apples and hawthorn. At one unmanaged fruit orchard, where there were many apples, plums were not infested. The same situation occurred with pears in this orchard. In St.

Cloud, an early variety of pear was infested at high levels. The proximity to the apples seemed to play a role in infestations in pear, as isolated pears were not infested.

It may not be surprising that pear, plum, and cotoneaster are apple maggot hosts in Washington, as they are hosts in other parts of the U.S. However, it cannot be assumed apple maggot uses the same hosts in every region in the U.S. For example, apple maggot has established in rose in New England, but extensive rose collections in areas with high apple maggot populations in southwestern Washington have yet to yield apple maggot pupae. This is significant because such information, when correlated with habitat type and fly ecology factors, may lead to predictions of apple maggot host usage in areas where fly has become recently established.

Whether established populations of apple maggot exist on the new hosts remains to be determined. Establishment requires that fly populations be able to sustain themselves on the host for many generations without the need for any other hosts. It is possible flies may not be able to do this on some of the new hosts and that they must periodically infest nearby apples or hawthorns to maintain their genes in the fly population.

Several factors may affect the use by apple maggot of Asian pear, pear, bitter cherry, and cotoneaster in the major apple-growing regions in central and eastern Washington. High tree abundance, high availability of normal hosts, and high fly populations probably will increase chances they are used. They may also be used in situations where flies cannot access or do not readily infest apples or hawthorns, e.g., where apple or hawthorn trees are removed as a control measure, infested fruit are introduced by humans into backyards with no normal hosts, hawthorn fruit drop off trees early, or where less susceptible, hard varieties of apples occur. In these situations, traps placed in the new hosts will maximize chances of detecting apple maggot in areas where flies are a threat to the apple.

**Table 1. Complete updated list of apple maggot developmental hosts in Washington, as confirmed by studies in 2004 and 2002-2003.**

| Common Name | Scientific Name | Host Status |
|---|---|---|
| 1) Apple | *Malus domestica* | Common host, used less than haws? |
| 2) Black Hawthorn | *Crateagus douglasii* | Common host |
| 3) Ornamental Hawthorn | *Crateagus monogyna*[a] | Common host |
| 4) Crabapple | *Malu* hybrids | Common host |
| 5) Asian Pear | *Pyrus serotina*[b] | Common host, but rarer than other hosts |
| 6) Bitter Cherry | *Prunus emarginata*[b] | Rare host; tree mostly in wild areas |
| 7) Common Pear | *Pyrus communis*[c] | Moderately common host; used locally |
| 8) Plum | *Prunus domestica*[c] | Moderately common host |
| 9) Cotoneaster | *Cotoneaster horizontalis*[c] | Rare host; residential yards; urban areas |

[a]Possibly other species as well; species are difficult to identify.
[b]New hosts identified in 2002-2004.
[c]New hosts in Washington identified in 2002-2004.

***Significance to the Industry and Potential Economic Benefits.*** The research is significant for the apple industry because it may help reduce the possibility of quarantines being imposed in areas now free of apple maggot, specifically by identifying which trees can be infested and populated by the fly and therefore should be treated. Areas where apple maggot is now a threat include the Nile and Naches areas, which have many alternate fruit tree hosts and are adjacent to the Yakima Valley. If an apple maggot is found within ½ mile of an apple orchard, the orchard is threatened and inspection of the fruit is required, at costs in time to the grower. Shipments need to be accompanied by official certificates showing that fruit are free of infestations. In addition, apple maggot establishment near or

in commercial orchards may increase numbers of spray applications that lead to additional costs to growers.

**Table 2. Trees sampled and numbers of apple maggot (AM), snowberry maggot (SBM), rose maggot (RM) or western cherry fruit fly (WCFF) pupae recovered from various fruit collected in five regions in Washington State, WA, June to September 2004.**

| Tree | No. Trees | No. Fruit | No. Pupae | % Trees Pos. | Likely Species |
|---|---|---|---|---|---|
| | | Vancouver (Clark Co.) | | | |
| Ornamental Hawthorn | 3 | 7,702 | 739 | 100.0 | AM |
| Black Hawthorn | 4 | 3,539 | 40 | 50.0 | AM |
| Apple | 6 | 425 | 23 | 83.3 | AM |
| Crabapple | 3 | 1,752 | 234 | 66.7 | AM |
| Asian Pear | 7 | 413 | 10 | 42.9 | AM |
| Pear | 10 | 720 | 80 | 30.0 | AM |
| Plum | 14 | 863 | 24 | 21.4 | AM |
| Cherry Plum | 4 | 378 | 3 | 25.0 | WCFF |
| Rose | 2 | 479 | 0 | 0 | ------ |
| Sweet/Sour Cherry | 31 | 8,638 | 945 | 67.7 | WCFF |
| Bitter Cherry | 5 | 5,980 | 669 | 100.0 | WCFF |
| Cascara | 3 | 1,402 | 1 | 33.3 | WCFF |
| Service Berry | 2 | 844 | 0 | 0 | ------ |
| | | Puyallup (Pierce Co.) | | | |
| Ornamental Hawthorn | 2 | 400 | 125 | 100.0 | AM |
| Apple | 7 | 345 | 208 | 100.0 | AM |
| Asian Pear | 1 | 8 | 12 | ------ | AM |
| Plum | 2 | 60 | 0 | 0 | ------ |
| Black Berry | 2 | 100 | 0 | 0 | ------ |
| | | Ellensburg (Kittitas Co.) | | | |
| Black Hawthorn | 19 | 3,800 | 0 | 0 | ------ |
| Apple | 194 | 4,850 | 0 | 0 | ------ |
| Crabapple | 151 | 15,700 | 0 | 0 | ------ |
| Pear | 96 | 2,400 | 0 | 0 | ------ |
| Plum | 55 | 2,612 | 0 | 0 | ------ |
| Rose | 12 | 2,400 | 10 | 25.0 | RM |
| Snowberry | 3 | 600 | 0 | 0 | ------ |
| Elderberry | 10 | 2,000 | 0 | 0 | ------ |
| Apricot | 11 | 300 | 0 | 0 | ------ |
| | | Nile Valley (Yakima Co.) | | | |
| Black Hawthorn | 40 | 10,402 | 15 | 22.5 | AM |
| Apple | 23 | 1,030 | 0 | 0 | ---------- |
| Pear | 6 | 320 | 0 | 0 | ---------- |
| **Table 2, continued** | | | | | |
| Plum | 4 | 319 | 0 | 0 | ---------- |
| Rose | 45 | 4,223 | 585 | 84.4 | RM |
| Bitter Cherry | 20 | 3,784 | 308 | 65.5 | WCFF |
| Choke Cherry | 20 | 5,592 | 0 | 0 | ---------- |
| Snow Berry | 32 | 5,343 | 178 | 50.0 | SBM |
| Elderberry | 18 | 3,103 | 0 | 0 | ------ |

| | | Yakima (Yakima Co.) | | | |
|---|---|---|---|---|---|
| Tree | No. Trees | No. Fruit | No. Pupae | % Trees Pos. | Likely Species |
| Black Hawthorn | 41 | 6,741 | 0 | 0 | ---------- |
| Apple | 12 | 233 | 0 | 0 | ---------- |
| Pear | 5 | 70 | 0 | 0 | ---------- |
| Rose | 15 | 1,316 | 65 | 73.3 | RM |
| Sweet/Sour Cherry | 9 | 1,800 | 1,338 | 100.0 | WCFF |
| Choke Cherry | 20 | 365 | 0 | 0 | ---------- |
| Snow Berry | 5 | 619 | 13 | 0 | SBM |
| Elderberry | 6 | 1,070 | 0 | 0 | ------ |

**Table 3. Mean numbers of adult apple maggot flies caught per unbaited sticky yellow panel trap at two sites 2 miles apart in western Washington June to August 2004. Numbers of trees are inside parentheses.**

| Site 1 Date | Cherry Plum (2) | Cascara (1) | Italian Plum (4) | Japanese Plum (2) | Asian Pear (2) | Common Pear (4) | Apple (3) | Bitter Cherry (1) | Crab-apple (1) |
|---|---|---|---|---|---|---|---|---|---|
| 25 June | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.3 | 0* | 0 |
| 7 July | 0* | 0 | 0 | 0 | 2.5* | 0.5* | 4.3* | 0* | 1 |
| 13 July | 1.0* | 0 | 0 | 0 | 6.0* | 0.2* | 1.3* | 0* | 2 |
| 21 July | 1.5* | 0 | 0 | 0.5* | 8.0* | 0* | 2.7* | 0* | 0 |
| 27 July | 1.0 | 0 | 0.2* | 0.5* | 4.5* | 0.2* | 3.7* | 1* | 1 |
| 4 August | 0 | 0 | 0.2* | 1.0* | 2.5* | 0.2* | 4.3* | 4* | 0 |
| 11 August | 1.5 | 0 | 0* | 0.5* | 4.0 | 1.0 | 7.7 | 0 | 1 |
| 18 August | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.7 | 0 | 0* |
| No. Fruit | 199 | ---- | 86 | ---- | 131 | 22 | 210 | 69 | 327 |
| Pupae | 3 | ---- | 0 | ---- | 9 | 0 | 15 | 11 | 0 |
| Likely sp. | WCFF | ---- | ---- | ---- | AM | ---- | AM | WCFF | -- |

| Site 2 Date | Cherry (5) | Bitter Cherry (1) | Service Berry (1) | Cascara (6) | Crab-apple (1) | Apple (1) | Common Pear (1) | Ornam Haw (2) | . Black Haw (2) |
|---|---|---|---|---|---|---|---|---|---|
| 16 June | 0 | 0* | 0* | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 June | 0 | 0* | 0* | 0 | 0 | 0 | 0 | 0 | 0* |
| 7 July | 0 | 0* | 0 | 0 | 1.0 | 0 | 0 | 0 | 0* |
| 13 July | 0* | 0 | 0 | 0.5* | 0 | 0 | 0 | 0 | 40.0* |
| 21 July | 0.8* | 0 | 0 | 0.5* | 1.0 | 3.0* | 0 | 0.5* | 47.5* |
| 27 July | 0.2 | 1 | 1 | 0.2* | 0 | 3.0* | 0* | 0.5* | 16.5* |
| 4 August | 0 | 0 | 0 | 0.7 | 0 | 0* | 0 | 6.0* | 15.5* |
| 11 August | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 15.0* | 3.0 |

**Table 3, continued**

| Site 2 Date | Cherry (5) | Bitter Cherry (1) | Service Berry (1) | Cascara (6) | Crab-apple (1) | Apple (1) | Common Pear (1) | Ornam. Haw (2) | Black Haw (2) |
|---|---|---|---|---|---|---|---|---|---|
| 18 August | 0 | 0 | 0 | 0 | 0* | 0 | 0* | 4.5* | 0 |
| No. Fruit | 173 | 54 | 331 | 1,402 | 257 | 40 | ---- | 1,694 | 1,907 |
| Pupae | 88 | 9 | 0 | 1 | 0 | 1 | ---- | 14 | 31 |
| Likely sp. | WCFF | WCFF | ----- | WCFF | ----- | ---- | ---- | AM | AM |

*Periods of fruit ripening.
Likely sp. refers to the pupae; western cherry fruit fly (WCFF); apple maggot (AM).

**Budget:**

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

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

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

**CONTIUING REPORT**

**YEAR 1/3**

**WTFRC Project #:** AE-04-427.doc
**Project title:** Control of Apple Maggot Using Bait Spray Insecticides and Traps
**PI:** Wee Yee
**Organization:** USDA-ARS, Wapato, WA
**Collaborator:** Mike Klaus, WSDA, Yakima, WA

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

**2004 Significant Findings:**
1) GF-120 bait sprays were not effective in reducing larval infestations in an unmanaged apple orchard with dense canopy, but were effective in isolated apple trees.
2) Residues of GF-120 on apple leaves exposed to central Washington conditions in July through August for 3 days were highly active against apple maggots, those exposed for 7 days were less slightly active, and residues exposed for 14 days were least active.
3) Use of GF-120 sprays and use of ammonium carbonate baited red spheres were equally effective in reducing larval infestations in apple at two sites; however, in no case were infestations eliminated.
4) When GF-120 and red spheres were used together, there was no further decrease in larval infestations, perhaps because flies were more attracted to the red spheres than the bait.

**Materials and Methods:**
1) *Determine release of ammonia and other volatile release rates from bait sprays*. Release rates of ammonia and other attractants from GF-120 (0.02% spinosad, protein, ammonium acetate, sugar, DowAgroSciences, Indianapolis, IN) are being determined in the laboratory. Several label dilutions (GF-120: water volumes) will be tested. These will be 1:1.5 (i.e., 4 ml GF-120 and 6 ml water) and 1:5, equivalent to 40 and 17% GF-120. One or 3 ml of each will be tested (depending on detectable ammonia). Fresh bait will be applied on plastic dishes and placed inside an ammonia-collecting apparatus. Other volatiles will examined using gas chromatography. Collections will be made at 0, 3, and 7 days post exposure in the laboratory. Ammonia, total protein, and sugar content of the GF-120 will be determined. The baits Nu-Lure and Mazoferm will also be tested if they elicit fly responses.

2) *Determine attraction to bait sprays and feeding behaviors on baits*. Fly responses to the 40, 20, 5, and 0% GF-120 concentrations in 1or 3 ml of solutions will be applied on artificial plastic leaves in one-hour assays. Droplets of 20% sugar water will serve as a control. Individual flies will be released inside gallon-size containers and feeding and resting times on or near bait droplets recorded. In the field, bait spray will also be applied on apple leaves. Observations will be made of flies arriving at droplets and their feeding and resting times. Representative flies will be collected and held in containers and returned to the laboratory to determine mortality. Flies on leaves from control trees will be used for comparison. Comparisons using Nu-Lure and Mazoferm will also be made.

3) *Determine effects of bait sprays in different habitat types*. In 2004, two tests were conducted to determine the effects of four rates of GF-120 on adult fly and larval populations in two habitat types in the Vancouver area in western Washington. One habitat was an unmanaged apple orchard with dense canopy and trees about 12 ft tall and 12-15 ft wide. The other was an area with isolated and

scattered apple trees in residential yards and trees 12 ft tall and about 8 ft wide. In each habitat, GF-120 sprays were applied weekly on individual trees. Rates were 1) 40% GF-120 in 75 ml spray; 2) 40% GF-120 in 225 ml spray; 3) 17% GF-120 in 180 ml spray; 4) 17% GF-120 in 540 ml spray. An 5) unsprayed control was also included. Sprays were applied using a hand-held squirt bottle beginning in mid-June and continued weekly until early September for 8 or 9 applications.

A single ammonium carbonate baited sticky yellow panel was hung in all trees. Traps were checked once a week and all flies were removed. At least 50 apples from each tree (depending on fruit load) were collected from each tree in mid or late August and the larvae reared out. There were five replicates for five treatments in each habitat. Further tests are planned for 2005, and 2006.

The residual activity of GF-120 on apple leaves under central Washington conditions was determined by exposing the residues to apple maggot flies in the laboratory. 'Fuji' apple trees at the USDA farm in Moxee were sprayed with 40% GF-120 on 27 July for test 1 and on 17 August for test 2. Leaves were removed at 0, 3, 7, and 14 days after treatment and then exposed to flies collected in the field in Puyallup in western Washington. Unsprayed leaves were used for controls. Leaves were transported from Moxee to Puyallup and tested the same day. There were 10 flies per replicate. A single leaf was exposed to the 10 flies and mortality was recorded daily (except weekends) for 10 (test 1) or 24 days (test 2). There were three replicates in test 1 and five replicates in test 2.

4) *Determine effects of bait sprays and trapping methods on apple maggot control*. To test the hypothesis that removing apple maggot flies through trapping will result in reduced infestations after treatment with GF-120, one test was conducted single apple trees in an orchard in Puyallup and another on single scattered and isolated feral apple trees in Vancouver using a combination of traps and GF-120 sprays. In Puyallup, treatments were (1) a control, (2) weekly GF-120 sprays (17% GF-120 at 540 ml/tree), (3) 6 red spheres, and (4) a combination of (2) and (3). There were 11 applications of GF-120. In Vancouver, a similar test was conducted, except treatments were: (1) yellow panel only; (2) yellow panel + GF-120 sprays; (3) 6 red spheres only; and (4) 6 red spheres + GF-120 sprays. There were 7 applications of GF-120. At both sites, each trap was baited with a 10 g ammonium carbonate lure. Traps were checked weekly and the numbers of flies were counted. At least 50 apples from each tree were collected in July through October (depending on test and apple variety) and placed in tubs. Apples were held for at least 2 months. In Puyallup, apples were also cut to determine numbers of larvae inside the fruit after 2 months.

**Results and Discussion:**

1) *Determine release of ammonia and other volatile release rates from bait sprays*. Studies are currently being conducted in the Wapato laboratory to determine ammonia release rates from GF-120. Analyses of Nu-Lure and Mazoferm, as well as for the ammonium hydroxide lure, are also planned.

2) *Determine attraction to bait sprays and feeding behaviors on baits*. Tests to determine attraction to GF-120 bait, Nu-Lure, Mazoferm, and ammonium hydroxide solutions will be conducted in winter 2005 after apple maggots emerge from collections made in August through October 2004. Tests will be conducted in laboratories in Vancouver and Puyallup.

3) *Determine effects of bait sprays in different habitat types*.

The effects of sprays differed in the two apple habitats in 2004. There were no differences in numbers of adult flies caught on traps in either habitat, but while numbers of larvae per fruit in the orchard habitat were similar among all treatments and the control, the numbers of larvae in the isolated tree habitat were lower in the treatments than in the control (Table 1). It is possible that in the orchard setting, with trees close to one another and with branches interlocking, flies move more frequently among trees than in the isolated tree setting, where flies need to move farther among trees. The sprays apparently do not protect fruit from flies that are ready to lay eggs, perhaps explaining in part why infestations were greater in the orchard habitat. In both habitats, there were early and late apple varieties, which may have influenced infestation rates. 'Early Transparent' apples seemed to be

attacked the most. Further tests controlling as much as possible for variety are planned for 2005 and 2006.

The residual activity of GF-120 under central Washington conditions in 2004 was determined. There were significant differences in mortality of apple maggot flies exposed to GF-120 residues aged for varying lengths of time on apple leaves (Table 2). Mortality at day 1 after exposure was greatest to GF-120 applied fresh, at 0 days, but GF-120 aged for 3 and 7 days were also effective, although it took longer to cause 100% mortality. Residues aged for 14 days were least effective, and although they killed 100% of flies in test 1, they did not kill all the flies even after 24 days of exposure in test 2 (Table 2). Based on these tests, fresh GF-120 was most effective, but 3-day old residues were nearly as effective in killing flies before they can oviposit. If sprays are applied early enough, shortly after flies emerge and before flies are gravid (about 7 days), then spray intervals of 7 days may be sufficient. There was no rain during the aging in the field, but rain would probably reduce the length of activity. In field tests below, rain may have reduced residual activity, reduced mortality, and raised infestations.

4) *Determine effects of bait sprays and trapping methods on apple maggot control*.

In 2004 in Puyallup, there was a significant reduction in numbers of larvae in apples when trees were trapped used 6 baited red spheres alone, sprayed with GF-120 alone, or when spheres and GF-120 were combined, as compared with the control (Table 3). Presumably, the baited red spheres alone removed enough gravid flies to reduce larval infestations, while GF-120 sprays killed enough flies to reduce infestations. The combination of the two surprisingly did not further reduce infestations, perhaps because flies were more attracted to the red spheres than to the bait spray, and were caught before they fed on the bait. When ammonia-baited spheres were absent, the flies apparently fed on the bait and suffered high mortality, resulting in reduced infestations. Red spheres did not eliminate infestations in one season, but they may increase the effectiveness of sprays by reducing populations, making it easier to eliminate populations the following. Trees were relatively small at 5-13.5 ft tall and 4.5-11.5 ft wide, and the 540 ml sprays resulted in very thorough coverage of the trees.

In 2004 in Woodland, there were significant reductions in larval infestations in apple when 6 red spheres were used, when 6 red spheres were combined with weekly GF-120 sprays, and when 1 yellow panel was combined with weekly GF-120 sprays, as compared with when single yellow traps alone were used (Table 3). Possibly results may have even been more dramatic had no yellow panels been placed in the control, for some female flies that would have oviposited were removed from the population in these trees. Because the test was conducted in isolated trees, larval infestations in the single yellow trap treatment may have reduced due to lack of many immigrating flies. Trees were relatively large at 12.5-20 ft tall and 12-20 ft wide, so coverage was not as high as in Puyallup. However, as in Puyallup, the results indicate that either baited red sphere traps or GF-120 can reduce larval infestations.

***Significance to the Industry and Potential Economic Benefits*:** The results are significant to the apple industry in that they show GF-120 can potentially be used to manage apple maggot in feral or backyard trees, which at present seem to be the greatest threat to commercial apple orchards. GF-120 is a safer alternative to the organophosphates used in the past to control the flies and thus is more desirable to use near residential areas. Results also show the potential of mass trapping to reduce larval infestations, suggesting baited red spheres may be useful in feral and backyard trees. By suppressing fly populations, the risks of flies spreading into commercial apple-growing areas and of these areas being placed under quarantine are reduced. When a fly is caught $\leq$ ½ mile from an apple orchard, the orchard is considered threatened. Apples from such an orchard need to be inspected and permits need to be issued for their movement. Fly establishment may also result in increased spray application costs.

**Table 1. Effects of GF-120 spray rates on numbers of apple maggot flies trapped on ammonium carbonate-baited yellow panels and larval infestations ± SE of apple in two habitat types in western Washington, 2004.**

| | Habitat 1: Unmanaged Apple Orchard with 15 feet spacing | | | | |
|---|---|---|---|---|---|
| | Mean No. Flies per Yellow Panel Trap | | | | |
| Date | 40% GF-120 75 ml (n = 4) | 40% GF-120 225 ml (n = 5) | 17% GF-120 180 ml (n = 5) | 17% GF-120 540 ml (n = 6) | Control (n = 4) |
| 21 June | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 28 June | 1.3 ± 1.0 | 0.8 ± 0.3 | 1.5 ± 0.4 | 2.2 ± 1.1 | 1.7 ± 0.9 |
| 6 July | 2.2 ± 1.1 | 2.5 ± 0.8 | 1.7 ± 0.6 | 2.3 ± 1.6 | 1.8 ± 1.5 |
| 14 July | 2.3 ± 0.7 | 2.7 ± 0.8 | 2.3 ± 0.6 | 2.5 ± 1.3 | 2.8 ± 1.5 |
| 20 July | 1.2 ± 0.8 | 3.7 ± 1.0 | 2.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 28 July | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 3 August | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 11 August | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Total Flies | 14.5 ± 5.1a | 20.8 ± 5.9a | 12.8 ± 3.1a | 15.7 ± 6.8a | 13.0 ± 5.1a |
| Larvae/Fruit | 0.010 ± 0.007a | 0.202 ± 0.168a | 0.008 ± 0.005a | 0.012 ± 0.007a | 0.032 ± 0.016a |

| | Habitat 2: Isolated Apple Trees in Residential Areas | | | | |
|---|---|---|---|---|---|
| | 40% GF-120 75 ml (n = 4) | 40% GF-120 225 ml (n = 5) | 17% GF-120 180 ml (n = 5) | 17% GF-120 540 ml (n = 6) | Control (n = 4) |
| Total Flies | 5.2 ± 2.0a | 7.6 ± 3.0a | 2.2 ± 0.8a | 7.2 ± 3.4a | 15.5 ± 8.0a |
| Larvae/Fruit | 0.028 ± 0.021ab | 0.006 ± 0.004a | 0.010 ± 0.006a | 0.000 ± 0.000a | 0.058 ± 0.023b |

Means followed the same latter within rows are not significantly different (ANOVA, $P > 0.05$).

**Table 2. Mean cumulative mortality ± SE of apple maggot flies from Puyallup, WA, exposed to residues of 40% GF-120 (1:1.5 dilution) aged for various days on 'Fuji' apple leaves in the field in Moxee, central WA, in the laboratory, 2004.**

| Test 1: 27 July to 5 August | | | | | |
|---|---|---|---|---|---|
| Days after | | Age of GF-120 Residues on Apple Leaves | | | |
| Exposure | Control | 0 Day | 3 Days | 7 Days | 14 Days |
| **Table 2, continued** | | | | | |
| 1 | 0.0 ± 0.0a | 36.7 ± 3.3b | 36.7 ± 6.7b | 30.0 ± 5.8bc | 20.0 ± 5.8c |
| 2 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 80.0 ± 11.6b | 26.7 ± 8.8d |
| 3 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 90.0 ± 10.0b | 26.7 ± 8.8c |
| 4 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 96.7 ± 3.3b | 26.7 ± 8.8c |
| 7 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 70.0 ± 5.8c |
| 8 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 70.0 ± 5.8c |
| 9 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 90.0 ± 5.8c |
| 10 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ±0.0b |

Test 2: 17 August to 10 September

| Days after | | | Age of GF-120 Residues on Apple Leaves | | |
|---|---|---|---|---|---|
| Exposure | Control | 0 Day | 3 Days | 7 Days | 14 Days |
| 1 | 0.0 ± 0.0a | 54.0 ± 8.1b | 22.0 ± 5.8c | 22.0 ± 3.7c | 2.0 ± 2.0a |
| 2 | 0.0 ± 0.0a | 64.0 ± 8.1b | 28.0 ± 2.0c | 32.0 ± 2.0c | 4.0 ± 2.4a |
| 3 | 0.0 ± 0.0a | 78.0 ± 4.9b | 52.0 ± 3.7c | 66.0 ± 5.1bc | 10.0 ± 7.8a |
| 4 | 0.0 ± 0.0a | 82.0 ± 5.8b | 66.0 ± 4.0b | 76.0 ± 6.8b | 12.0 ± 7.4c |
| 7 | 0.0 ± 0.0a | 100.0 ± 0.0b | 90.0 ± 4.5b | 94.0 ± 2.4b | 20.0 ± 2.6c |
| 8 | 0.0 ± 0.0a | 100.0 ± 0.0b | 98.0 ± 2.0b | 96.0 ± 2.4b | 26.0 ± 3.6c |
| 9 | 0.0 ± 0.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 96.0 ± 2.4b | 26.0 ± 3.6c |
| 10 | 2.0 ± 2.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 26.0 ± 3.6c |
| 11 | 2.0 ± 2.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 30.0 ± 3.0c |
| 14 | 6.0 ± 4.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 38.0 ± 2.4c |
| 15 | 8.0 ± 3.7a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 42.0 ± 1.6c |
| 16 | 8.0 ± 3.7a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 50.0 ± 3.0c |
| 17 | 10.0 ± 4.5a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 52.0 ± 2.4c |
| 18 | 18.0 ± 8.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 58.0 ±12.4c |
| 21 | 24.0 ± 7.5a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 58.0 ± 2.4c |
| 22 | 28.0 ± 8.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 62.0 ±14.6c |
| 23 | 38.0 ± 8.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 64.0 ±15.0c |
| 24 | 38.0 ± 8.0a | 100.0 ± 0.0b | 100.0 ± 0.0b | 100.0 ± 0.0b | 64.0 ± 5.0c |

Test 1: 3 replicates; Test 2: 5 replicates; 10 flies (5 males and 5 females) per replicate.
Means followed the same latter within rows are not significantly different (ANOVA, $P > 0.05$).

**Table 3. Effects of GF-120 sprays and red sphere and yellow panels traps on mean adult and larval apple maggot infestations of apple ± SE at two sites in western Washington in 2004.**

| | Puyallup (Pierce County) Mean No. Flies on Traps | | | |
|---|---|---|---|---|
| | | 6 Red Spheres + | | |
| Date | 6 Red Spheres | GF-120 | GF-120 | Control |
| 18 June | 8.8 ± 1.2 | 1.0 ± 1.0 | -------- | ------- |
| 25 June | 14.4 ± 2.5 | 8.6 ± 2.5 | -------- | ------- |
| 3 July | 22.4 ± 4.2 | 20.0 ± 3.7 | -------- | ------- |
| 9 July | 20.2 ± 3.8 | 28.0 ± 5.3 | -------- | ------- |
| 16 July | 36.2 ± 7.9 | 28.0 ± 4.4 | -------- | ------- |
| 23 July | 36.6 ± 6.3 | 27.2 ± 6.8 | -------- | ------- |
| 30 July | 31.0 ± 9.1 | 37.8 ± 10.1 | -------- | ------- |
| 9 August | 26.4 ± 5.6 | 18.0 ± 4.3 | -------- | ------- |
| 16 August | 16.2 ± 1.7 | 9.4 ± 4.8 | -------- | ------- |
| August | 7.4 ± 0.8 | 3.2 ± 1.8 | -------- | ------- |
| 3 September | 0.8 ± 0.6 | 0.2 ± 0.2 | -------- | ------- |
| 10 September | 0.0 ± 0.0 | 0.0 ± 0.0 | -------- | ------- |
| Total Flies | 220.4 ± 33.1a | 180.2 ± 33.2a | No flies removed | No flies removed |
| Larvae/Fruit | 0.059 ± 0.043a | 0.096 ± 0.034a | 0.011 ± 0.007a | 0.580 ± 0.144b |
| | | Woodland, WA (Clark County) | | |

| | | Mean No. Flies on Traps | | |
|---|---|---|---|---|
| | | 6 Red Spheres + | 1 Yellow Panel + | |
| Date | 6 Red Spheres | GF-120 | GF-120 | 1 Yellow Panel |
| 28 June | 56.0 ± 42.1 | 20.2 ± 9.6 | 5.2 ± 2.9 | 10.7 ± 5.0 |
| 6 July | 18.2 ± 10.2 | 10.0 ± 2.4 | 2.2 ± 0.8 | 10.3 ± 1.2 |
| 12 July | 12.0 ± 7.2 | 4.0 ± 1.3 | 1.2 ± 1.0 | 4.7 ± 0.3 |
| 19 July | 5.5 ± 1.7 | 1.8 ± 1.2 | 1.2 ± 1.0 | 1.3 ± 0.9 |
| 26 July | 6.0 ± 2.2 | 4.5 ± 1.0 | 1.2 ± 0.2 | 3.0 ± 1.7 |
| 2 August | 7.5 ± 3.5 | 1.2 ± 0.5 | 0.8 ± 0.2 | 0.0 ± 0.0 |
| 9 August | 7.8 ± 3.5 | 2.8 ± 0.2 | 0.2 ± 0.2 | 1.7 ± 0.9 |
| Total Flies | 113.5 ± 56.8a | 44.5 ± 15.0a | 12.2 ± 3.9a | 41.7 ± 9.9a |
| Larvae/Fruit | 0.17 ± 0.15a | 0.02 ± 0.01a | 0.03 ± 0.01a | 1.80 ± 0.87b |

In Puyallup, 5 replicates of treatments and the control.
In Woodland, 4 replicates/treatment and 3 replicates of control; all traps were baited with 10 g ammonium carbonate lures.
Means followed the same latter within rows are not significantly different (ANOVA, $P > 0.05$).

**Budget:**

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

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

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

**CONTINUING REPORT** **Year 2/3**
WTFREC Project #AE-03-330 WSU Project #13C-3643-3386

**Project title:** Biology, migration, and management of Western flower thrips in apple Orchards

**PI:** Elizabeth H. Beers, Entomologist
**Organization:** WSU Tree Fruit Research and Extension Center, Wenatchee, WA
**Co-PIs and affiliations:**
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WSU Tree Fruit Research and Extension Center, Wenatchee, WA
David Horton, Research Entomologist, USDA-ARS, Wapato, WA
Gene Miliczky, Research Associate, USDA-ARS, Wapato, WA
Vince Jones, Associate Entomologist
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John Dunley, Associate Entomologist
WSU Tree Fruit Research and Extension Center, Wenatchee, WA
Rich Zack, Assistant Entomologist
WSU Dept. of Entomology, Pullman, WA

**Contract administrator:** Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

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

**Significant findings:**

1. **Populations of thrips in apple flowers were highest on the orchard border next to sagebrush steppe, indicating significant migration from dry uncultivated areas into the orchard. Results from 2004 indicate a large decrease in population within 30 feet of the border. The relationship is reflected in thrips damage to fruit.**
2. **Milk established a detectable mark on 52% of thrips in sagebrush steppe habitat located next to an orchard.**
3. **In the second year of comparisons between thrips populations in weedy and herbicide-treated blocks, very little reduction in thrips was measured. Only one site had significantly less fruit injury in the herbicide-treated block.**
4. **Sampling revealed that very few thrips eggs were laid in fruit during bloom. Most eggs were laid after petal fall. A Carzol timing trial indicated that sprays were most effective (least amount of damage) a week after petal fall, after which they were ineffective.**

**Methods:**

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

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

Eight orchards with a history of thrips damage were selected from Monse to Moxee in the central fruit-growing region in Washington. Each orchard had an edge bordered by native vegetation. In 2004, two orchards were located in Bridgeport, two in Moxee, and one each in Brewster, Monse, Durey, and Cowiche, WA. Samples were taken at six distances: border row (0), 30, 60, 100, 200, and 300 feet into the orchard from the native habitat at pink, open king bloom, full bloom, and at 100% petal fall. Twenty-five flower clusters at the appropriate phenological stage were collected at each location and time. Up to 25 trees were sampled in a broad band or row at each distance. Plant samples were washed in soapy water and thrips were strained out. All insect specimens were stored in 50% ethanol. Both adult and immature thrips were counted. Ten sticky cards were placed in the native vegetation during bloom to measure the relative population pressure of thrips available to migrate. Data were analyzed as repeated measures in an ANOVA with each distance being a repeated measure in each orchard. If the distance effect was significant at $P < 0.1$, paired contrasts of adjacent distances (such as edge vs. 100 feet) were examined for significant differences. Contrasts were used to determine where the populations ceased to change significantly.

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

**This experiment was conducted at Smith Tract 10, a 0.7-acre block of mature 'Granny Smith' near Orondo, WA. The site is bordered by an extensive area of native vegetation on the east and north sides. On the south side lies a road and a cherry orchard, with an apple orchard on the west side. A sample area of 0.5 acres of native habitat was marked adjacent to the orchard, extending approximately 50 m from the orchard border.**

**On 9 April, the sample area in the native vegetation was sprayed to drip (handgun) with a 17.5% milk solution in water. The orchard floor (drive row and herbicide strips) was sprayed with a solution of 10% egg whites in water. This application was made at 286 gpa using a small boom sprayer. After the material had dried, the flowers of four arrowleaf balsamroot plants in the native vegetation and four dandelion plants in the orchard were immediately frozen with dry ice to collect thrips. Negative control samples were collected from balsamroot flowers at least 50 m from the sprayed vegetation. A sample of the mixture in the tank was also taken. The treatments were repeated on 13 April. The egg white application was unchanged, but the milk concentration was increased to 32.5%. After the material had dried, tank mix samples and thrips were collected as before. During spray applications and sample collections, protective clothing was worn to prevent any cross-contamination of the plots.**

**Samples were taken from both the orchard and the native vegetation on 16 April, when the orchard was in full bloom. Balsamroot flowers were collected from the native vegetation as before. One hundred apple flowers were collected from each of six locations within the orchard, and dandelions were collected from the same locations. As before, protective clothing was used to prevent cross-contamination and all samples were immediately frozen with dry ice. Flowers were examined under a microscope, and thrips were collected on a toothpick with a tiny drop of sticky material. Each insect was placed in the bottom of a 1.5 ml microcentrifuge vial. Each insect will be tested for the presence of milk and egg white proteins with ELISA.**

**Starting in September, all the sagebrush and snow buckwheat plants in the native habitat area were treated with a 500 ppm rubidium chloride solution at 8 gpa. Six applications (3 to 7 days apart) were made until signs of bloom had begun. Plant samples were taken before and after each treatment and were tested for presence of rubidium. After bloom was near completion, in mid-October, adult and second instar thrips were collected from sagebrush flowers. These will be tested for rubidium to measure the success of the marking technique. The rubidium mark will track the migration of overwintered adults from the sagebrush areas n 2005.**

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

**Four orchards were selected with the following cultivars and locations: 'Braeburn' in Quincy, Pateros and Brewster and 'Granny Smith' in Bridgeport. Each orchard was approximately 5-10 acres. One-half of each block received regular herbicide treatments as well as spot treatments to reduce broadleaf weeds over time. The other half received herbicides only in the herbicide strips beneath the trees (the growers' usual management regime). This was the second year of the management regimes. The plots will be managed and sampled for an additional year to measure the reduction in WFT over time. At approximately 3-5 m intervals, ten 1-$m^2$ areas were randomly selected and marked in the drive row of each treatment block. Within these areas, dandelion plants were counted once per month beginning in April. The number of dandelions in flower was also recorded. Each month beginning in April, dandelion plants were collected at random locations within the drive row. Four plants without flowers and four plants with flowers (if available) were selected. In May during full bloom and before insecticides were applied, 25 open apple blossoms were selected from each of eight trees within the drive row. In June, July and August 10 buds were collected from each of eight trees. Plant tissue samples were washed with soapy water to dislodge adult and immature thrips. Specimens were slide-mounted for identification of *Frankliniella occidentalis* (WFT).**

**3. Susceptibility of apple bloom stages to WFT damage.**

***Thrips exclusion***. Treatments consisted of various timings of Carzol combined with exclusion cages. Starting at tight cluster (ca. 2 April), exclusion cages measuring 45×10 cm were placed on 100 branches of 'Delicious' trees in block 29S, TFREC. Each treatment had ten replicates (cages). Each selected branch had 7-10 flower clusters. Treatments were on approximately half the trees in the block due to scattered bloom. Treatments and replicationss were assigned randomly to each branch (CRD). Cages were slipped over the branches and left in place, but open, until they were needed. In the first treatment, the branches were sprayed with the equivalent of 1 lb of Carzol per acre at an equivalent of 200 gpa (0.5 lb/100 gal). Material was mixed in a 16-oz spray bottle and applied until runoff. The cages were tied shut and left in place until the next treatment, about 3-4 days in the early treatments and 7 days in the later treatments. Treatment times were at tight cluster (5 April), pink (9 April), king bloom (13 April), full bloom (16 April), petal fall, or when fruit measured 2.6 mm in diameter (20 April), then when fruit measured 2.9 mm (23 April), 5.6 mm (27 April), 10.9 mm, (4 May), and 15.8 mm (11 May). One set of cages remained on the branches during the entire time and was sprayed every 3-4 days, and a set of 10 sampled branches had no cage or Carzol treatments. All king bloom fruit was harvested on 24 and 25 May.

***Thrips phenology***. At periodic intervals corresponding to the developmental stages of the apple bloom, 100 blossom clusters, or after petal fall, 100 king fruit were sampled. One sample per tree was taken on trees scattered throughout the block that were not being used for any other experiment. Two samples were taken at tight cluster (2 April, 6 April), then a sample was taken at pink (9 April), king bloom (13 April), full bloom (14 April), petal fall, or at 2.6 mm diameter (19 April), at 2.9 mm (23 April), 5.6 mm (27 April), 10.9 mm (4 May), and 15.8 mm (11 May). Plant tissues were washed in soapy water which was then sieved to collect insects. In addition, bean pods were exposed to adult thrips to test the staining technique. The plant tissue was trimmed so that only the king bloom fruitlet or fruit remained, and this was stained to reveal thrips eggs. Direct observation of the pods or apple fruit was found to be unsatisfactory. An acid fuschin staining technique was chosen instead. Some modifications were made to adapt the technique to apple fruit. Samples in the clearing solution were heated in a double boiler under a fume hood for one hour to soften the tissue. After clearing, fruit were trimmed or sliced so that they were 0.5 mm thick, including the skin. Any remaining tissue

from the inside of the fruit that did not include skin was discarded. The skin was placed between two glass microscope slides and pressed flat. The thin tissue was studied under a dissecting scope under transillumination to reveal the (darker) eggs in the plant tissue.

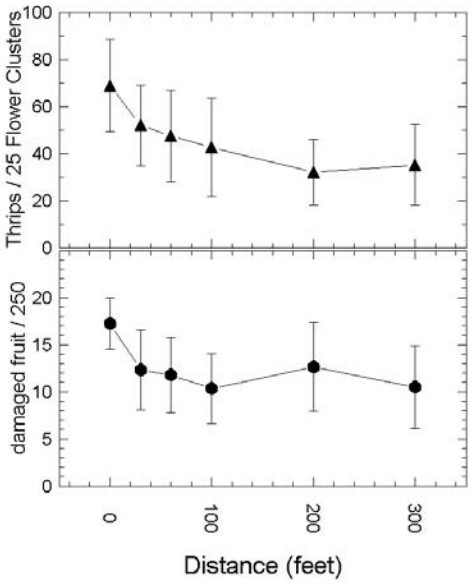


**Fig. 1.** (Top) Mean thrips collected in samples from pink to petal fall in eight orchards in 2004. (Bottom) Number of fruit damaged by thrips out of 250

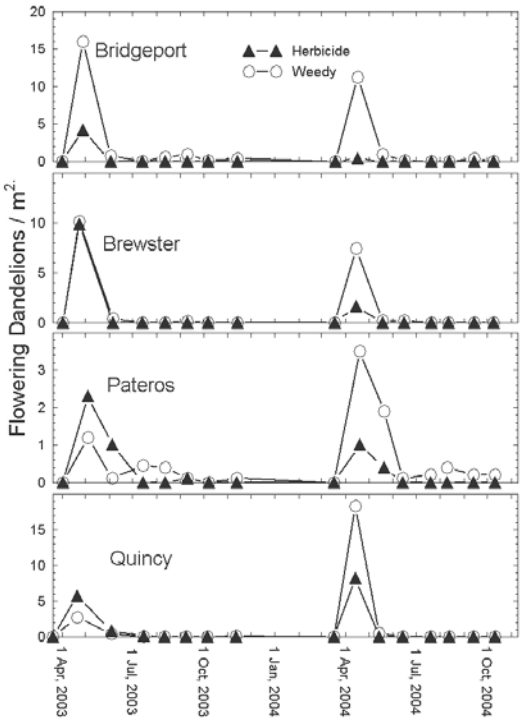


**Fig 2.** Mean dandelion populations in paired herbicide-treated (herbicide) and non-treated (weedy) blocks within four orchards, 2003-2004.

**Results:**

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

Thrips populations changed significantly with greater distance away from the native habitat in samples taken at full bloom ($P$=0.0007) and in summed samples ($P$=0.007). Significant decreases occurred within 30 feet of the edge of the orchard at full bloom ($P$=0.035) and in summed samples ($P$=0.013). The same trend was found in fruit injury, which changed significantly as distance from the orchard increased ($P$=0.013). A significant decrease occurred within 30 feet of the border ($P$=0.018).

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

**At the time of this report, all vegetation samples have been processed and approximately 1,700 thrips have been isolated and stored in preparation for ELISA. Thrips from balsamroot samples taken the day of the first and second sprays have been tested for the presence of milk protein. After the first spray 38.5% of thrips were found marked, and after the second spray the percentage had increased to 51.7%. The percentage of thrips with a positive mark for milk protein will be mapped at each sample site within the orchard to see if a population gradient exists near the orchard border. The percentage of thrips with a positive mark for egg white protein will be calculated to see if thrips are living on the orchard floor before flying up to open apple flowers. This experiment will be further refined and repeated next year at the same site.**

**2. Management of resident WFT populations by reducing alternative hosts within the orchard.** Dandelions and other broad-leaf weeds have proven difficult to control. The only effective compound available is 2,4-D, which can be toxic to apple trees. At orchard rates, plants take 4-6 weeks to die and often recover. Seeds already in the orchard will continue to germinate. One site had fewer

dandelions in flower in the herbicide blocks in the first year (Fig. 2). Other sites began with little difference or more dandelions in the herbicide block. By the spring of 2004, all sites had fewer flowering dandelions in the treated block. No significant differences in thrips populations have been found in the apple trees between the two treatments in either year. Thrips fruit injury, while not different at any site in 2003, was significantly different in the Bridgeport orchard in 2004 (based on binomial 95% CI). The herbicide-treated block had 2.0% fruit damage and the weedy block had 5.8%.

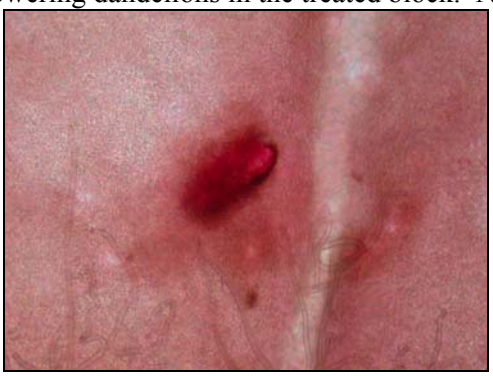

**Fig. 3.** *F. occidentalis* egg in skin of apple fruit.

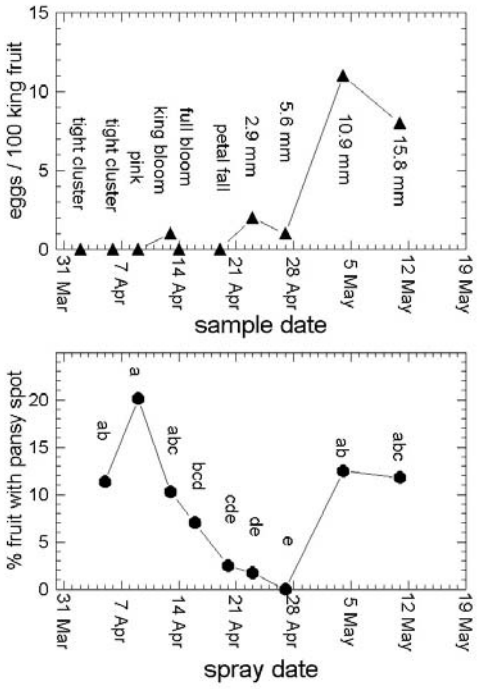


**Fig. 4.** (Top) Eggs in king fruit found per 100-fruit sample, and (Bottom) fruit injury resulting from Carzol applications at nine timings.

### 3. Susceptibility of apple bloom stages to WFT damage.

The stained eggs of Western flower thrips were visible just under the apple skin (Fig. 3). Thus the staining procedure provided a successful method for detecting oviposition. Adult thrips were found in low numbers from tight cluster on; however, they increased in the blossom clusters as petals opened. There was a marked increase from king bloom to full bloom. Adults remained abundant until petal fall, then decreased rapidly. A low population remained on fruit clusters up to 15.8 mm, or 21 days after petal fall. Although eggs must have been laid on or near flower clusters well before bloom, judging from the presence of immatures at pink very few eggs were laid on king fruit during bloom. The majority were laid on king fruit shortly before 10.9 mm, or 14 days after petal fall. Immatures peaked just after petal fall, indicating much egg laying during bloom on unknown tissue. The timing of adults and immatures on flower or fruit clusters had little relationship to the timing of egg detection on king fruit. Carzol applications made after bloom were more effective than those before or during bloom (Fig 4.). Applications made when fruit was 10.9 mm or larger (2 weeks or more after petal fall) were less effective than earlier applications. This corresponded to an increase in oviposition that occurred some time between 5.5 and 10.9 mm fruit size (1-2 weeks after petal fall).

**Budget:**

| | |
|---|---|
| **Project title:** | Biology, migration, and management of Western flower thrips in apple orchards |
| **PI:** | Elizabeth H. Beers |
| **Project duration:** | 2003 through 2005 |
| **Current year:** | 2005 |
| **Project total (3 years):** | $116,552 |
| **Current year request:** | $44,663 |

| Item | Year 1 (2003) | Year 2 (2004) | **Year 3 (2005)** |
|---|---|---|---|
| Salaries (0.6 FTE, 0.0625 FTE)[1] | $21,300 | $22,378 | **$25,349** |
| Benefits [2] | 7,029 | 7,609 | **9,220** |
| Wages [3] | 3,900 | 900 | **5,400** |
| Benefits (11%-yr 3) | 429 | 144 | **594** |
| Equipment [4] | 600 | 600 | **600** |
| Supplies [5] | 1,500 | 1,500 | **1,500** |
| Travel [6] | 2,000 | 2,000 | **2,000** |
| Miscellaneous | - | - | **-** |
| Total | $36,758 | $35,131 | **$44,663** |

[1]Salaries: Steve Cockfield (0.6 FTE); Randy Talley (0.0625 FTE).

[2]Benefits - year 1 benefit rate 33%; year 2 benefit rate 34%; year 3 benefit rates: Cockfield 36%, R. Talley 41%. Increase in rate is due to the increase in health insurance benefit contribution WSU makes on behalf of the employee.

[3]Time-slip wages.

[4]Computer.

[5]Cell phone charges are allowed on this grant.

[6]Local travel to plots for sampling.

**CONTINUING PROJECT REPORT** **YEAR 2/3**

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

**Project Title**: Biological control of leafrollers through habitat modification

**PI:** Tom Unruh
**Organization**: USDA-ARS
**Address** 5230 Konnowac Pass Rd.
Wapato, WA 98951
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**CO-PIs** Jay Brunner, WSU-TFREC, Wenatchee
Dave Horton, USDA-ARS, Wapato

**OBJECTIVES:**

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

**SIGNIFICANT FINDINGS**

- **High parasitism by C. florus in spring was again seen near gardens at several orchards**
- **Sprays reduced Strawberry leafroller, Ancylis, and thus C. florus, at some gardens**
- **Ancylis, and thus C. florus, were reduced at some gardens due to predation**

**Objective1.** No gardens were planted or expanded in 2004. In common garden experiments begun in 2002 and assessed in 2004, locally collected Fragraria virginiana (from Trout Lake) was found to rank highest in cover and spreading followed closely by several accession of the F. chiloensis. This was surprising becaaus I wild habitats where collected this form is a very spotty under fir and pine tree canopies. Additional evaluations of these for harboring Ancylis will be complete this winter. At our other gardens, started with much lower abundance of strawberry, strawberry appears to be slowly disappearing because of the combined pressures of encroaching over-story of roses and weed growth. At all gardens roses are prospering once they make it through year 1.

Several sites showed reduced or inadequate abundance of the strawberry leafroller and therefore *C. florus*, thus several sites received supplemental infestations of A. comptana in August or September as identified in Table 1. We believe this low Ancylis stems from poor establishment when gardens were planted. See objective 4 for discussion of abundance of Ancylis populations. Two additional gardens are planned for early in 2005.

Table 1. 34 gardens: Ancylis found (SLR) and C.florus parasitism; Ancylis added (S); //////=no data

| Garden Name | Planted | Location | Management | SLR | SPRING C. florus | SUMMER C. florus | S |
|---|---|---|---|---|---|---|---|
| arwhd1 | 2002 | Brewster | conventional | NO | //////// | NONE | y |
| arwhd2 | 2002 | north/Brewster | conventional | NO | //////// | //////// | Y |
| arwhd3 | 2002 | north/Brewster | conventional | NO | //////// | //////// | Y |
| arwhd4 | 2002 | north/Brewster | conventional | NO | //////// | //////// | Y |
| arwhdW | wild | north/Brewster | conventional | NO | //////// | //////// | |
| brooks1 | 2001 | Wapato | organic | YES | LOW | LOW | |
| brooks2 | 2001 | Wapato | organic | YES | LOW | MEDIUM | |
| brooks3 | 2001 | Wapato | organic | YES | LOW | MEDIUM | |
| Dalles1 | 2002 | OR | conventional | NO | //////// | NONE | Y |
| Dalles2 | 2002 | OR | conventional | NO | NONE | NONE | y |
| dela | 2002 | Zillah | conventional | YES | LOW | LOW | y |
| djm | 2003 | George | conventional | YES | NONE | NONE | y |
| MF1 | 2002 | OR | conventional | NO | NONE | NONE | y |
| MF2 | 2002 | OR | organic | NO | NONE | NONE | y |
| Mox.a | 2002 | Moxee | experimental | YES | //////// | MEDIUM | |
| Mox.chr | 2002 | Moxee | experimental | YES | //////// | //////// | |
| Mox.f | 2002 | Moxee | experimental | YES | //////// | HIGH | |
| Mox.org | 2002 | Moxee | experimental | YES | //////// | //////// | |
| Mox.w | 2002 | Moxee | experimental | YES | //////// | HIGH | |
| ostenson | 2002 | George | organic | YES | LOW | NONE | y |
| plathbins | 2003 | Parker | conventional | NO | //////// | //////// | |
| plathbo | 2002 | Parker | conventional | YES | MEDIUM | HIGH | |
| plathlp | 2000 | Parker | conventional | YES | LOW | HIGH | |
| plathgu | 2003 | Parker | conventional | NO | //////// | NONE | |
| plathrv | 2002 | Parker | conventional | YES | HIGH | HIGH | |
| plathscr | 2002 | Parker | conventional | NO | ///////// | //////// | |
| plathup | 2000 | Parker | conventional | YES | HIGH | HIGH | |
| sf1 | 2002 | Vernita | conventional | YES | ///////// | HIGH | |
| sf2 | 2002 | Vernita | conventional | YES | ///////// | MEDIUM | |
| sf3-50 | 2002 | Vernita | conventional | ? | ///////// | ///////// | y |
| stemilt1 | 2002 | Wenatchee | conventional | NO | ///////// | ///////// | Y |
| stemilt2 | 2002 | Wenatchee | organic | NO | NONE | NONE | y |
| wvc | 2002 | Wenatchee | organic | YES | NONE | HIGH | |
| yarl | 2000 | Wapato | experimental | YES | HIGH | HIGH | |

**Objective 2.**
Parasitism was measured at 21 sites one or more times in 2004 season. Attempts to measure parasitism in Arrowhead (Okanagon) and the Dalles generally failed because of very poor recovery of deployed insects. In Arrowhead we ascribe this to a combination of overhead sprinklers and pesticide use. At the Dalles we suspect that our lab colony of OBLR doesn't "like" cherries and to pesticide use. We are currently still collecting pesticide data. Examination of Table 1 suggests that parasitism by C. florus in the spring or summer was dependent on the presence of Ancylis in adjacent gardens. In the 5 cases where no parasitism by C. florus was observed, Ancylis was not found. However, presence of Ancylis did not ensure observable parasitism in both generations, nor did it insure high parasitism. Usually we associated high parasitism with older gardens (Plath sites, YARL) and organic (WVC and Moxee). Despite these patterns, parasitism was more variable in 2004 than in 2003. Specifically, at several sites there was a slump in summer. We suspect insecticide disruption of our assessments or of parasite activity.

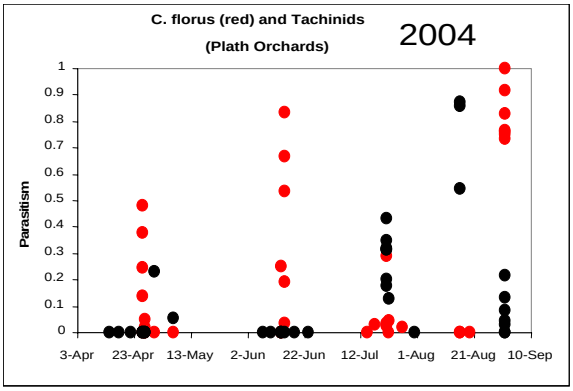



Figure 1. Parasitism near various gardens at Plath orchards in Parker on 5 sample dates. Note slump of C. florus parasitism in July-August samples at most sites. Codling moth sprays were applied in most blocks (Success).

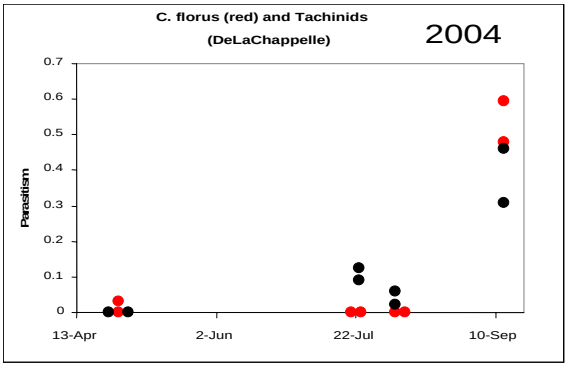



Figure 2 shows and organic orchard with lower C. florus activity in 2004 compared to 2003. Significant use of Entrust for codling moth was used.

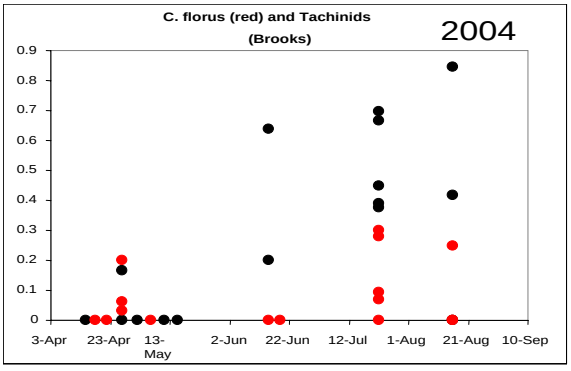



Figure 3 shows pattern when population is low in garden. Site also used direct sprays to control leafrollers. Pattern is consistent with wasps coming into orchard in fall from surrounding habitats and not from gardens.

Some disruption of parasitoid activity was associated with pesticide (Success) applications in some blocks in 2004 as also seen in 2003. The following figures (1-3) show that parasitism continues to show a pattern of increase through the season, but the important summer generation parasitism slumped at most sites. This was not observed in any previous year. Finally, reduced Ancylis populations at well established gardens (see objective 3) may also account for this slump. We know in areas where C. florus is well established (Wapato, Parker, Yakima) that fall parasitism is always high, even in the absence of gardens. It is spring (April-May) and summer (July-August) parasitism that is critical

**Objective 3.** Seasonal pheonolgy of Ancylis was not critically monitored in 2004 because of time spent on assessments of parasitism. However, midsummer samples at 4 gardens indicated much lower Ancylis levels than expected and compared to previous years. Winter evaluations of overwintering density will be conducted inJanuary and February. Observations made in 2004 also suggest there may be significant predation of leafrollers in gardens by the hornet Vespula. Studies in 2005 will focus on this aspect.

Objective 4. Web site is completed and should be online by time of meeting. No field days were held.

**Objectives for 2005:**

1. **Conduct parasitism assessments at 20 orchard sites 4 times during the growing season and if leafroller abundance is significant conduct fruit damage assessment in transects from gardens**

2. **Plant 1 – 2 new gardens in organic orchards (conventional orchards are well represented)**

3. **Conduct biweekly samples will be collected from gardens to assess phenology of-, parasitism rate of-, and species parasitizing-, *Ancylis* in roses and strawberries. .**

4. **Redouble efforts to host local field days that demonstrate the how to plant a garden and what to look for in the garden to know it is working for you. Continue updating web site.**

**BUDGET**

**Proposed Project Duration:** 3 years (2003-2005)

**Current year request** **2004: $51,700**

**Budget:**
Following a failure in 2003, we succeeded in getting a competitive grant (Western SARE) to continue garden studies for 2005-2007. 2005 represents the final year of the WTFRC and the IFAFS funding; however, when these funds are coupled with SARE the project can support 2 technicians to continue and extend this effort through 2007. The effort is labor intensive.

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

[1]Salary represents 1.7 GS-5 Technician equivalents

**CONTINUING PROJECT REPORT** **YEAR 1/2**

**Project Title**: Optimizing the use of the codling moth granulovirus.
**PI:** Lawrence A. Lacey[1]
**Co-PI:** Steven Arthurs[1]
**Cooperator:** Robert Behle[2], Rob Fritts, Jr.[3]
**ORGANIZATIONS:** [1]USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA, [2]USDA-ARS, Peoria, IL, [3]Certis USA, Clovis, CA
**Contract Administrator:** Janet Tsukahira

**OBJECTIVES - 2005**

1. Investigate the potential of several adjuvants for protecting CpGV from solar degradation using laboratory bioassays.
2. Select the most promising adjuvants for field testing in 2005.
3. Continue to assess the shelf life of commercial formulations at various temperatures.

**SIGNIFICANT FINDINGS – 2004**

- In replicated single tree plots, season-long treatments of CpGV at 3 rates (1, 3 and 6 oz acre) and 3 application intervals (7, 10 and 14 days) resulted in significantly fewer deep entries and surviving larvae but did not reduce the proportion of fruit damaged by codling moth.
- There was a statistical trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval.
- In replicated ½ acre plots, CpGV provided > 90% larval mortality at 1, 2 and 3 oz/acre, but was not as effective as Guthion in protecting fruit.
- The larvicidal activity of three commercial CpGV formulations (Carpovirusine, Cyd-X and Virosoft) was maintained for more than 88 weeks under storage conditions of 36˚F. Cyd-X and Virosoft stored well at 77˚F for more than 80 weeks and at 95˚F for 24 and 44 weeks respectively.
- The efficacies of 3 commercial CpGV formulations were significantly reduced (52-77%) by exposure to UV light (10,800 KJ/$m^2$ in a solar simulator).
- Bioassay procedures to screen adjuvants providing possible UV protection of CpGV formulations were developed.

**METHODS**

*UV protection studies, laboratory screening (Objective 1).*

Because exposure to solar radiation limits the activity of CpGV in the Pacific Northwest, we established a bioassay system in the laboratory to assess various adjuvants for UV protection (sunscreens). In the procedure apples are sterilized, halved and the open end sealed using molten wax, aluminum foil and glue in preparation for virus treatments. Apples will be treated in a DeVries spray cabinet which is calibrated to deliver specific quantities of experimental virus formulations (standard and high dilution) and then subsequently exposed to a controlled dose of UV and other wavelengths of light equivalent to 10,800 KJ/$m^2$ in a solar simulator (Atlas) for 4 hours. Treated and control apples are then challenged with neonate codling moth larvae from a lab colony. Resulting fruit damage and larval mortality are measured in order to compare the activity of the virus treatments and hence quantify the most effective sunscreens for CpGV.

In addition to combining virus with adjuvants, microencapsulation of virus with UV protectants such as lignan will be undertaken in cooperation with the USDA-ARS in Peoria, IL. Experimental formulations will be tested in the laboratory first using the solar simulator as described above and the

larval challenge method described by Lacey et al. (2004). First tests will be conducted in single tree plots (10 replicate trees per formulation) as described below.

*Assess full-season virus programs adopting different formulation components in an experimental orchard (Objective 2).*

This study will be conducted at the USDA experimental orchard near Moxee, WA. Virus applications will be made to individual trees (Red Chief) using a Stihl SR420 backpack airblast sprayer with a large tarpaulin and a one-tree buffer used to confine treatments. Virus treatments will comprise (Cyd-X, Certis, USA) together with different formulation additives selected as most promising from the laboratory studies applied in a complete randomized block design. Virus rates will cover the range labeled for use with spray intervals based on the persistence of treatments observed in 2003 and 2004 (Arthurs and Lacey, 2004).

For each treatment, ten randomly selected trees will be sprayed at a 100 gal./acre plus Nufilm17 at 8oz/acre. Control trees will be sprayed with Nufilm17 plus water. Initial virus treatments will be made at 5% egg hatch and continued until ≈ 95% (Beers et al. 1993). CM injury will be assessed from 50 fruit per tree at the end of the first generation. Damaged fruit will be removed to the laboratory to assess both larval mortality and proportion of deep entries (> ¼ inch depth). Cardboard bands will be placed around trees to capture surviving larvae. The process will be repeated for the second larval generation. This will allow us to compare the performance of the different formulation under different climatic conditions (early season and late season).

*Storage studies (Objective 3)*

The Cyd-X, Carpovirusine and Virosoft formulations are being stored long-term at 2, 25 and 35˚C (35-95˚F). Starting summer 2003 the potencies of the viruses have been regularly assessed by bioassaying a low dilution ($10^{-3}$) and a high dilution ($10^{-5}$) of the products against neonate larvae using the procedures described in Lacey et al. (2002).

**RESULTS AND DISCUSSION**

*Optimal spray strategies - study 1.*

This study compared full-season virus programs adopting different application rates and frequencies of virus (Cyd-X, Certis USA) in single tree plots in an experimental orchard. Figure 1 shows fruit injury, proportion of deep entries and larval mortality at harvest for trees treated with different treatments (data shown at harvest). Virus applications did not reduce fruit damaged by CM, but the majority of damage was in the form of shallow stings (< ¼") and larval mortality was high (>80% in all treatments). There was a statistical trend of fewer deep entries and higher larval mortality rates with increasing rate of CpGV and shorter application interval. Rates of larval mortality were supported by the number of larvae captured in tree bands (data not shown).

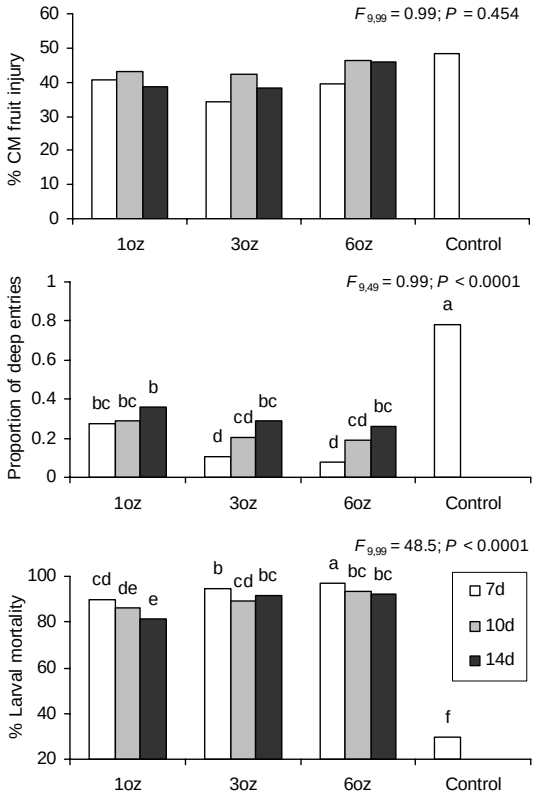



Figure.1. Fruit damage, deep entries and CM mortality at harvest following different treatments of Cyd-X in individual tree plots. (Data shown for Delicious, Moxee experimental orchard). Letters indicate Fishers LSD at $P < 0.05$.

*Optimal spray strategies - study 2.*

This study compared different rates of Cyd-X in a 21 acre commercial Delicious block. Figure 2 compares fruit injury, larval mortality and moth activity following 6 weekly applications of Cyd-X together with Guthion and untreated areas. There was less CM damage in virus plots compared with untreated areas, but more compared with Guthion-treated areas. Most damage was observed higher in the canopy (data not shown). Rates of CM mortality in virus-treated plots were similar to those observed in individual trees sprayed with equivalent rates of virus in the previous study. Data from interception traps showed fewer moths in virus-treated and Guthion-treated plots compared with untreated areas. Despite this, the virus study was terminated before harvest when fruit damage approached 10%. The heavy infestation (pheromone-baited traps averaged 70 moths/week) and untreated areas may have contributed to the high damage level.

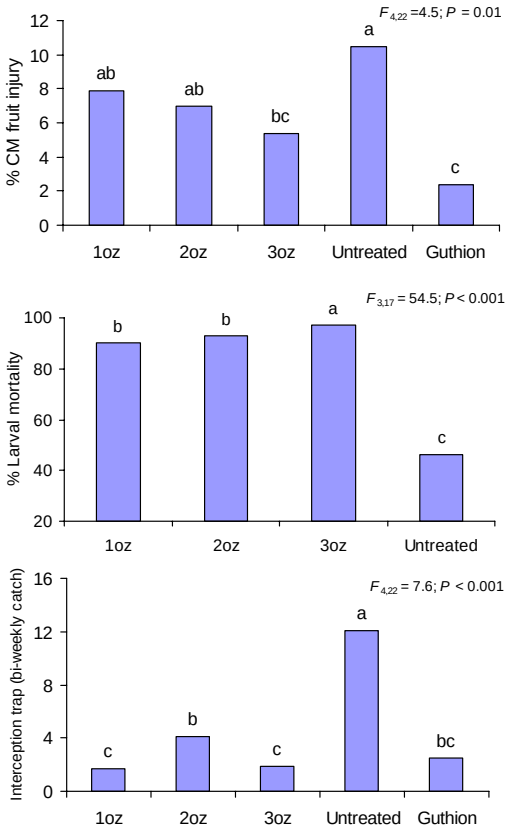



Figure 2. Fruit damage, CM mortality and interception trap catches following different treatments of Cyd-X in ½ acre blocks in a 21 acre commercial orchard (data for 1st generation). Letters indicate Fishers LSD at $P < 0.05$.

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

*Storage studies*

The larvicidal activity of three commercial CpGV formulations (Carpovirusine, Cyd-X and Virosoft) was maintained for more than 88 weeks under storage conditions of 36˚F. Cyd-X and Virosoft stored well at 77˚F for more than 80 weeks and at 95˚F for 24 and 44 weeks, respectively (Table 1). The bioassays will continue into 2005.

*UV protection studies*

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

Table 1. Storage of codling moth granulovirus formulations at 36, 77 and 95˚F. Bioassays of $10^{-5}$ dilutions of virus on artificial medium were conducted against neonate larvae using methods described by Lacey et al. (2002).

| | Weeks in storage after which significant loss of activity occurs | | |
|---|---|---|---|
| | 36˚F | 77˚F | 95˚F |
| Carpovirusine | 80+ | 8-12 | 4-8 |
| Virosoft | 80+ | 80+ | 44-48 |
| Cyd-X | 88+ | 88+ | 24-28 |

+ end point not yet determined

Table 2. Mean CM mortality on apples treated with standard rate of CpGV (1000-fold dilution of 3 commercial formulations) and exposed to a 10,800 KJ/$m^2$ simulated sunlight.

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

**PROPOSED SCHEDULE OF ACCOMPLISHMENTS**

Optimal use strategies for CpGV (application rate and spray frequency) were evaluated in 2004 (year 1). For 2005, we propose to focus more on the formulation components of the virus. There is considerable evidence that a number of adjuvants may improve the efficacy of CpGV through enhanced environmental persistence and larval uptake. Laboratory studies (January through May) will identify the most promising sunscreens using the procedures outlined above. Those with good potential will be compared in field tests against natural CM infestations at the Moxee experimental farm in (May through October) using larval challenge of treated fruit (Lacey et al. 2004). Storage studies will continue during 2005 until larvicidal activities of the formulations decline to an unacceptable level.

**REFERENCES**


Arthurs, S.P. and Lacey, L.A. 2004. Field evaluation of commercial formulations of the codling moth granulovirus: persistence of activity and success of seasonal applications against natural infestations of codling moth in Pacific Northwest apple orchards. Biol. Contr. 31: 388-397.

Beers, E.H., Brunner, J.F., Willett, M.J. and Warner, G.M. 1993. "Orchard Pest Management: A Resource Book for the Pacific Northwest" Good Fruit Grower, Yakima, WA.

Lacey, L. A., S. P. Arthurs, A. Knight, K. Becker, and H. Headrick. 2004. Efficacy of codling moth granulovirus: effect of adjuvants on persistence of activity and comparison with other larvicides in a Pacific Northwest apple orchard. J. Entomol. Sci. 39: 500-513.

Lacey, L.A., Vail, P.V. and Hoffmann, D.F. 2002. Comparative activity of baculoviruses against the codling moth *Cydia pomonella* and three other totricid pests of tree fruit. J. Invertebr. Pathol. 80: 64-68.

**BUDGET**

**Title:** Optimizing the use of the codling moth (CM) granulovirus: effects of application rate and frequency of spraying on control of codling moth larvae in experimental and commercial orchards with supportive laboratory studies on product storage and formulation.

**PI:** Lawrence A. Lacey

**Project duration**: 2004-2005 (2 years)

**Current year:** 2005

**Project total:** $67,000

**Current year request** $33,500

| Current year request: | year 1 (2004) | year 2 (2005) |
|---|---|---|
| Salaries and wages (includes benefits) | | |
| Salary, technician, partial support for GS-5 | $25,000 | $25,000 |
| Wages, summer help, GS-3, 2 FTE (3 mos.) | $7,000 | $7,000 |
| chemicals, plasticware, misc. materials | $1,500 | $1,500 |
| **Total** | $33,500 | $33,500 |

**CONTINUING PROJECT REPORT** **YEAR 2/3**

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

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

**Objectives**:

Project objectives:

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

2005 Objectives/goals.

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

**Significant Findings**:

1. Beta farnesene attracted males throughout the season, and attracted females in the second flight.
2. A new GC-EAD study of wild codling moth antennal responses to diluted apple volatiles revealed significant neurophysiological responses to a small number of compounds.
3. Field testing of those EAD active volatiles demonstrated the attractiveness of a novel combination of esters.

**Methods for 2005**:

1. Beta farnesene optimization. Field tests will be conducted in apple orchards to determine the optimum release rate and trap design for capture of codling moths. The chemical will be dispensed from vials, rather than septa, to provide a higher release rate range, and using an interception type trap design. The trap design test will include the delta wing, bucket, smart and pane traps. A second trap design test will evaluate variations of the pane trap to include an opaque version and a different adhesive material. Trapping tests will be conducted in both commercial and experimental orchards. Beta farnesene is purchased form Sigma Chemical Company and purified by HPLC.

2. Ester blend optimization. Field tests will be conducted in apple orchards to determine the minimum number of blend compounds needed for attractiveness, to determine the best release rate, and to evaluate trap designs for capturing attracted codling moth. The first test will compare the ester compounds singly and together. The second test will compare the kairomones (pear ester, beta farnesene, and ester blend) singly and together. The third test will compare a broad range of release rates of the ester blend. The fourth test will compare the ester blend in the chemicals will be dispensed from the delta, wing, bucket, pane and smart traps. Rubber septa and vials will provide a broad release rate range to be tested. Until the trap comparison test is conducted, tests will use the wing trap. Trapping tests will be conducted in both commercial and experimental apple orchards.

3. Season-long comparison of kairomones. Three lures will be used to bait traps that will be maintained in apple orchards from mid-April until October. These lures are pear ester, beta farnesene, and the ester blend. Lures will be replaced every two weeks, and traps will be checked weekly. Three replicates of this trap set (9 traps) will be placed at each of 6 sites. Three sites will include pheromone mating disruption for codling moth, while 3 will not.

.

**Results and Discussion**:

1. Testing of blends. Blends of compounds evaluated included E,E-alpha farnesene, beta farnesene, linalool, ocimene, and hexyl hexanoate. In these field tests conducted in the first flight, female codling moths were attracted by alpha farnesene, and males were attracted by beta farnesene. Other compounds either did not seem to enhance codling moth attraction, or were inhibitory ocimene, linalool). Female response to blends that included alpha farnesene and to alpha farnesene alone were not consistent, similar to results in 2002 and 2003 and similar to 2004 results with beta farnesene below, and to results with pear ester reported by others. Possible confusing variables include competition from foliage and fruit odors that change with variety, pest levels, and season, as well as competition with other tested lures, and interaction with pheromone used in mating disruption.

2. GC-EAD of apple volatiles. GC-EAD results using wild codling moth antennae exposed to dilutions of a volatile collection from apples infested in the field, revealed responses to 3 compounds at the greatest dilution in the series. A preliminary field-test of two blends of these compounds with and without beta farnesene revealed female and male attraction to the new ester blend. This test provides evidence of a third kairomone, one isolated from apple volatiles, that is attractive to male and female codling moth.

3. Testing of beta farnesene. Trapping experiments with beta farnesene followed results of 1 above that much of the attraction of male codling moths to previous blends can be explained by their response to beta farnesene. A comparison of 5 trap types showed significantly higher numbers of codling moths on pane traps compared to wing, Universal, AM, or Multipher traps. A comparison of doses of beta farnesene in rubber septa showed best capture of males in traps baited with the highest dose tested, (10 mg), while female response did not increase with chemical dose. A comparison of the sex ratio of moths captured in traps baited with beta farnesene during the spring and summer flights revealed a very strong male bias in captures in the spring (94:06, n = 106), but a weak male bias (60:40, n = 139) in the summer. Previous tests in Europe indicated a response by males only.

**Budget**:

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

**PI**: Peter J. Landolt

**Project Duration**: 2003-2005
**Current Year**: 2005

**Project Total (3 years)**: $53,200

**Current Year Request**: $18,100

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

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

Additional support for this project is contributed by the IFAFS/RAMP projects, which will fund another student for the field work.

**CONTINUING PROJECT REPORT** **YEAR 2/3**
**WTFRC Project #AE-03-322**


**Project Title:** Evaluation of a codling moth larval aggregation pheromone as an IPM tool
**PI:** Gary Judd
**Organization:** Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97, Summerland, B.C. CANADA V0H 1Z0
**Co-PI and affiliation:** Gerhard Gries, Department of Biology, Simon Fraser University, Burnaby, B.C. CANADA V5A 1S6


**Objectives:**

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

**Significant Findings – 2004**

- In collaboration with Phero Tech Inc., Delta, BC, we completed development and preliminary testing of what is now a commercially available larval trap based on corrugated cardboard bands and an inexpensive proprietary pheromone-impregnated polyurethane matrix release system
- Using this trap we determined that under field conditions the optimal dose of our synthetic eight-component pheromone was 10,000 cocoon-spinning larval hour equivalents (CSLHE)
- In no-choice, replicated orchard experiments pheromone-impregnated bands containing 10,000 CSLHE or 23 nanograms (ng) of synthetic pheromone / linear foot caught **1.9**× more overwintering larvae than non-baited bands
- No trap tested, pheromone-baited or otherwise, was effective at trapping diapausing larvae in wooden harvest bins
- Attack rates and percent parasitism of CM larvae by the biological control agent, *Mastrus ridibundus*, are both impacted by CM larval aggregation and aggregation pheromone

**Methods – 2004:**

*Laboratory Bioassays - Larval Pitfall Olfactometer*

In developing the larval trap we continued to make use of our larval pitfall bioassay (Fig. 1) to examine response of larvae to various release devices loaded with different pheromone doses and blends (8 *vs* 5 component) rates. Synthetic pheromone equivalents and release devices were compared in paired tests by loading cardboard strips with test stimuli. For each replicate, one 5$^{th}$-instar larva was placed in the center of the olfactometer and its pupation site recorded 18-24 h later. A minimum of 30 larvae were tested in each experiment. Perforated Eppendorf tubes were placed in each vial to prevent contact of the larva with the test stimuli at the bottom of left and right tubes. Different doses, number of pheromone components and release devices were evaluated in this laboratory assay. Paired frequency data were analyzed using $\chi^2$ tests.

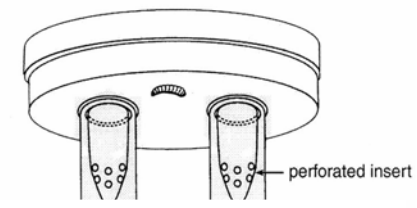



**Figure 1.** Larval pitfall bioassay

*Single-tree Choice Bioassay*

To test pupation site preferences and prototype pheromone release materials in the field, maple trees (*Acer* sp.) were scraped of potential pupation sites and banded with an open-fluted corrugated cardboard band. Treatment stimuli were randomly assigned to one half of each trunk band and control stimuli to the other half. Each band was divided into 6 equal 2.5-cm-wide cardboard strips, 3 such strips were on the treatment side and 3 strips on the control side. Each centre strip housed the test stimulus. Bands were affixed to the trunk with metal wire, 45 cm above the ground. For each replicate, twenty, 5$^{th}$-instar mass-reared male only, female only, or male and female larvae were placed on a modified milk carton collar placed at the base of the first lateral branch crotch. Experiments were started at 2200 h PST and numbers of larvae cocooning at treatment or control sites were recorded 10-12 h later. These test procedures were suitable for testing early prototypes. Experiments were conducted at SFU because proximity to the manufacturer expedited trap development. Tests were done in spring 2004.

*No-choice Orchard Experiments*

In August 2004 several thousand prototype larval traps were produced and field tested in Kelowna, BC. Five mixed-variety apple orchards were divided into plots of equal size. Four banding treatments were randomly assigned to each of five, 25-tree plots (125 trees / treatment). Three pheromone treatments, 10,000 CSLHE, 1000 CSLHE, and 100 CSLHE load rates were compared to non-baited control bands. To control for differences in tree-trunk circumference and potential variation in trapping surface area across treatments, all bands were limited to 50 cm in length regardless of tree girth. Bands were applied in early August and removed in early October. Within-orchard larval distributions and tree to tree variation in larval density are being mapped using banding data. This banding information will lead into studies of the role of aggregation and mating.

**Results and Discussion - 2004:**

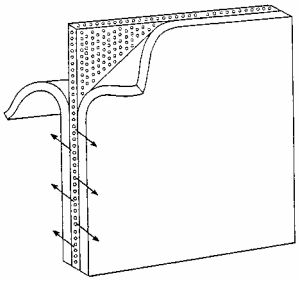
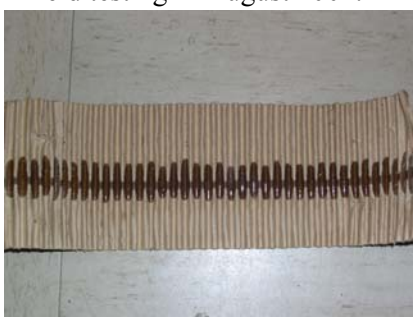


**Figure 2.** Original larval trap

**Pheromone Formulation and Trap Development:** Early in 2004, Hercon Environmental, Emigsville, PA, whose pheromone release technology we were going to incorporate into our trap design (Fig. 2), made a corporate decision to reduce staff and withdraw their collaboration services on a number of projects including potential development of a CM larval trap. We immediately switched our emphasis to pheromone release-technology owned by Phero Tech Inc. (Delta, BC) with whom we were developing the commercial trap. Before traps could be made it was necessary to build prototype manufacturing equipment to produce traps. While this set-back delayed development, in the end we produced a trap that was ready for field testing in August 2004.



We have coupled a simple corrugated cardboard band that can be cut to various sizes, sandwiched between a peel and stick adhesive backing with an inexpensive, pheromone-impregnated polyurethane matrix applied as a ribbon in the center of the band replacing the Hercon laminate system (Fig. 2). Preliminary studies suggest the polyurethane matrix delivers a complex pheromone blend at rates appropriate for 6 – 8 weeks of field use. Phero Tech is currently conducting detailed lab aging studies and volatile capture analysis to determine exact release rates and determine shelf life for traps.

**Pitfall Olfactometer Bioassays:** In laboratory pitfall bioassays, pheromone-impregnated (1000 CSLHE) polyurethane release materials (as above) adequately mimicked (Fig. 3B) natural pheromone emitted from 20 cocooned larvae by attracting/arresting similar numbers of mature larvae seeking pupation sites (Fig. 3A). About 85% of larvae assayed responded to the best pheromone treatment.

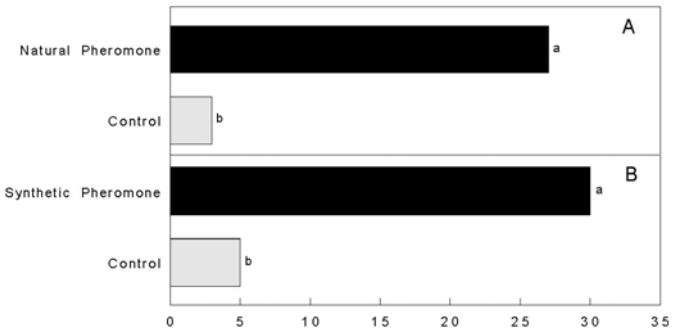



**Figure 3.** Relative attractiveness of synthetic and natural pheromone sources in pitfall bioassays

**Single-tree Bioassays.** In two-choice, individual tree bioassays, significantly (*t*-test $P < 0.05$) more mass-reared marked and released larvae cocooned in those halves of bands impregnated with synthetic pheromone than in non-baited control halves (Fig. 4). On these smooth barked trees pheromone-impregnated traps recaptured 50% of the released larvae, whereas non-baited cardboard recaptured ca. 39%, similar to previously reported values (Judd *et al*. 1997). Preliminary field aging studies suggest the polyurethane matrix plus pheromone release system should be suitable for at least one generation under field conditions.

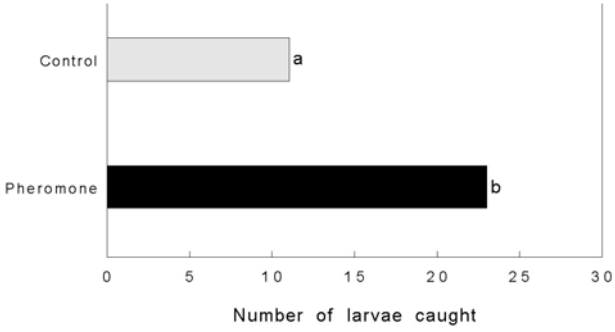



**Figure 4.** Attractiveness of synthetic pheromone sources in single-tree choice bioassays

**Orchard Experiment.** In no-choice, replicated orchard trials, catches of naturally overwintering wild larvae increased linearly with increasing pheromone doses (Fig. 5). Bands impregnated with the highest dose of synthetic pheromone (10,000 CSLHE) attracted / arrested significantly more larvae than bands impregnated with the lowest dose (10 CSLHE). Most importantly, we found that pheromone-impregnated bands containing an adequate dose caught significantly more larvae than non-baited control bands (Fig. 5). A pheromone dose of 10,000 CSLHE represents ca. 23 ng of chemical per linear foot of banding material. This means that the cost of traps is virtually limited to the cost of banding materials and pheromone application. We have provided definitive proof that the best pheromone-impregnated cardboard bands tested were **1.9×** more attractive than non-baited control bands, providing the first evidence that

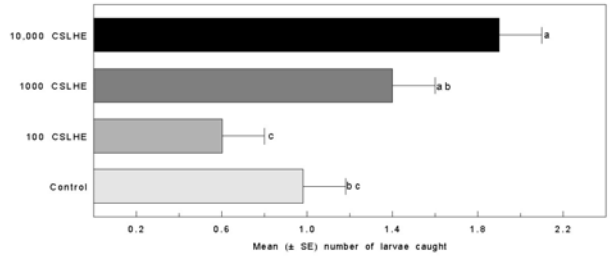



**Figure 5.** Relative attractiveness of pheromone-impregnated bands of different doses in no-choice orchard bioassays. Means followed by different letters are significantly different (ANOVA and SNK-multiple comparison tests $P < 0.05$)

pheromone-baited bands are more sensitive monitoring tools than cardboard bands alone (Judd *et al*. 1997). However, given pheromone is so inexpensive, we intend to test even higher release rates under no-choice orchard conditions to see if this differential can be increased. Phero Tech is currently upgrading mass-production facilities and developing a marketing strategy. Data on distributions of larvae within orchards are currently being analyzed to support 2005 studies on the impact of aggregation on mating probability and the combined impact of banding and mating disruption.

**Bin Bioassays.** Several mark-release-recapture experiments failed to identify a trap design, placement or pheromone dose that was effective in recapturing mass-reared larvae released into wooden bins. It appears the type and sheer number of possible sites for larvae to spin cocoons in wooden bins makes them exceptionally attractive for overwintering larvae. Several trap designs and placements in bins proved ineffective as no larvae were recaptured in eight replicated trials. We have one or two trap designs and placements still to test before accepting failure to achieve this objective. In 2005 we will run trapping tests with plastic bins as these are normally less attractive to larvae (Higbee *et al*. 2001) and should make trapping more efficient. We are also exploring the idea of combining pheromone into attract-n-kill paste.

**Larval Aggregation and Biological Control.** In field-cage assays, large larval aggregations (30 larvae) elicited significantly more (*t*-test, $P = 0.003$) parasite contacts (50.9%) than small (3 larvae) aggregations (39.0%), but the mean number of CM larvae parasitized was not significantly different (*t*-test, $P = 0.136$) in small and large aggregations. However, the mean percent parasitism declined as larvae became more aggregated, with large (20 larvae) aggregations experiencing a lower rate (11.9%) of parasitism than small (5 larvae) aggregations (36.3%). When larval density was held constant (10 larvae / tree), more parasites made contact with trees having aggregated larvae than trees with uniformly distributed larvae
(*t*-test, $P = 0.0011$), however, in the same way individual schooling fish are less susceptible to predation, larvae in the center of stacked aggregations, were parasitized (0%) significantly (*t*-test, $P = 0.0001$) less often than larvae on the perimeter (14%) of such aggregations. When placed in cone traps, large aggregations of larvae did not attract more parasites than small aggregations of larvae (*t*-test, $P = 0.1957$). These preliminary data suggest aggregation of CM larvae may reduce parasitism rates in egg-limited parasitoids like *M. ridibundus*. These data open the door on many possibilities for using CM larval aggregation pheromone to manipulate rearing and perhaps field efficacy of *M. ridibundus* as a biological control agent. However, these results raise an important warning that in-orchard use of attract-n-kill larval bait stations might be detrimental to CM larval parasitoids using the same semiochemical signals.

**References Cited:**

**Bezemer, T.M. and Mills, N. J.** 2001. Host density responses of *Mastrus ridibundus*, a parasitoid of the codling moth, *Cydia pomonella*. Biol. Control 22: 169-175.

**Higbee, B., Calkins, C.O. and Temple, C.A.** 2001. Overwintering of codling moth (Lepidoptera: Tortricidae) larvae in apple harvest bins and subsequent moth emergence. J. Econ. Entomol. 94: 1511-1517.

**Judd, G.J.R., Gardiner, M.G.T. and Thomson, D.R. 1997.** Control of codling moth in organically-managed apple orchards by combining pheromone-mediated mating disruption, post-harvest fruit removal and tree banding. Entomol. Exp. Appl. 83: 137-146.

**Jumean, Z., Rowland, E., Judd, G.J.R. and Gries, G**. 2004. Male and female *Cydia pomonella* larvae produce and respond to aggregation pheromone. Can. Entomol.131:1-3.

**Budget**

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

**BUDGET** with current year highlighted

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

[a]Travel for student help from Simon Fraser University in Vancouver to Okanagan field sites during field season

**Continuing Project** **Year 1/3**

**WTFRC Project #AE-04-428** **WSU Project #13C-3643-8366**


| | |
|---|---|
| **Project title:** | The importance of dispersal in biological control and IPM |
| **PI:** | Vincent P. Jones, Associate Entomologist |
| Organization: | **WSU Tree-Fruit Research and Extension Center** |
| **Address, phone, e-mail:** | 1100 N. Western Avenue, Wenatchee, WA 98801; (509) 663-8181 ext. 273; vpjones@wsu.edu |
| **Co-PIs and affiliations:** | Jay F. Brunner, WSU-TFREC<br>Tom Unruh, USDA-ARS, Wapato<br>Dave Horton, USDA-ARS, Wapato |
| **Contract administrator:** | Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691 |


**Objectives:**

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

**Significant findings:**

- **The marking procedure used for the ground cover worked exceedingly well; 97.6% of all insects collected in the ground cover were marked. In the canopy above the ground cover, we found an average of 23% of the predators were marked as having visited the ground cover.**
- **Our *Colpoclypeus florus* collections were very sparse this year due to low overwintering populations and overspray from the adjacent orchard.**
- **OBLR movement from post-harvest cherries appears to contribute significantly to the OBLR populations in adjacent apple blocks.**

*Objective 1.* **This year we set up plots at USDA's Moxee Experimental Farm to test whether we could detect the movement of pear psylla predators from the ground cover to the canopy of pear psylla infested trees. The various ground covers were planted in early spring, and the egg marker sprays began 10 June after predators were first detected and ended 9 August after plots became overgrown with lamb's-quarter. Applications of 20% egg solution at 20-25 gallons per plot were made at roughly weekly intervals with a weed sprayer mounted on a four-wheeler. Only the ground cover was treated.**

Insect collections were made by beating foliage from the ground cover or trees over a sticky panel and picking off the insects thought to be psylla predators. Each insect was identified, placed separately into a micro-centrifuge tube, and tested for the presence of the egg marker.

**Results***:* At the time of this report, we have processed about half of the samples. To this point, our data has been extremely clean, with 97.6% of the predator samples collected from the ground cover scoring positive. This means that the marker and the application method (using a four-wheeler mounted weed sprayer) are extremely efficient, which will reduce the possibility of false negatives (i.e., an insect originating in the ground cover and collected in the canopy but scoring negative for the mark) essentially to less than 2.5%.

We found that 23% of all psylla predators collected in the canopy, across all dates, scored positive for egg. For the dates we have completed, *Anthocoris, Deraeocoris*, and spiders were the most abundant in the tree canopy and were marked 21.4, 19.4, and 14.6% of the time, respectively. There were small numbers of lacewings, coccinellids, *Lygus*, Nabids, and *Orius* collected, all of which had at least one representative marked; but until more samples are processed, percentages could be very misleading as to the movement from the ground cover. Even so, 80% of the coccinellids were marked, and 58.3% of the lacewings were marked. We will have more data processed by the presentations this winter.

*Objective 2*. **We treated four rose patches with marker in the early spring to determine movement patterns of the OBLR parasitoid, *C. florus*, from the rose patches to the adjacent orchards. Unfortunately, this past year was a very poor season for *C. florus* and there were virtually no parasitoids collected from any of these orchards in the spring, even when we used sentinel larvae. In addition, at two of the sites, the grower applied leafroller sprays to the adjacent orchards in the spring, further affecting the parasitoids.**

**In the fall (when *C. florus* is at peak population levels), we placed sentinel larvae in an orchard adjacent to a treated rose patch and were able to find parasitoids in ≈80% of the leafroller retreats. These adults are currently being checked for markers, and that information will be available at the research review.**

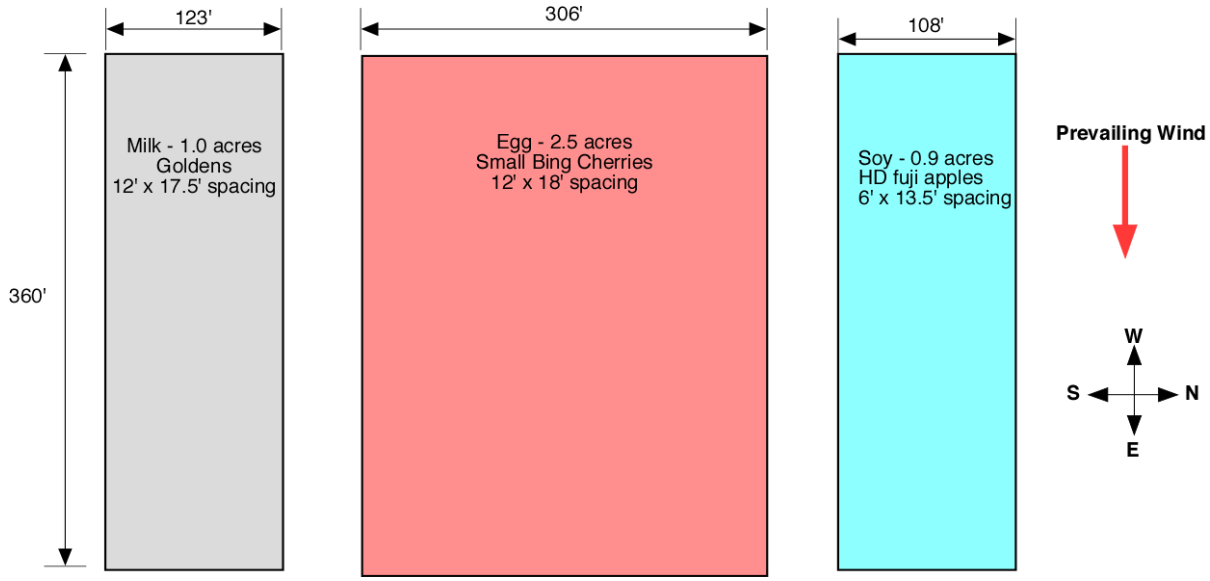
*Objective 3*. **This year we ran a test to examine the dispersal of OBLR from cherries (after harvest) to adjacent apple blocks. The test was set up 29 July at a large orchard in Mattawa, WA. The trial consisted of 3 blocks: a central Bing cherry block, a block of large Goldens on the south side of the cherries, and a high density trellised Fuji planting on the north side of the cherry block (below). We treated the cherries with egg whites (10%), the Goldens with milk (20% + 0.5% glycerol + water softener), and the Fujis with soy milk (20%). All treatments were applied using an airblast sprayer at ≈150 gpa.**

Four transects were set up with traps spaced at ≈50 feet apart in the Goldens and Bings and 41 feet apart in the high density Fuji planting. Over the four transects, a total of 15 traps were placed in the two smaller apple blocks, 5 in each of the untreated areas between the treated areas, and 30 in the egg treated area. For convenience, the untreated areas are named mid-X (between the Goldens and Bings) and mid-Y (between the Bings and Fujis). Each was roughly 50 feet wide.

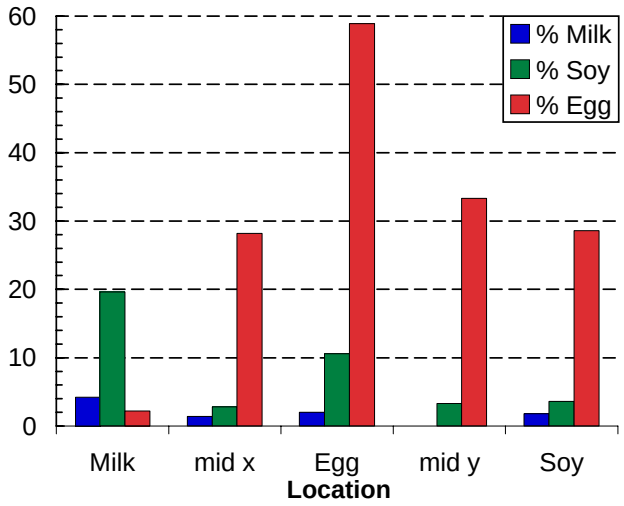
Results:

We captured an average of 6 OBLR moths per trap over the first 11 days after the markers were applied. The Golden (Milk) block contributed little to the number of moths caught in either of the other two blocks. The moths originating in the Fuji (Soy) block were picked up about 20% of the time in the Goldens and 10% of the time in the Bings (Egg) (figure right). The largest number of insects that were marked came from the Bings (Egg). This location is twice as large as the other two plots but, even so, the percentage of insects marked with egg in the Fujis (where soy was sprayed) was ≈28% or 7-fold higher than the recovery of soy-marked moths in the same area. Our data clearly suggest that the Bings were a major source of OBLR for the adjacent apple blocks.

The relatively low level of recovery of milk and soy-marked insects is likely the result of the grower applying Raynox in both blocks of apples 3 days after the markers were sprayed. In the lab, we have found Raynox to interfere with our ability to detect the mark. In the field, we think that it may "seal" the marker onto the leaves and reduce the probability that insects that walk on the residue will acquire the mark. The egg section was not treated with the Raynox so insects flying into that area and walking across the foliage would acquire the egg mark in much greater numbers than the other two areas where insects walking on the foliage would only have been able to acquire the mark for the 3-day period before the Raynox was sprayed.



Next year we will make sure that none of the blocks are treated with Raynox over the test period, and we also plan to investigate areas where abandoned (or untreated) blocks are adjacent to managed blocks. We will test for both CM and LR movement patterns, depending on the populations in each block.




Budget:

**Project title:** The importance of dispersal in biological control and IPM
**PI:** Vincent P. Jones
**Project duration:** 2004-2006 (3 years)
**Current year:** 2005
**Project total (3 years):** **$**140,145
**Current year request:** **$**46,160

| **Item** | Year 1 (2004) | **Year 2 (2005)** | Year 3 (2006) |
|---|---|---|---|
| Salaries[1] | 20,487 | **21,306** | 22,158 |
| Benefits (30%-yr 1; 34% yrs 2-3) [2] | 6,146 | **7,244** | 7,534 |
| Wages[3] | 11,000 | **11,000** | 10,000 |
| Benefits (16%-yr 1; 11% yrs 2-3) | 1,760 | **1,210** | 1,100 |
| Supplies[4] | 3,200 | **3,200** | 3,200 |
| Travel[5] | 3,200 | **3,200** | 3,200 |
| Total | 45,793 | **47,160** | 47,192 |

[1] Callie Eastburn, Associate in Research (.50 FTE).
[2] Benefits – increase from year 1 to years 2 and 3 is due to the increase in health insurance benefit contributions WSU makes on behalf of the employee.
[3] Time-slip employees.
[4] Lab supplies. Cell phone charges are allowed.
[5] Travel to research plots.

**Continuing Project** **Year 1/3**
WTFRC Project #AE-04-429 WSU Project #13C-3643-7366

**Project title**: Mechanisms underlying mating disruption

**PI**: Vincent P. Jones, Associate Entomologist
**Organization**: Tree Fruit Research and Extension Center
**Address, phone, e-mail**: 1100 N. Western Avenue, Wenatchee, WA; (509) 663-8181 ext. 273; vpjones@wsu.edu
**Co-PI and affiliation**: Jay F. Brunner, Entomologist and Director
Tree Fruit Research and Extension Center

**Contract administrator:** Mary Lou Bricker (mdesros@wsu.edu) (509) 335-7667; or Tom Kelly (kellytj@wsu.edu) (509) 335-3691

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

**Significant findings:**
- One-day-old CM males were more likely to find calling females than four-day-old males. In addition, all of the females found by one-day-old males were inseminated, whereas only 1/3 of those located by four-day-old males were inseminated.
- One-day-old OBLR males were also more likely to find calling females than four-day-old males, but females were rarely inseminated. This appears to be a result of CM males (run at the same time in the wind tunnel) flying upwind towards CM females, then after landing visually orienting to the OBLR females and preventing the OBLR males from mating.
- Density-dependent mating is facilitated by the visual search behavior (described above) and may be the mechanism by which MD breaks down at high population levels.
- The age of male codling moths mated to four-day-old female codling moths dramatically affected reproductive output of the females. This appears to be primarily from a tendency of older males to not transfer sperm packets. This result corroborates our work in the lab wind tunnels.

**Results:**
***Objective 1.*** We initially proposed and tried to use the field wind tunnels to investigate female and male mate choice. Although we obtained some data this summer, it was highly variable because of inconsistent airflow (wind outside the tunnel causes extreme variation within the tunnel) and because of high temperatures, which inhibited moth flight or killed older male moths.

To bypass these problems, we rebuilt the wind tunnel in my lab and began the studies there in mid-September. Initially, we set up the studies to run codling moth and OBLR simultaneously. Two male moths (of each species), one aged one day old and the other four days old, were marked with different color fluorescent dusts and placed in a small cage on a platform in the downwind side of the wind tunnel to acclimate at least 1.5 hours before dusk. At dusk, males were released simultaneously. The female moths were tethered ≈8 cm apart on a small platform located in the center of the tunnel, 100 cm upwind of the males. Digital video recorders were trained on the females and were started at

dusk when males were released. The next morning, the females were collected, observed under a fluorescent light to determine which male (if any) contacted the female, and frozen for later dissection. Dissection allowed us to determine if a sperm packet was actually passed and allowed us to account for mating that happened more than 1.5 hours past dusk (when the digital tape ran out).

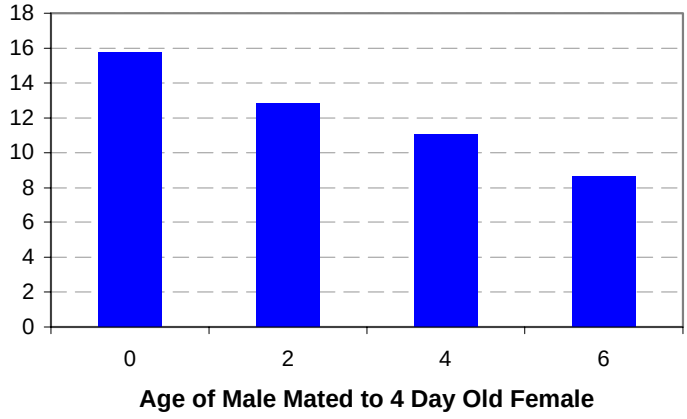
**Our initial data show that codling moth males aged one and four days were both successful in finding the female in our laboratory wind tunnel. Over the 29 days of data we have at the time of this report, females called 22 times and males were able to find and make contact with the calling female 15 times. Of the 15 female contacts, 9 were from one-day-old males and 6 were from four-day-old males. Dissection showed that all of the females contacted by one-day-old males received sperm packets, but only 2 of the 6 females marked by the four-day-old males received sperm packets. At present, the number of replicates is too low for sweeping conclusions, but these data tend to agree with our observations in objective two that older males mated with females of a given age tend to have a greater percentage of sterile eggs laid. Thus, at this point, it appears that older males are able to find and couple with younger females, but the frequency of passing a sperm packet during the mating process is greatly reduced.**

Over the 29 days of data, the OBLR are similar in terms of the number of females calling (22) and the percentage of males flying upwind towards the female. We found that 10 of the one-day-old males contacted the calling female, but only 4 of the four-day-old males contacted the female based on the videos and the passing of the fluorescent dust. However, only 2 of the female OBLR marked by one-day-old males and only 1 of the 4 females marked by four-day-old males had sperm packets present in the oviduct. While this result seems perplexing at first, further observation showed that male codling moth fly sooner. Upon landing on the platform, CM males moved over to the calling OBLR female ≈15% of the time and attempted to mate. It is important to note that the OBLR and CM do not share any pheromone components, so that the attempted mating is not chemically mediated. The likely explanation is that after landing on the platform visual factors take precedence and that the larger sizes of OBLR females are more attractive to male codling moth. Early studies on CM behavior had shown that visual cues during mating are important, and this visual search does provide a mechanism by which within-tree mate finding is possible when the pheromone channel is blocked by mating disruption.

Unfortunately, our marking system was set up so that OBLR and CM of the same age were marked with the same color dust so we could not distinguish the number of times that cross-species mating was attempted. Our further studies will be performed separately to eliminate the interference between the two species.

***Objective 2.*** This experiment compared the reproduction of four-day-old females paired with 0, 2, 4, or 6-day-old males (the converse of our previous work where 1- to 2-day-old males were paired with females that were 0, 2, 4, or 6 days old). Similar to the previous experiment (Objective 1), we found that increasing the male age at which mating occurred drastically dropped the reproductive output of the females (≈20, 30 and 45% reductions from the control) (Fig. 1). We also determined

**Fig. 1 .** Effect of male age on reproductive capacity of 4-day-old females



what proportion of the population did not lay any eggs. We found that the proportion of females that laid no eggs at all increased from ≈8, 12, 17, and 28% when females were mated to males that were 0, 2, 4 or 6 days old.

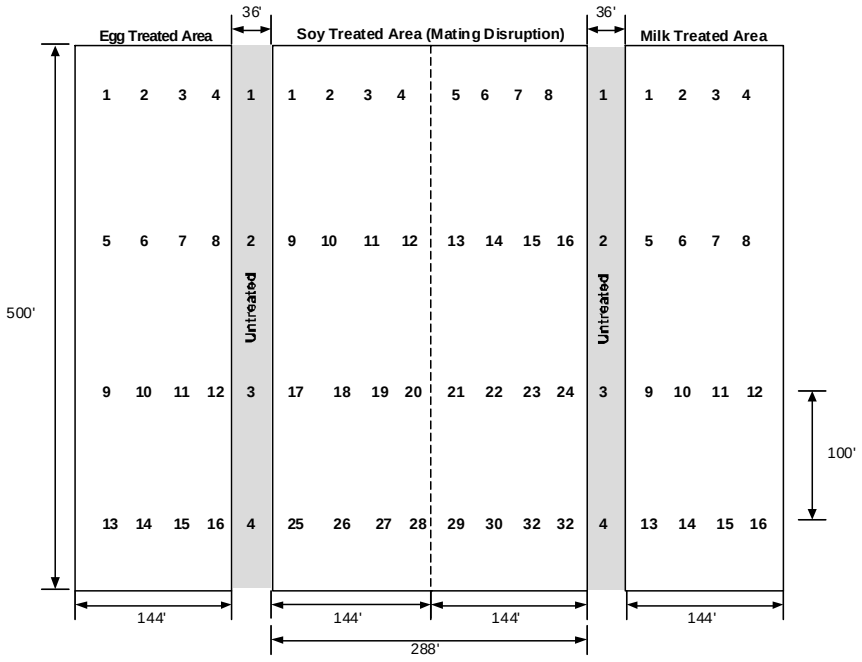
These results give strong support to the idea that females and males alike should have mechanisms that allow them to be "choosy" with respect to age of their potential mates. Mating with an older male or female dramatically reduces the potential progeny, and our studies strongly suggest that delay of mating is a very important part of mating disruption in the field.

***Objective 3.*** This work has started, but results will not be available until the spring.

***Objective 4.*** A 7.4-acre block of Red Delicious in Quincy with a high population of CM present was used for this experiment. We applied Isomate C+ dispensers to the central 3.3 acres of the plot at full rate. The entire orchard was under normal pesticide treatments over the full duration of the test. We ran 3 trials:

1. An initial trial was conducted with half the MD area marked with soymilk and a 1.65-acre plot adjacent to the MD area, but separated by 2 tree rows (36 feet), treated with the egg marker. The markers were applied on 7 May, 144 degree-days from the start of male flight (Fig. 2). Pheromone trap collections were made at 3, 7, 10, 14, and 19 days after application; traps in the MD area received 10x lures, and in the non-MD area 1x lures were used.
2. The entire MD area was treated with the soy milk marker, the 1.65 acre plot previously treated with egg was re-treated with egg, and a 1.65 acre plot on the other side of the MD area was treated with the non-fat milk marker. The treatments were applied on 26 May at ≈298 degree-days and corresponded to the latter part of the first flight (Fig. 2). Trap collections occurred at 0, 2 ,6, 12, and 19 days after treatment; traps in the MD area received 10x lures, in the non-MD area 1x lures were used. We also added some DA lures in this trial (in separate traps) to determine female movement.

**Fig. 2. Plot map of trials to determine if moths move away from or towards MD areas. First trial consisted of the egg treated plot and the soy treated plot up to the dotted line (from the left). Second and third trials used the entire area.**




3. The same as number 2, but applied on 12 July, at 1090 degree-days, which was 11% of the second flight. Trap collections occurred at 0, 2, 6, 12 and 19 days after treatment. In this experiment, we substituted the normal pheromone lures with the combo DA/pheromone lures so that both male and females might be captured.

**Results**:

During the first field study, 72% of the moths collected in the MD area (treated with soy) tested positive for soy, and 28% of the moths tested positive for egg whites. In the area treated with egg whites, 46% of the moths tested positive for soy and 54% for egg whites. These figures suggest that moths initially in the MD area move back and forth equally between the MD and non-MD blocks. However, there appears to be a significant decrease in male movement into the MD area from the non-MD area. This suggests that the net movement of males is towards the non-MD area. However, because we didn't mark the other side of the MD area with another marker, we could not be sure that the net movement towards the non-MD area was not related to other factors (such as wind patterns).

**In the second study, we did mark the other side of the MD area so that wind patterns could be taken into account. We have processed the samples for the egg section but have not yet completely processed the samples from the soy or milk sections. We have also not processed any of the samples for the third test at this time. We expect to have those samples processed by the review in January.**

**Budget:**

**Project title:** Mechanisms underlying mating disruption
**PI:** Vincent P. Jones
**Project duration:** 2004-2006 (3 years)
**Current year:** 2005
**Project total (3 years):** $157,548
**Current year request:** $52,514

| **Item** | Year 1 (2004) | **Year 2 (2005)** | Year 3 (2006) |
|---|---|---|---|
| Salaries[1] | 22,724 | **23,634** | 24,579 |
| Benefits (32%-yr 1; 35% yrs 2-3)[2] | 7,272 | **8,272** | 8,603 |
| Wages[3] | 12,800 | **12,800** | 12,800 |
| Benefits (16%-yr 1; 11% yrs 2-3) | 2,048 | **1,408** | 1,408 |
| Supplies[4] | 3,200 | **3,200** | 3,200 |
| Travel[5] | 3,200 | **3,200** | 3,200 |
| Total | 51,244 | **52,514** | 53,790 |

[1] Nik Wiman, Associate in Research (0.618 FTE).
[2] Benefits – increase from year 1 to year 2, is due to the increase in health benefits contributions WSU makes on behalf of the employee.
[3] Time-slip wages.
[4] Lab supplies, Cell phone charges are allowed.
[5] Travel to research plots.

**CONTINUING PROJECT REPORT** **YEAR 2/3**

WTFRC Project AE-03-335

**Project title**: Feeding stimulants to increase efficacy of insecticides

**PI**: Maciej A. Pszczolkowski, Kansas State University, Dept. of Entomology, 123 West Waters Hall, Manhattan, KS, 66506

**Cooperator(s)**: Dr. Carol A. Sheppard, Harmony Borkhard-Wier, Sarah Overbee, Shannon Reive, Scott Stewart

**Contract Administrator** (Name/email address/Phone #): Sherry Figge, Kansas State University, Dept. Entomology, 123 West Waters Hall, Manhattan, KS 66506, (785) 532-4754, sfigge@ksu.edu

**Objectives**: The studies previously done and published by PI and Co-PI in codling moth neonates (1,2) indicated that monosodium glutamate (MSG) stimulates feeding in neonates of this species. Therefore MSG can be successfully used for enhancement of insecticide formulations by either increasing their efficacy and/or maintaining their efficacy at reduced toxic ingredient amounts (3-6). MSG is moderately rain-fast, however (5). To solve the problem of moderate MSG rain-fastness, PI and Co-PI suggested use of trans-1-aminocyclobutane-1,3- dicarboxylic acid (*trans*-ACBD). *trans*-ACBD has superior rain-fastness, and feeding stimulatory and pesticide enhancing properties (7), however it is difficult to synthesize, and expensive because of its low-scale production for laboratory use only. The general objective of this project was to design or suggest another chemical substance that has feeding stimulatory properties similar to monosodium glutamate, but is cheaper than *trans*-ACBD.

To that end, we selected twelve chemicals that, similar to *trans*-ACBD, have amino and carboxylic acid groups attached to either cyclic or aliphatic (acyclic) hydrocarbon chain. There are three groups of such chemicals: (1) derivatives of aminocyclobutanecarboxylic acid, (2) derivatives of L-aminophosphono acids, and (3) derivatives of D-aminophopshono acids. Additionally, we selected for tests three other chemicals that are reported to have activity similar to that of *trans*-ACBD in vertebrate experimental systems. The following chemicals were tested in our study:

<u>derivatives of aminocyclobutanecarboxylic acid</u>

1. 1-Aminocyclobutane-1-carboxylic acid (ACBC)
2. *cis*-1-Aminocyclobutane-1,3-dicarboxylic acid (*cis*-ACBD)
3. *trans*-1-Aminocyclobutane-1,3-dicarboxylic acid (*trans*-ACBD)

Note: *trans*-ACBD was already tested during our previous research on codling moth feeding. The rationale for re-testing this drug in the present study was to evaluate the fidelity of our testing procedure in the new laboratory using insects that were shipped from Washington State to Kansas State.

derivatives of L-aminophosphono acids

1. L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4)
2. L-(+)-2-Amino-5-phosphonopentanoic acid (L-AP5)
3. L-(+)-2-Amino-6-phosphonohexanoic acid (L-AP6)

derivatives of D-aminophopshono acids

1. D-(-)-2-Amino-4-phosphonobutyric acid (D-AP4)
2. D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5)
3. D-(-)-2-Amino-7-phosphonoheptanoic acid (D-AP7)

chemicals with *trans*-ACBD activity in vertebrates

1. L-Serine-O-phosphate (O-phospho-L-serine)
2. (L)-(+)-a-Amino-3,5-dioxo-1,2,4-oxadiazolidine-2-propanoic acid (quisqualate)
3. (RS)-(Tetrazol-5-yl)glycine

The effects of the aforementioned drugs on feeding initiation and intensity have been studied. Our proposal included using computer software for chemical structure analysis and comparison in the drugs tested. Because the project ends in March 2005, this task is just being undertaken during the upcoming three months. For now (December 2004), comparison of the structures of the chemicals increasing feeding behavior in codling moth has been done visually, without computer aid. However, even this tentative comparison clearly suggests a cheaper alternative to *trans*-ACBD.

There were no and will be no deviations from the original objective or schedule. The project will be finished in March 2005.

**Significant findings**:

- ACBC initiates and increases feeding at concentrations 0.1 mg/ml and 1 mg/ml
- Feeding stimulatory activity of the derivatives of L-aminophosphono acids increases together with decrease in length of aliphatic hydrocarbon chain of these acids
- The derivatives of L-aminophosphono acids have no effects on feeding behavior
- (RS)-(Tetrazol-5-yl)glycine initiates and increases feeding at concentrations 0.1 mg/ml and 1 mg/ml

**Methods**:

*Insects*

Codling moth adults (*C. pomonella)* originating from USDA-ARS at Yakima, WA, USA, were held at 25$^{o}$C, 70-80%RH, under a 16L:8D photoperiod. Wax paper was provided as an oviposition surface. The circadian hatch began approximately 6h into the photophase. The neonates used to test materials were collected on the first day of hatching, 1 h post-hatch.

*Chemicals*

All tested substances were purchased from Tocris Cookson, (St. Louis, MO), and dissolved in double distilled water, containing 0.02% Triton X-100.

Bioassay technique

Circular sections of uniform size (12 mm of diameter) were removed from Honeycrisp™ (U.S. patent No. 7197) foliage, avoiding the rib area. Test solutions (10 μl) were distributed evenly, using a strip of polyethylene foil, over the upper surface of the excised sections that subsequently were allowed to air dry. To prevent any loss of tested solutions throughout the drying procedure, the sections were placed, dry side down, on small plastic cubes, so the solution could form a meniscus, and surface tension prevented any run-off over the edges of the treated section. Next, the same procedure was employed for treatment of the sections' lower surfaces. Treated sections were placed in bioassay stations and each section was infested with one, 1h old neonate larva. To prevent dehydration, the bioassay stations were placed in Petri dishes with wet filter paper placed on the bottom of each dish. The surface area of each leaf disc that was consumed was determined using a stereo microscope equipped with an ocular square mesh reference scale (No. 12-561-RG2, Fisher Scientific, Pittsburgh, PA, USA). Thirty-three fragments of leaves, chosen randomly from mid-rib areas of the leaves, were also measured visually and then dried and weighed to establish a relationship between optical measurement and weight of foliage consumed. Based on this determination, consumed areas of the leaf were converted to an estimated dry weight of leaf. Remaining details of this bioassay are described elsewhere (2,5,8).

*Experimental design*

Every chemical was tested at the following concentrations: 0.0001, 0.001, 0.01, 0.1, and 1 mg/ml. Control leaves were treated with 0.02% Triton X-100 in double distilled water.

To evaluate the effects of tested drugs on feeding initiation, the following procedure was used. Sixteen experimental larvae were individually exposed to the aforementioned test solutions in bioassay stations. Control larvae (N=16 for each concentration of each substance) were exposed to 0.02% Triton X-100. The number of larvae feeding was monitored at 15 min intervals for 3h-period. This procedure was repeated 5 times. The results were expressed as average (mean ± SEM) length of time before commencement of feeding

To evaluate the effects of tested drugs on feeding intensity, 8 larvae were individually exposed to each concentration of each substance, and this procedure was repeated 4 times. Amounts of leaf tissue consumed by neonates were estimated 24h after infestation of each bioassay station, and expressed as average (mean ± SEM) leaf consumption.

Statistics

No set of data on feeding induction passed normality tests ($P>0.05$). Here, mean times needed for induction of feeding were compared between groups exposed to respective sugars or sugar substitutes or to control solutions (0.02% Triton X-100) using Kruskal-Wallis test.

All data sets on leaf consumption passed tests for normality with $P<0.05$ (GraphPad InStat®, GraphPad Software Inc., San Diego, CA), and were subjected to regression analyses.

Regardless of the test used, results were regarded as significantly different at $P<0.05$.

**Results and discussion**:

*Derivatives of aminocyclobutanecarboxylic*

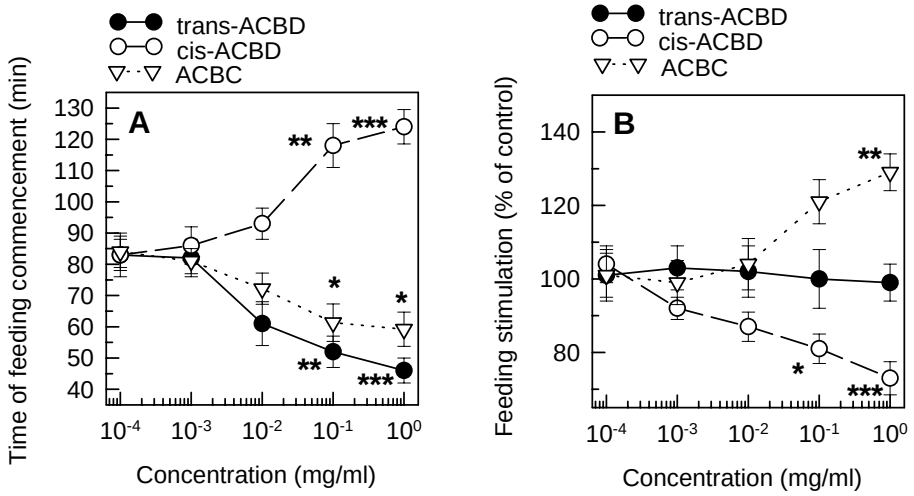



**Fig. 1**. The effects of aminocyclobutanecarboxylic acid derivatives on initiation (A) and intensity of feeding (B) by codling moth neonates. Each datum point refers to mean ± SEM obtained for ninety (A) or thirty-two (B) larvae. The time of feeding commencement in controls varied between 81.9 ±8.1 and 85.8±7.2 min. The amount of foliage ingested in control groups varied between 129.4 ± 11.8 and 136.6 ± 12.1 μg larva$^{-1}$ . * P<0.05, ** P<0.01, *** P<0.001

*Trans*-ACBD initiated feeding, but had no effect on feeding intensity (Fig. 1A,B), a result which corroborates well with our previously published data (9).In contrast, *cis*-ACBD significantly decreased feeding by codling moth neonates (Fig. 1B), as well as delayed feeding commencement (Fig. 1A). This result is in concert with anecdotal evidence reported by other authors in exotic coleopterans (10). ACBC accelerates feeding commencement by over 30 minutes and stimulates feeding by approximately 30% in comparison to control (Fig. 1 A, and B, respectively). These findings demonstrate three facts. First, hydrogen and carboxylic acid groups have to be attached to cyclobutyl ring of aminocyclobutanecarboxylic acids in *trans*- and not in *cis*- conformation to accelerate feeding commencement in codling moth neonates. Secondly, *cis*- conformation of amino and carboxylic acid groups provides effects deterrent to codling moth neonates. Consequently, feeding stimulatory activity of cyclobutanecarboxylic acids may be modified by substitutions in the cyclobutyl ring. Thirdly, the results with ACBC show that only one pair of amino and carboxylic acid groups, on one side of the cyclobutyl ring, is needed for acceleration of feeding commencement and increasing amounts of foliage consumed (please refer to Table 1 for chemical structures of tested chemicals).

*Derivatives of L-aminophosphono acids*

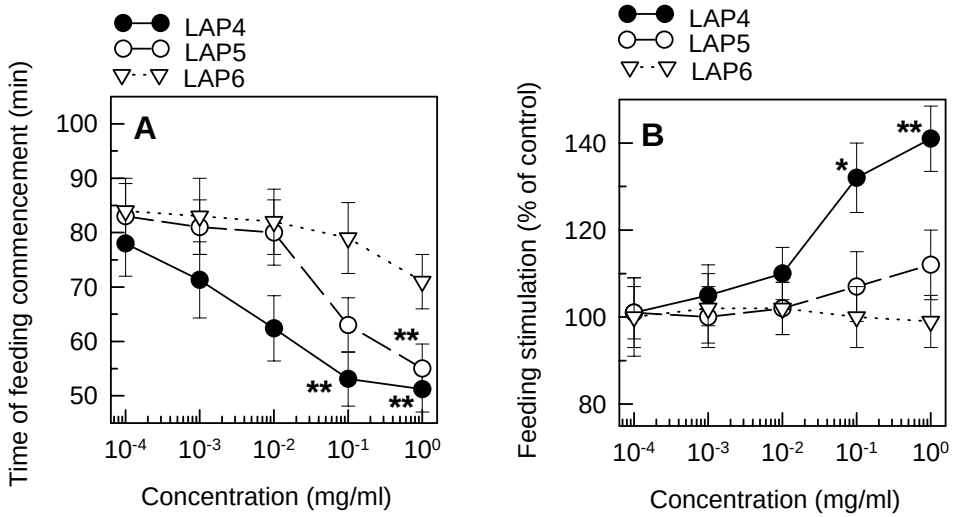



**Fig. 2.** The effects of L-aminophosphono acid derivatives on initiation (A) and intensity of feeding (B) by codling moth neonates. Each datum point refers to mean ± SEM obtained for ninety (A) or thirty-two (B) larvae.The time of feeding commencement in controls varied between 82.3 ±7.2 and 84.1±8.1 min. The amount of foliage ingested in control groups varied between 127.6 ± 109 and 137.5 ± 11.8 μg larva$^{-1}$ * P<0.05, ** P<0.01

An interesting correlation between the molecular structure and effects on feeding was observed in this group of chemicals. The longer the aliphatic hydrocarbon chain of these acids is, the less feeding stimulatory activity of a given chemical is. For instance, LAP-4, which has 3-carbon aliphatic hydrocarbon chain, both accelerated feeding commencement (Fig. 2A) and significantly increased leaf consumption (Fig. 2B) at concentrations 0.1 and 1 mg/ml. However, LAP-5, which has 4-carbon aliphatic hydrocarbon chain, only accelerated feeding at 1mg/ml concentration (Fig. 2A), and had no effects on feeding intensity (Fig. 2B). LAP-6, which has an even longer aliphatic hydrocarbon chain consisting of 5 carbon molecules, had no effects on feeding (Fig. 2 A,B).

*Derivatives of D-aminophopshono acids*

It is noteworthy that none of these acids influenced codling moth neonates feeding behavior (Fig. 3). This finding, together with data presented on Fig.2, demonstrates that amino and carboxylic acid groups have to be attached to aliphatic hydrocarbon chain of aminophosphonoacids in L- and not in D- conformation, to initiate or stimulate feeding in codling moth neonates (Table 1 summarizes this finding in reference to chemical structures of tested drugs). Please note that in this study DAP-7 was used. There is no DAP-6 being produced and sold thus far.

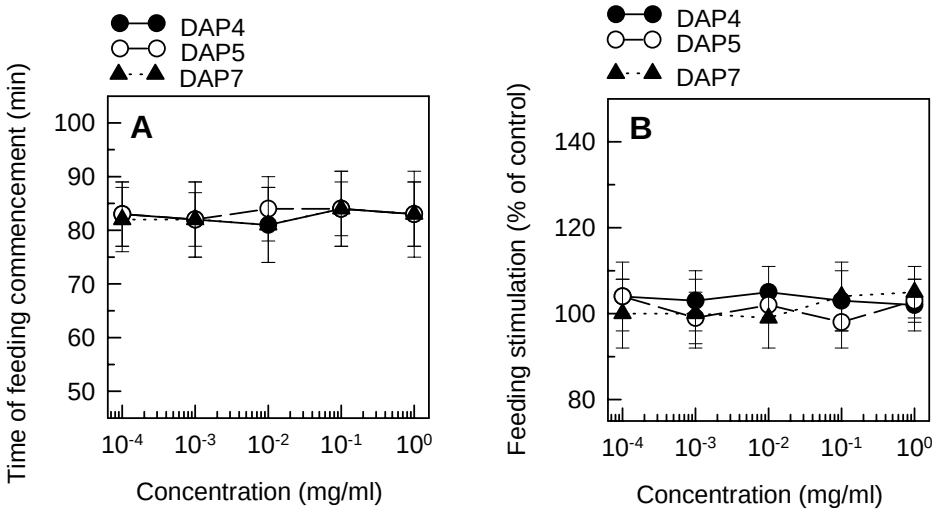



**Fig. 3**. The effects of D-aminophosphono acid derivatives on initiation (A) and intensity of feeding (B) by codling moth neonates. Each datum point refers to mean ± SEM obtained for ninety (A) or thirty-two (B) larvae. The time of feeding commencement in controls varied between 83.4 ±6.3 and 86.1±6.3 min. The amount of foliage ingested in control groups varied between 126.1 ± 10.3 and 129.9 ± 11.0 μg larva$^{-1}$ . No statistically significant differences found.

*Chemicals that have trans-ACBD activity in vertebrates*

In this group of chemicals, only RS-tetrazol-5-yl-glycine both accelerated feeding commencement (Fig. 4A) and increased feeding intensity (Fig. 4B). O-phospho-L-serine only accelerated feeding commencement (Fig. 4A), and quisqualate had no effects. The low activity of O-phospho-L-serine and absence of activity from quisqualate, corroborates very well the data presented in Fig. 3, in that both chemicals have their amino and carboxylic acid groups in D- conformation, suggesting that this conformation does not interact with neonates' sense of taste. Stimulation of feeding behavior by RS-tertazol-5-yl-glicyne corresponds well with the fact that this chemical has very high *trans*-ACBD- like activity in vertebrate experimental systems.

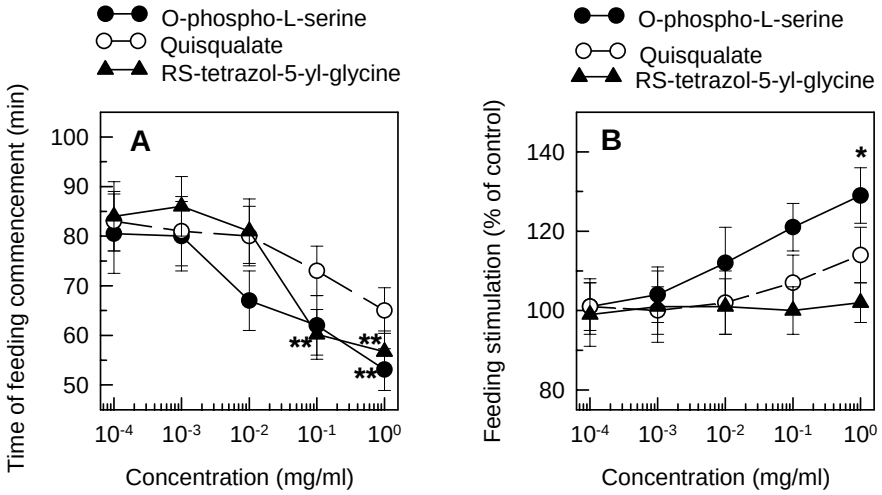
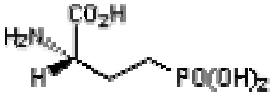
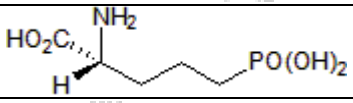
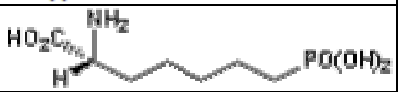
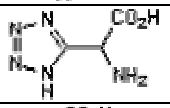
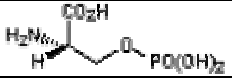
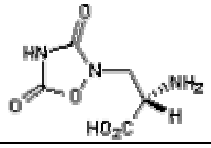



**Fig. 4**. The effects of chemicals that have *trans*-ACBD activity in vertebrates, on initiation (A) and intensity of feeding (B) by codling moth neonates. Each datum point refers to mean ± SEM obtained for ninety (A) or thirty-two (B) larvae. The time of feeding commencement in controls varied between 83.4 ±6.3 and 86.1±6.3 min. The amount of foliage ingested in control groups varied between 126.1 ± 10.3 and 129.9 ± 11.0 μg larva$^{-1}$ . * $P<0.05$, ** $P<0.01$.

*Modification of feeding behavior versus chemical structure of tested chemicals*

The trends we observed here are illustrated with Table 1. Generally, it seems that chemicals that possess feeding stimulatory properties have amino and carboxylic acid groups either attached in L-conformation to short aliphatic hydrocarbon chain, or to cyclobutane ring with no further groups attached. These findings inform the direction of the search for cheap and effective alternatives of *trans*-ACBD. The finding that RS-tertazol-5-yl-glicyne stimulates feeding has little practical output, since this chemical is as expensive as *trans*-ACBD. One could employ ACBC as an alternative to *trans*-ACBD. ACBC is more than 3 times cheaper than *trans*-ACBD and has only 6-fold higher water solubility, equaling 12g/l. This is substantially less than MSG water solubility, which equals 789g/l. Therefore, one should expect that ACBC will prove rain-fast under field conditions. The other direction indicated by our findings is that use of a chemical with a short aliphatic hydrocarbon attached to amino and carboxylic acid groups in L-conformation should be explored further. Such a chemical, namely L-aspartic acid, has a water solubility only 2-fold higher than *trans*-ACBD. Additionally, the retail prices of L-aspartic acid are only 4 times higher than those of MSG ($60 versus $15 per kg), and over 30,000 times lower than those of *trans*-ACBD.

**Table 1. Comparison of effects on feeding versus chemical structure in tested chemicals**

| Name | Chemical structure | Effect on feeding initiation | Effect on feeding intensity |
|---|---|---|---|
| ACBC |  | + | + |
| *trans*-ACBD |  | + | 0 |
| *cis*-ACBD |  | - | - |
| LAP-4 |  | + | + |
| LAP-5 |  | + | 0 |
| LAP-6 |  | 0 | 0 |
| DAP-4 |  | 0 | 0 |
| DAP-5 |  | 0 | 0 |
| DAP-7 |  | 0 | 0 |
| RS-tertazol-5-yl-glicyne |  | + | + |
| O-phospho-L-serine |  | + | 0 |
| Quisqualate |  | 0 | 0 |

+ feeding behavior stimulation, - feeding behavior inhibition, 0 no effect on feeding behavior

*Introductory experiments with L-aspartic acid*

We have evaluated potential feeding stimulatory properties of L-aspartic acid in preliminary feeding assays. This compound accelerated feeding commencement by approximately 30 minutes, and increased leaf consumption by approximately 37% (Table 2). These values are very similar to those that were found for MSG and *trans*-ACBD in our previous studies (2,7).

**Table 2. Effect of L-aspartic acid on initiation and intensity of feeding by codling moth neonates**

| Behavior observed | Control† (N=20) | 1 mg/ml L-aspartic acid (N=24) |
|---|---|---|
| Feeding commencement (minutes) | 84.2±6.2 | 49.8±3.2 ** |
| Leaf consumption ($\mu g\ larva^{-1}$) | 124.3± 10.6 | 176.5±13.5 *** |

† 0.02% Triton-X in water

** P<0.01, ***P<0.001

*Significance to the industry and potential economic benefits*

Our previous publications show LD50 analysis indicating that addition of MSG or *trans*-ACBD to Spinosad or *Bacillus thuringiensis*- based commercial pesticide formulations increases their effectiveness 2-3 fold (6,7). Our current research indicates that L-aspartic acid may serve as a potential substitute for MSG and *trans*-ACBD. Although we have no absolute numbers or values to illustrate the economic impact of our current accomplishments, our findings stand to have an enormous significance for the apple growing industry of the Northwest, since application of appropriate feeding enhancers can reduce the amounts of insecticides used for codling moth control, thereby improving the quality of fruit and safety of pesticide application. We observed the same degree of feeding stimulation in larvae exposed to MSG or *trans*-ACBD (2,7) and in larvae exposed to L-aspartic acid (Table 2). Therefore, we may expect that the latter compound will have pesticide-enhancing potential similar to that of MSG and *trans*-ACBD. We believe that L-aspartic acid should be further studied in terms of its effects on feeding, rain-fastness and potential for enhancement of orally active insecticides. A proposal of such studies will be the subject of a separate application.

References:


1. Pszczolkowski M.A., Matos, L.F, Bushman S.M, and Brown J.J .(2001). Effects of glutamate receptors agonists and antagonists on feeding by economically important insect pest. Acta Neurobiol. Exp. 61: 237.
2. Pszczolkowski M.A., Matos, L., Zahand. A, and Brown J.J. (2002). Effect of monosodium glutamate on codling moth larvae fed apple leaves. Entomol. Exp. Appl. 103:91-98.
3. Pszczolkowski M.A., Matos, L.F, Bushman S.M, and Brown J.J .(2001). Feeding enhancements for insecticide targeting neonate lepidopteran larvae. In: 6th International Symposium on Adjuvants for Agrochemicals ISAA 2001. Ed. Hans de Ruiter. Amsterdam, The Netherlands. pp.420-425.
4. 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. Ann. Entomol. Soc. Amer. 95:603-607.
5. 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.
6. Pszczolkowski, M.A., Brunner,J.F.,Doerr, M.D., and Brown, J.J. (2004). Enhancement of *Bacillus thuringiensis* with monosodium glutamate against larvae of obliquebanded leafroller (Lep.:Tortricidae). J. Appl. Entomol. 128:474-477.

7. Pszczolkowski M.A. and Brown J.J. (2004). Enhancement of spinosad toxicity to *Cydia pomonella* neonates by monosodium glutamate receptor agonist. Phytoparasitica 32:342-350.
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9. Pszczolkowski M.A., Zahand. A, Bushman S.M, and Brown J.J (2003). Effects of calcium and glutamate receptor agonists on leaf consumption by lepidopteran neonates. Pharm. Biochem. Behav. 74:389-394
10. Allan, R.D., Hanrahan, R.J., Hambley, T.W., Johnston, G.A.R., Mewett, K.N. and Mitrovic, A.D. (1990) Synthesis and activity of a potent N-methyl-D-aspartic acid agonist, trans-1-aminocyclobutane-1,3-dicarboxylic acid, and related phosphonic and carboxylic acids. J. Med. Chem. 33:2905-2915.

Project title: **Feeding enhancements for insecticides targeting neonate lepidopteran larvae**
PI: Maciej Pszczolkowski
Project duration: 2004-2005 (one year)
Project total (one year): $15,000
Current year request: $ 15,000

| Item | |
|---|---|
| Salaries[1] | 1640 |
| Benefits[2] | 492 |
| Wages[3] | 7533 |
| Equipment[4] | 1920 |
| Supplies[5] | 2765 |
| Travel[6] | 650 |
| Total | 15000 |

1. Dr. Pszczolkowski's salary (training and supervision of two student workers throughout the duration of the project)
2. Benefits of Dr. Pszczolkowski, according to the policies of Kansas State University
3. Time-slip wages + benefits of two student technical assistants at average labor of 40h/month/person, average salary 7.00$/hour, throughout the project duration.
4. Economy- grade stereo dissecting microscope and illuminator
5. Costs of insect shipments, apple tree purchase and greenhouse, materials for feeding stations, small laboratory supplies, access to crystallographic database
6. 50% of coverage of domestic conference fee and travel