# Washington Tree Fruit Research Commission

Apple Entomology Research Review

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# FINAL REPORT FOR 2000

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

PRINCIPAL INVESTIGATOR:	Diane G. Alston, Department of Biology, Utah State University (USU)
<b>CO-INVESTIGATOR:</b>	Anchalee Stark, Technical Research Assistant, USU
FUNDING HISTORY:	Funded in 2000 (Year initiated): \$15,000

# **SIGNIFICANT FINDINGS:**

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

# **OBJECTIVES:**

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

# **PROCEDURES:**

# PC Survey

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

# PC Host Range

The potential host range for PC in Utah was investigated by placing field-collected adults on green fruits of commercial, ornamental, and wild fruit trees in cages in the laboratory. One each active male and female were placed together on 5-10 fruits attached to a twig kept in floral preservative inside cages kept

at room temperature. Cages were replicated 10 times each. Fruits were evaluated 5-8 days later for the number of feeding and oviposition scars, and eggs and larvae inside.

### PC Control

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

## Adult Trapping

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

## Exclusion of PC from Trees

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

PC Control on the Ground

This objective was not addressed in 2000.

## **RESULTS AND DISCUSSION:**

### PC Survey

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

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

Region	# Sites Surveyed	# Sites Positive for PC
1 – Tremonton to northern	170	14
border of Brigham City 2 – Northern border of	168	14
Brigham City to Weber Co.	414	85
3 – Weber Co. line to North		
Ogden	60	0
4 – Cache Co.: Wellsville to		
Mendon and Avon to		
Paradise	15	_0
Total	657	99 (99/657=15.1%)

# PC Host Range

In the field, PC larvae have been found in apple, apricot, cherry (sweet and tart), peach, and plum (cultivated and wild). No PC injury or larvae have been found in pear fruit in the field. Results of

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

Number per cage (5-10 fruits) after 5-8 days							
Fruit Host	# Feeding Scars	<b># Oviposition Scars</b>	# Eggs	<pre># Larvae Leaf Feeding?</pre>			
Cultivated Stone	e Fruits						
Apricot	40.6	13.8		9.2	Yes		
Sweet Cherry	9.7	8.3	0.9	2.4	Yes		
Tart Cherry	17.8	17.1	6.3	0.6	Yes		
Peach	2.7	0.8	0.9	0	Yes		
Mean number per cage (5-10 fruits) after 5-8 days							
<u>Fruit Host</u>	Feeding Scars	<b>Oviposition Scars</b>	Eggs	Larvae Leaf Feeding?			
Cultivated Pome	e Fruits						
Apple	9.4	8.6	4.8	1.0	Yes		
Pear	2.4	0.4	0.2	0	Yes		
Wild and Ornamental Fruits							
Wild Plum	21.1	7.8	6.1	0.2	Yes		
Black Hawthorn	n 7.0	3.0	2.2	0.6	No		
Ornamental Cra	bapple 3.4	0	0	0	No		

### PC Control

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

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

# Adult Trapping

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

# Exclusion of PC from Trees

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



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

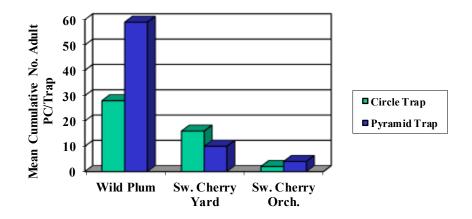


Figure 2. Comparison of adult plum curculio capture in circle and pyramid traps at three sites from May 2 – September 8, 2000.

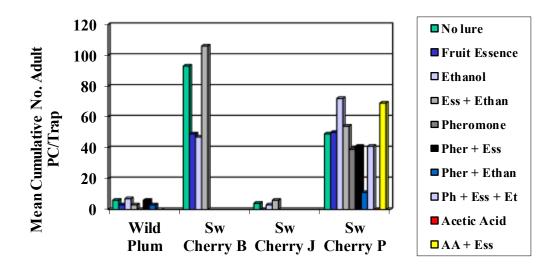


Figure 3. Comparison of adult plum curculio attractants in circle traps at one wild plum and three sweet cherry home yard sites from May 2 – September 29, 2000. The acetic acid and acetic acid + essence lures were only in place from June 9 – September 29.

# **CONCLUSIONS:**

• Where is PC? The PC population in northern Utah has been delimited to an approximately 50 sq. mile narrow strip along highways 38 and 89 from Honeyville to Willard, centered on Brigham City, in Box Elder County. PC is unlikely to be found beyond this area because of large stretches of vegetation without host trees (dominated by sage brush) surrounding the infested area. Survey traps and fruit injury samples conducted in surrounding areas of Box Elder County and in other fruit producing counties of northern Utah (Cache, Davis, Weber, Utah Counties) have all been negative for PC.

- What is PC's primary habitat and host fruit? 74% of the sites positive for infestation with PC are in Brigham City home yards. In the majority of these (69%), sweet cherry is the host tree. Therefore, the primary target for reduction and elimination of PC should be removal and treatment of home yard trees, especially sweet cherry.
- What is the potential for PC to attack native, naturalized or ornamental hosts? In the laboratory, PC demonstrated the ability to attack a native western U.S. pome fruit, black hawthorn (*Crataegus douglasii*). PC did not lay eggs in ornamental crabapple (*Malus* spp.). A non-native, naturalized host, American plum (*Prunus americana*), is a common host of PC in the infested area. All cultivated stone and pome fruits are potential hosts for PC, although peach and pear do not seem to be as readily attacked as others.
- Can PC be controlled with insecticides? The primary control of PC in the eastern U.S. is insecticides. Control of PC with diazinon or other insecticides appropriate for home yard use shows promise. Applying the insecticide at petal fall and repeating treatment 10-14 days later may improve the efficacy of diazinon over a single spray applied 1-2 weeks after petal fall.
- Adult attractants and trapping. No attractants evaluated show consistent increase in capture of adults in traps. Continued development and improvement of adult attractants, along with increased knowledge of PC host-finding behavior is needed.
- **PC population reduction program.** Removal of PC infested trees is the best approach to reduce and eliminate PC in northern Utah. Application of well-timed insecticides is an additional tool that has been implemented to a degree. Beyond these approaches, development of tactics for long-term population suppression is needed, such a biological control, mass trapping, removal or disturbance of overwintering sites, and others.

#### **PROGRESS REPORT 2000-01**

<b>PROJECT NO:</b>	5802
TITLE:	The interaction of mite feeding and water stress on apple photosynthesis and productivity.
YEAR INITIATED: TERMINATING YEAR:	2000-2001 CURRENT YEAR: 2001-02 2003-2004
PERSONNEL:	Elizabeth Beers, Entomology, WSU Wenatchee Leo Lombardini, Horticulture, WSU Wenatchee Frank J. Peryea, Soil Science, WSU Wenatchee Don Elfving, Horticulture, WSU Wenatchee Larry Schrader, Horticulture, WSU Wenatchee John Dunley, Entomology, WSU Wenatchee <b>Steve Welter, Entomology, UC Berkeley</b> Steve Drake, Postharvest, USDA-ARS, Wenatchee Jim McFerson, WA-TFRC, Wenatchee

Funding History: Funded 2000-01 (year initiated): \$28,746

#### **OBJECTIVES:**

- 1. Determine if water stress significantly increases the negative effect of mite feeding damage on 4 cultivars of apple. (2000-2002)
- 2. Determine diurnal pattern of CO<sub>2</sub> assimilation on mite-damaged and/or water stressed trees. (2000)

#### **PROCEDURES:**

- 1. Create mite-infested and mite-free trees in a uniform block of 5<sup>th</sup> leaf trees containing plots with the cultivars 'Oregon Spur', 'Golden Delicious', 'Fuji BC 2' and 'Royal Gala'. For each level of mite damage, create 2 levels of water stress (optimum water, stressed). Count mites *in situ* at biweekly intervals to calculate the cumulative feeding damage (mite days/leaf or cm<sup>2</sup>). Equalize crop load early in the season. Measure gas exchange with a portable IRGA on the entire tree canopy at intervals throughout the season. Count leaves, get average leaf area and use to obtain a measure of m<sup>2</sup> canopy surface area in order to calculate photosynthetic rate. Measure fruit size shortly after June drop and again at harvest. Measure fruit quality parameters and storageability of fruit.
- 2. Measure whole canopy photosynthesis at 2 h intervals during the photophase three times during the period of maximum water stress and mite infestation (July/August).

#### **Results and Discussion:**

Trees were hand-infested with mites from a laboratory colony of twospotted spider mite, *Tetranychus urticae* Koch. Repeated inoculations were performed on the high mite treatment plots. The low mite plots were sprayed with a combination of acaricides twice during season to prevent population development. Mites were counted weekly *in situ* with Optivisors (10x magnification) to prevent loss of leaf surface area, a critical component of the equation for calculating whole canopy photosynthesis. Mite days were accumulated to provided a summation of mite feeding stress according the following formula:

$$\Sigma CMD = ((p_1 + p_2)^*.5)^*(d_2 - d_1)$$

where  $p_1$  is the mite population (in average mites/leaf) at  $d_1$  (date 1) and  $p_2$  is the population on  $d_2$  (date 2).

Mite populations rose after an initial slow increase due to cool weather in the first half of the season. The cumulative mite days (CMD) did not exceed 500 mite days/leaf in the high mite plots, a moderate degree of stress (Fig. 1). Of the two high mite level plots, there was a slightly higher CMD level in the water stress treatment, indicating the possibility of a feedback mechanism, that is, that population growth is favored on water stressed plants.

The population was composed primarily of *T. urticae* in the plots with high mite levels (76-79%). The predominance of this species was expected because of the early season inoculations. The proportion of *T. urticae* in comparison to the European red mite (*Panonychus ulmi* (Koch)) was lower in the plots with low mite levels (46-59%). These plots were sprayed with acaricides, and it is possible that the acaricides killed a higher proportion of *T. urticae* than *P. ulmi*.

**Water Stress:** An above-ground microsprinkler irrigation system was superimposed on the existing solid-set impact system in the test orchard. Each 6-tree plot in each of the 9 rows of the orchard could be irrigated individually; however, the entire 3-row x 6-tree plot was treated as a unit. In addition, Sentek® Enviroscan soil moisture probes were installed in two the four replicates, with sensors located at 4 soil depths in each probe (20, 30, 40, and 60 mm). Capacitance data were downloaded weekly from the solar-powered datalogger. The Enviroscan monitors allowed real-time visualization of both amount of soil moisture and movement of irrigation pulses through the soil profile.

An arbitrary irrigation schedule was chosen the first season in order to assess soil/tree response. The highwater plots were watered weekly (8 hour sets), and the low-water plots were watered every other week. There was very little difference in the seasonal average soil moisture between the high and low moisture treatments (Table 1).

Mites	Water	Sensor depth	n	Moisture (mm)
High	High	20	2	27.9 a
Low	High		2	32.3 a
High	Low		2	30.7 a
Low	Low		2	32.1 a
High	High	30	2	31.8 a
Low	High		2	30.8 a
High	Low		2	31.1 a
Low	Low		2	30.6 a
High	High	40	2	32.4 a
Low	High		2	31.4 a
High	Low		2	29.5 a
Low	Low		2	29.2 a
High	High	60	2	32.7 a
Low	High		2	31.0 ab
High	Low		2	29.6 b
Low	Low		2	29.4 b

Table 1. Enviroscan soil moisture measurements, seasonal average, Blk. 29s, TFREC, 2000.

**Whole canopy photosynthesis:** (WCP) was measured on 'Oregon Spur' trees every three weeks on the following dates: July 26-28; August 16, 17, 21; September 6, 7, 11; October 2, 4, 5. On each sampling date, four trees (one tree/treatment) were measured approximately between 10 a.m. and 4 p.m. Total leaf area was estimated for each tree at each 3-week interval.

Leaf area data were analyzed as percent increase and as leaf area expansion rate (LAER, expressed as mm<sup>2</sup> of leaf area 'produced' by 1 cm<sup>2</sup> of leaf area per day). All trees indicated a similar leaf area growth rate for most of the period of investigation. The only exception was detected during the September measurements in which plants that received less water had a significant increase in percent growth and LEAR.

WCP data have not yet been analyzed statistically, but a visual examination of the daily trends seems to indicate that there was not a treatment effect on the photosynthetic and transpiration rate during the July and August measurements. The mite level seemed not to induce major effects on photosynthesis and transpiration rate. Statistical analysis of the daily trends will be performed shortly.

# **Conclusions:**

Conclusions at this point in time are only preliminary pending analysis of the photosynthesis data. There appeared to be little detectable effect of either mite or water treatments in the first season; however, the level of stress was most likely not severe enough in either case to cause a change. Both the levels of water and mite stress will be increased in the 2001 growing season.

## **PROGRESS REPORT**

<b>PROJECT NO:</b>	4095		
TITLE:	Development of bait-based monitoring systems for codling moth leafrollers and lacanobia fruitworm.		
YEAR INITIATED: TERMINATING YEAR:	2000-01 <b>CURRENT YEAR</b> : 2001-02 2003-04		
PERSONNEL:	Jay F. Brunner, Entomologist, and Mike Doerr, Research Associate, WSU-TFREC, Wenatchee Peter Landolt, USDA-ARS, Yakima		
FUNDING HISTORY:	\$30,158		

# Allocated FY 2000-01 \$30,158 Request for FY 2001-02 \$20,960

## **SIGNIFICANT FINDINGS:**

- Development of a "dry" food-bait attractant that can be placed into the same monitoring device as a pheromone lure was an important innovation in 2000. These attractants appear to be useful in monitoring a variety of noctuid pests including lacanobia fruitworm under all management practices, conventional or mating disruption.
- □ Standard load pheromone lures used to monitor codling moth in non-pheromone treated orchards were evaluated in the first and second generation. An experimental lure utilizing "flex" lure technology (Pherotech, Inc.) maintained a relative attractancy of approximately 50% of the septa changed every 12 days. Last Call-CM (Attract and Kill technology, IPM Technologies) was also evaluated as a possible low cost, long-acting lure. The Last Call-CM droplet caught moths for 60 days.
- High-load pheromone lures used to monitor codling moth in mating disruption orchards were evaluated during the second codling moth generation. The "bubble" lure (CM Superlure, Pherotech, Inc.) attracted equivalent numbers of moths as a 10X septum (Pherotech, Inc.) through 28 days. After that time the relative attractancy diminished.

# **OBJECTIVES**:

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

# **PROGRESS:**

# Lacanobia food-bait lures - Methods

A food-bait lure was developed by Dr. Peter Landolt for monitoring noctuid pests in orchards. The foodbait lure was a blend of acetic acid and isoamyl alcohol believed to be attractant specifically to noctuids. In previous tests the attractant was placed in "wet" traps where the moths were collected in a drowning solution. This was the first test of the noctuid attractant in a "dry" lure. Because of this development, the food-bait lure could be used in standard monitoring traps. This allowed for a direct comparison of the food-bait to a pheromone lure with all other conditions being equal. This attractant was deployed in general purpose bucket-style traps (Unitrap, Pherotech, Inc.) and placed in side-by-side comparisons with bucket-style traps baited with a lacanobia fruitworm pheromone lure (Scenturion, Inc.) Twelve orchards ranging from the Royal Slope area of Washington to Brewster were monitored. Orchards selected for study were thought to have lacanobia fruitworm populations based on previous studies. Furthermore, each orchard was chosen because of its history of reduced insecticide inputs. This was important in order to limit disruption to normal phenological development. One of the test orchards was also a lacanobia fruitworm mating disruption site (Lacanobia MD, 400 dispensers/a, Pacific Biocontrol). A possible benefit of food-bait lures should be the ability to monitor plots with the same lure regardless of the presence of mating disruption products. Each of the bucket-style traps had a small piece (2x1 inch) of "kill-strip" (Hercon Vaportape, DDVP toxicant strip, Great Lakes, IPM) placed in the collection area. A fresh kill strip was placed at the start of each of the two generations. The pheromone lures were replaced every 6 weeks and the food-bait lures every 4 weeks. The traps were monitored every week and the total numbers of lacanobia fruitworm, bertha armyworm and spotted cutworm males and females were recorded.

#### Results

Lacanobia fruitworm were collected at each of the sites in both the pheromone and food-bait traps. Adult lacanobia phenology was closely correlated between the two lure technologies. The food-bait lures attracted lacanobia males and females in a 50:50 ratio average over the entire season. There did not appear to be a significant sex ratio difference either at the beginning or end of the flight period. The food-bait trap was not specific to the lacanobia fruitworm and attracted many noctuid species. Included in the samples were bertha armyworm and spotted cutworm. This monitoring system may be ideal for monitoring all three of these important orchard pests although a certain skill level was required to differentiate the important pests from various other noctuids present. The mating disruption product shut down pheromone trap catch for both the first and second generations. However, the food-bait traps in both treatments attracted lacanobia males and females at equal levels throughout both generations.

### Discussion

The development of a "dry" food-bait attractant that can be placed into the same monitoring device as a pheromone lure was an important innovation in 2000. These attractants appear to be capable lures for monitoring a variety of noctuid pests under all management practices. These lures should prove to be valuable tools for both research of male and female behavior as well as the practical application of developing consistent treatments and reliable thresholds based on trap catch. If this technology can be developed for all lepidopteran pests, thresholds could be developed that would be consistent whether mating disruption was used or not. It remains important to determine ideal release rates as well as the area of attractancy for non-pheromone baits to best utilize this new technology.

## **Codling moth pheromone lures**

#### **Methods: Non-pheromone-treated orchards**

The experimental design was a randomized complete block. Tests were conducted in 6 non-pheromonetreated blocks at the Tree Fruit Research Center. The number of male moths captured in traps baited with the different lures was recorded every 2-3 days. To minimize position effects, traps were rotated each time they were inspected. Red septum lures were replaced after each complete rotation (12 days) during both generations. The traps used for this test were delta-style sticky traps (Scenturion, Inc.). Trap bottoms were replaced after a cumulative catch of 30 moths, more often if dirty.

**First generation:** An experimental standard load lure (flex lure technology, Pherotech, Inc.) was directly compared to the attractancy of a commercially available red septum loaded with 1 mg of codlemone (Pherotech, Inc.). This test included two red septum treatments. The first was a red septum changed at the

end of each rotation, and the second was a red septum that was changed every 28 days. The flex lure was not changed during the first generation. **Second generation:** Two experimental standard load lures were tested: a "flex" lure (Pherotech, Inc.) and a single droplet ( $50 \Box l$ ) of a commercially available attract-and-kill technology product (Last Call-CM, IPM Technologies, Inc.). The droplet of Last Call-CM was chosen as a potentially inexpensive alternative to commercially available lures. The Last Call-CM droplet "lure" was developed by placing a single droplet of Last Call-CM (cost approximately \$0.04) on a small piece of plastic and protecting it in a screen cage. We directly compared the attractancy of the two experimental lures and the commercially available red septum loaded with 1 mg of codlemone (Pherotech, Inc.). The standard 1X red septum lure was changed at the end of each rotation (12 days). The experimental lures were not changed during the entire test.

#### Results

In the **first generation** the relative attractancy of various standard load lures was tested during the first generation flight of CM. The "flex" lure maintained attractancy for the entire 60 days of the test. Across the whole test the flex lure attracted approximately 50% as many moths as the septum changed every 28 days and 33% as many moths as the septum changed every 12 days. In the **second generation** the flex lure attracted approximately 40% as many moths as the red septum; this difference was statistically significant. There was no significant difference between the Last Call-CM treatment and the red septum across the whole test.

## **Methods: Pheromone treated orchards**

The experimental design was a randomized complete block. Tests were conducted in 6 pheromone-treated blocks (Isomate C Plus at 300 dispensers/acre, Pacific Biocontrol Corp.) at the Tree Fruit Research and Extension Center. The number of male moths captured in traps baited with the different lures was recorded every 2-3 days. To minimize position effects, traps were rotated each time they were inspected. Red septum lures (10X) were replaced after each two completed rotations (14 days). The traps used for this test were delta-style sticky traps (Pherocon VI, Trécé, Inc. and Scenturion, Inc.). Trap bottoms were replaced after a cumulative catch of 30 moths, more often if dirty. Two high load lures were tested in the second CM generation: a bubble lure (CM Superlure, Pherotech, Inc.) and an experimental lure utilizing membrane technology (cookie-1148, Scenturion, Inc.). We directly compared the attractancy of the two lures and the commercially available red septum loaded with 10 mg of codlemone. Both long-life lures were fresh at the start of the second-generation test. All lures, with the exception of the red septum, were not changed during this test.

#### Results

The "bubble" lure was as attractive as a 10X septum (Pherotech, Inc.) through 28 days. After that time the relative attractancy diminished. The "cookie" lure attracted similar numbers of moths as the bubble lure through 28 days with the exception of the 21-day rotation where it attracted significantly fewer moths than the other lures. At 35 days, the red septum attracted significantly more moths than the long-life lures. There were no significant differences at day 42 or 49. All lures attracted statistically equivalent numbers of moths as the 10X septum changed regularly at the end of the flight, a potentially significant factor in management situations.

#### Discussion

It continues to be important to monitor the effectiveness of pheromone lures for attracting codling moth, especially with new technologies developing annually. Each new technology represents a potential for changes in treatment thresholds based on trap catch. Testing this year indicated that the flex lure attracted fewer moths than a red septum changed frequently. However, the flex lure maintained relative attractancy for the entire 60 days in both first and second generation tests. These data suggest that the flex lure could be an effective monitoring tool, but thresholds would need to be adjusted. The use of Last Call-CM as a

low cost alternative lure was encouraging. Monitoring codling moth in mating disruption orchards continues to be a limiting factor in developing reliable treatment thresholds based on trap catch. The pheromone monitoring system is not as efficient during the summer months. A stable long-life lure should reduce variation in trap catch, and the lures tested were an improvement to the standard red septum. However, these lures did not increase the attractancy of the monitoring system relative to a red septum changed frequently. These results were slightly different from those noted in previous tests where the "bubble" lure did attract more moths than the red septum. This may be an indication of variation that could be expected upon widespread implementation of this lure technology. It did not appear that either of the lures tested was sufficient to cover an entire generation, and thus a lure change would be required to completely cover the second generation. The potential for long term lures to reduce costs of both product and labor is significant.

#### **PROGRESS REPORT**

<b>PROJECT NO:</b>	6093		
TITLE:	Enhancing biological control of leafrollers through ground cover management and augmentation of alternate hosts for leafroller parasites		
YEAR INITIATED: TERMINATING YEAR:	1999-00 <b>CURRENT YEAR:</b> 2001-02 2001-02		
PERSONNEL:	Jay F. Brunner, Entomologist, WSU-TFREC, Wenatchee Kent Mullinix, Wenatchee Valley College/WSU, Wenatchee Nancy Decker, Associate in Research, WSU-TFREC, Wenatchee <b>Collaborators:</b> Tom Unruh, USDA-ARS, Wapato Keith Pike, Entomologist, WSU Prosser Don Elfving, Horticulturist, WSU-TFREC, Wenatchee David Granatstein, Area Extension Agent, WSU-TFREC, Wenatchee Elizabeth Beers, Entomologist, WSU-TFREC, Wenatchee		
<b>FUNDING HISTORY:</b> Allocated FY 2000-01: Request for 2001-02:	\$26,202 \$10,320 (+\$41,300 from IFAFS/RAMP)		

JUSTIFICATION: (See NEW 6093 in 1999-00)

# **OBJECTIVES:**

1. Determine the effects of cover crops in an apple orchard on the biological control of leafrollers and aphids and on other components of the arthropod community.

- 2. Determine the effects of cover crops on apple tree vigor, fruit size and fruit quality.
- 3. Determine management strategies for an alfalfa cover.

# **SIGNIFICANT FINDINGS:**

Second-year data were collected for all important apple pests and some natural enemies. For the winter of 1999-00, the overwintering beneficial insect population was three times higher in the alfalfa treatments than in the grass treatments.

- All plots were monitored with pheromone traps for codling moth and leafrollers throughout the season. Sentinel leafroller larvae (OBLR and Xenotemna pallorana) were placed in plots to assess the occurrence of parasites. A Braconid, Apanteles sp., and Tachinids were detected in all plots from early August through late September. Initially, parasitism by Tachinids was >60% for both treatments but dropped to a range of 26-39% during September. Parasitism by Apanteles was 26-33% during August and September (both treatments). This represents an increase in the presence of Apanteles since 1999 when only 7% parasitism was observed (in alfalfa treatments only). Colpoclypeus florus was observed in both treatments from early September through early October. Parasitism by C. florus was 47% in the alfalfa treatments and 56% in the grass treatments. During 1999, C. florus appeared on the same date; however, parasitism was lower (36%) and observed only in the alfalfa treatment. This parasitoid occurred naturally in the orchard without seeding C. florus from other sources. (Note: % parasitism refers to the % of total parasitism by the species indicated).
- In addition, this year, sentinel Xenotemna were set out within a .5 meter radius at five flagged sites in the transect row of each plot (50 larvae per plot). Larvae were collected once every seven days and brought to the lab for rearing out. During the summer, 7% of the seeded larvae were recovered from the alfalfa treatment. Parasitism by Tachinids was 3% in late June, and no parasitism was observed thereafter. Only 4% of the seeded larvae were collected from the grass treatment (from clover or dandelion leaves), and no parasitism was observed.
- During July of this year, 18 *Xenotemna* field-rearing cages were constructed of PVC and fiberglass netting. Three cages per plot were placed in the transect row and seeded with male and female *Xenotemna* adult moths (five females and five males per cage). Releases and reseeding were done on a weekly basis. Cages were moved weekly in an attempt to establish a population of *Xenotemna* in the orchard. During mid-August, weekly visual timed inspections of the cover were conducted in the transect rows to determine the presence/absence of *Xenotemna* egg masses. A total of 36 egg masses was found during the first inspection in mid-August with 22 egg masses found on alfalfa shoots and 14 egg masses found on clover or dandelion shoots in the grass plots. Weekly inspections continued through mid-September, but only four egg masses were found after the initial observation.
- Leafroller parasitism in the lower canopy was determined this year during early September. Parasitism of Pandemis by *Apanteles* sp. was 88% in alfalfa and 100% in grass.
- Spider mite and predatory mite populations were reduced this year (<1 mite per leaf) with the exception of apple rust mite counts, which increased slightly over 1999 counts. The peak count for ARM was two mites per leaf found in grass treatments during early July.

# **PLOT ESTABLISHMENT**

During the spring of 1999, two Fuji blocks (tree age five years) at the Wenatchee Valley College Auvil orchard were divided into six subplots (two treatments and three repetitions, alfalfa and grass). A "star" configuration with a transect from the border of each plot to the approximate center of each plot was designated as the "sample area." Thirty trees were selected within the star configuration and numbered 1-30.

## **ORCHARD PESTS - YEAR TWO OBSERVATIONS**

**Overwintering biodiversity:** Overwintering arthropods were trapped inside one-inch wide cardboard strips wrapped near the trunk base on each of 30 sample trees per plot during September of 1999. Bands were collected in late October and various species counted. During year one, the overwintering beneficial insect population was three times higher in the alfalfa treatments than in the grass treatments. Pest species collected were Noctuids, Lygus, Lygaeidae, winged green apple aphid, leafhopper and Arctiidae. Beneficial species collected were Aphididae, Hemerobiidae, Chalcidoidea, green lacewing larvae, Stethorus, Deraeocoris sp., Nabid nymph, and Syrphidae. Miscellaneous species included Collembola, weevil, Carabidae, Hymenoptera and Acarina. Total arthropod counts averaged 9.7 in grass treatments and 38.7 in alfalfa treatments.

Leafroller parasitism: In the spring, five tight cluster buds were collected from 30 trees in each plot and taken to the lab. A total of 29 buds of 900 buds examined was infested with Pandemis leafroller. Parasitism by Apanteles sp. occurred in 12.5% of infested buds. During 1999 no parasitism was observed. During early May, 100 LR infested shoots were collected from each plot and taken to the lab. Leafroller parasitism by Apanteles sp. was 3.3% in alfalfa treatments and 5% in grass treatments. During 1999, no parasitism was observed at this sample time.

**Codling moth and leafroller traps:** During early May, traps were placed in each treatment plot (two per replicate) to monitor the presence of two leafroller species, the obliquebanded leafroller (OBLR), Choristoneura rosaceana (Harris), and pandemis leafroller (PLR), Pandemis pyrusana. In addition to the above traps, live Xenotemna traps were introduced in all treatments. Three traps per plot were suggested; however, because the Xenotemna colony is diminished due to a laboratory mold, we set out only one trap per plot. A pheromone dispenser was constructed with 1-inch round hair rollers capped with 1-inch round plastic furniture caps. Twice weekly, a female moth was placed in the dispenser that adhered to the sticky paper in the delta trap. Pheromone traps were also placed to monitor codling moth activity and differences in densities. Traps were monitored weekly from June through early September. Peak PLR catches occurred in mid-June and again in late August. Total PLR trap counts for 2000 were five times higher than 1999 with no difference between treatments. Codling moth trap counts remained low with only 10 moths trapped all summer. Xenotemna trap counts totaled 11 with no difference between treatments. (Note: Sentinel Xenotemna were first released in early June. The first adults were released from cages in mid-July.)

Sentinel Xenotemna and parasitism: Beginning in mid-June, fourth instar Xenotemna larvae were set out within a 0.5 meter radius at five flagged sites in the transect row of each plot (50 larvae per plot). Larvae were collected once every seven days and taken to the lab for rearing out. Two weeks prior to setting out the first Xenotemna, all plots were mowed (every other row). The flail-type mower frayed the alfalfa tops that dried and became brittle in the hot weather prior to collection of the sentinel larvae. Initially, 50% of the sentinel larvae were recovered from the unmowed alfalfa rows, and none were recovered from rows mowed with the flail mower. A sickle–type mower was subsequently used which resulted in more successful recovery of sentinel larvae. Over the summer, 7% of the seeded larvae were recovered from the alfalfa treatment. Parasitism by Tachinids was 3% in late June, and no parasitism was observed thereafter. Only 4% of the seeded larvae were collected from the grass treatment, and no parasitism was observed. In both treatments, larvae were collected from clover or dandelion growth invading the dominant cover.

**Sentinel OBLR and parasitism:** Third instar OBLR were set out from early June through early October and collected seven days later. A Braconid, *Apanteles* sp., and Tachinids were detected in all plots from early August through late September. Initially, parasitism by Tachinids was >60% for both treatments but dropped to a range of 26-39% during September. Parasitism by Apanteles was 26-33% during August and September. Last year, parasitism by Apanteles was 7% (observed in alfalfa treatments only). *Colpoclypeus florus* was observed in both treatments from early September through early October.

Parasitism ranged from 47% in alfalfa plots to 56% in grass plots. During 1999, C. *florus* appeared on the same date; however parasitism was lower (36%) and observed only in the alfalfa treatment.

Leafroller infested shoots: Shoots high in the tree canopy were inspected (300 shoots per plot) to determine leafroller infestation levels from mid-May through late August. Infestation of leafrollers was 7% for both treatments in early spring but increased to dense infestations, 90% for both treatments by mid-July. Leafroller infestation appeared two months earlier in 2000, and densities were five times higher than in 1999.

**Leafroller parasitism in the canopy:** In early to mid-September, leafroller larvae were collected from the lower canopy of 30 sample trees per plot and taken to the lab for rearing out. Parasitism by *Apanteles* sp. was 88% in the alfalfa treatment and 100% in grass treatment. This sampling was not done in 1999. It will be important to determine parasitism levels during spring 2001 to assess overwintering parasitoid presence and levels.

**Western tentiform leafminer and parasitism:** Leaves were sampled during late June for western tentiform leafminer (WTLM) to determine the density of this pest and to assess levels of parasitism. WTLM appeared one and one-half months earlier this year, and parasitism by *Pnigalio flavipes* increased from 15% (both treatments) in 1999 to 76% this year in the alfalfa treatment and to 69% in the grass treatment. The average number of mines per leaf increased from <2 in 1999 to 3-4 mines per leaf in 2000.

White apple leafhopper: White apple leafhopper (WALH) density was determined by counting the number of nymphs on leaves (150-leaf sample per plot). Counts taken in mid-May and early September revealed densities of <1 WALH per leaf. WALH densities increased by approximately 50% in alfalfa treatments over the summer but remained the same in grass treatments.

**Mites:** Mites were sampled at biweekly intervals from early June through early September. Fifty leaf samples were collected from each replicate on each sample date, brushed in the laboratory and mites counted under magnification. The active stages and eggs of phytophagous and predaceous mite species were recorded. McDaniel mites (McD) and twospotted spider mites (TSSM) appeared first in late June, and counts increased until late August when predaceous mite counts peaked. In late July, TSSM incidence was three times higher in the grass treatments than in the alfalfa. European red mites (ERM) appeared in low numbers in early August, then disappeared. Typhlodromus occidentalis counts were three times higher in grass than in alfalfa at late August peak, and Zetzellia mali counts were almost two times higher in grass than in alfalfa at peak densities.

**Campylomma, Lygus, thrips and stink bug**: Another measure of arthropod predator activity within each treatment was made by four limb tap samples during the months of May and June. Campylomma densities increased six times over 1999 with two times more found in alfalfa than in grass. No Campylomma injury to fruit was detected. Thrips densities decreased from 1999 counts by 38% overall, with counts dropping 27% in the alfalfa treatment. No Lygus or stink bugs were observed. (Note: by August, high counts of adult Lygus were observed in sweep net sampling). Apple grain aphids and green apple aphids were found in equal numbers for both treatments with average counts of 175-178 aphids. (Note: no aphids were observed in 1999 limb tap sampling). Beneficial insects observed included lady beetle larvae and adults, parasitic wasps, arachnids, lacewing nymphs, syrphid flies and deraeocoris.

*Aphids*: Aphid densities were determined by counting aphid-infested leaves on five shoots in 30 trees per replicate. Shoots were examined for aphid infestation from late May through early August. By late July, densities of green apple aphid on more than five infested leaves per shoot reached a peak of 22% in alfalfa and 9% in grass treatments, subsiding to less than 1% for the remainder of the season for both treatments. Peak aphid densities occurred one month later this year and were three times higher. There was an abundance of lady beetles, damsel bugs, syrphid flies, and brown and green lacewing from late May onward. Numerous aphid mummies were evident by late June. Active rosy apple aphid colonies were initially observed in late June at peak densities for the season (7% infested shoots where >5 leaves per shoot were infested). RAA densities were two times higher in alfalfa than in grass. RAA infestations were below 4% by late July and declined to 0% by August 10th. Aphid infestations were not deemed injurious to the trees or to the crop. Beginning in late June, parasitized aphids were collected from alfalfa shoots and from apple leaves and sent to Keith Pike in Prosser for identification of parasitoid species. Aphid

predators observed in mid-July include lacewing larvae, parasitic wasps, deraeocoris, lygus, lady beetle larvae, damsel bug, arachnids, and lady beetles. Beneficial insect species and counts were the same for both treatments.

**Tree growth, biomass and nutrition:** Ammonium nitrate 34-0-0 was applied prebloom to all treatment plots at 0.175 lbs per tree, 45-50 lbs of actual N per acre. No other fertilizers were applied. Leaf analysis in July determined nitrogen (N) and zinc (Zn) levels for both treatments. Nitrogen levels in the alfalfa treatments ranged from 2.33-2.70% which is slightly higher than 1999 levels and still exceeding optimum N range. Nitrogen levels in the grass treatments ranged from 2.22-2.81%, raising N levels from below normal range in 1999 to above normal range this year. Zinc levels in the alfalfa ranged from 12-17 ppm, slightly higher than 1999 levels but still below normal range. Zinc levels in grass ranged from 10-17 ppm, essentially unchanged from 1999 levels and still below normal levels. During July, biomass samples were collected by clipping three 1-meter diameter areas at six inches above ground from each treatment plot. Composite samples were analyzed for total N and fresh and dry weights recorded. Results received from Cascade Analytical for alfalfa treatments ranged from 4.47-4.64% total nitrogen (excess N value) and were unchanged from 1999. Results for the grass treatments were 1.59-1.86% which represents a decrease from 1999 N values. The biomass fresh and dry weights for 2000 were so drastically different from 1999 as to consider the results an artifact due to sampling and/or analysis error.

During the second year, various weeds invaded the primary cover crops of alfalfa and grass. Analysis of the resulting cover composition was done by recording species observed within 10 randomly chosen 1meter areas per plot. The alfalfa treatment contained 48% alfalfa, 3% grass, 28% dandelion, 4% lambsquarter and 17% foxtail. The grass treatment contained .3% alfalfa, 40% grass, <9% clover, 21% dandelion, 2% lambsquarter and 28% foxtail. Reseeding will be done in early spring 2001 in an attempt to reestablish a more homogenous vegetation.

During April of 1999, 10 groups of five trees per treatment plot were chosen based on uniform characteristics. Trunk diameter measurements were taken at 20 cm above the graft union. In early spring of 2000, a large percentage of those trees was removed due to winter damage. New trees were selected and measurements taken during April of 2000. Comparison trunk growth data will be available by May of 2001. The impact of grass vs. alfalfa treatments on apple tree growth was evaluated by shoot growth assessments taken at the end of the summer growth period. Ten trees were preselected per plot, and one uniformly representative branch was chosen on each of the 10 trees. Shoots were measured and (cm) growth recorded for three shoot-type classifications: true terminal shoots, true lateral shoots, and bourse shoots. Comparison data will be available by the end of November, 2000.

*Cull analysis for October 1999:* One hundred randomly chosen apples were examined from the total cull bins harvested from each plot. The average percent damage for each treatment was as follows:

	Alfalfa	Grass
Codling moth	1%	1%
Aphid	0%	0%
Scale	0%	0%
Cutworm	1%	0%
Thrips	0%	0%
Lygus	0%	2%
Leafroller	11%	11%
Stink bug	23%	12%
Bitterpit	3%	3%

Russeting	10%	20%
Cull data will be avail	ilable for comparison by late	e November.

**Soils:** General fertility is assessed each April and September. To assess soil chemical and colloidal properties, samples were subject to a complete nutrient analysis (N, P, K, S, Mg, Ca, Mn, B, Zn, Mo, Cu, Fe) and measure of pH. Separate samples were taken from the drive row and the tree row at 12 inch depth using a 10-core composite for each treatment plot. Early spring soil (N) values in the tree row and drive rows were deficient for both grass and alfalfa treatments. September soil (N) values were deficient in the drive row for both treatments but exceeded optimum levels in the tree row for both treatments. Soil test results from September 1999 indicate that soil (N) was deficient in both the drive row and tree row for both treatments.

**Root boxes**: Six root boxes (one per plot) were installed in order to scan and view the interaction of the tree and cover crop root systems. Locations were established by choosing a healthy and uniform bearing tree. Measurements were taken 30 inches out into the row from the tree base, then 18 inches to the right of the tree, and a 7 inch wide rectangle was staked out. Each box was buried 37-38 inches deep with the lid level analogous with the slope of the land and about 1 inch below ground surface. Successful scanning of developing root systems was accomplished using Logitech Scan software with HP laptop computer and scanner powered by a marine battery. Apple and alfalfa root morphology are visible in the scanned images and have been sent to Pullman for further analysis. The second set of images will be available for comparison by late November.

**ANTICIPATED BENEFITS AND INFORMATION TRANSFER**: Increased biological control of leafrollers would reduce insecticide use in tree fruit crops. Information developed from the project will be reported at grower meetings.

#### FINAL REPORT

<b>PROJECT NO:</b>	4091		
TITLE:	Biology, behavior and management of cutworms		
YEAR INITIATED: TERMINATING YEAR:	1998-99 2000-01	CURRENT YEA	<b>R</b> : 2000-01
PERSONNEL:	Peter Landol Richard Zac	er and Mike Doerr, W t, USDA-ARS, Wapa c, WSU, Pullman ox, GRA, WSU, Pulli	
FUNDING HISTORY:	2000-01: \$2	1,270 20	001-02: \$0

#### **JUSTIFICATION:**

When chlorinated hydrocarbons were lost (DTT and then chlordane) as chemical controls in fruit orchards, many predicted that populations of cutworms and armyworms (Noctuidae) would soon increase and cause serious crop injury. This did not occur and for the past 20 years cutworms have been a relatively minor problem in most fruit orchards. With introduction of "soft" controls in apple orchards, especially mating disruption, critics again forecast increased problems with many pests as broadspectrum insecticide use declined. This has not occurred yet although it still could. What has happened, however, has been an increase in populations of cutworms and armyworms, especially in the central basin of Washington and northeastern Oregon. Armyworms often follow a cyclical pattern of population increase and rapid decline, and these pests have been only sporadic problems in selected orchards. Cutworms, however, have been more of an annual problem in orchards and in the last two years one species, *Lacanobia subjuncta* (aka - lacanobia fruitworm), has caused considerable damage to fruit. One grower in northeastern Oregon has reported a loss of approximately \$30,000 in one year to this pest.

There has been no active research on Noctuidae in Northwest fruit cropping systems for nearly 15 years. With the passage of FQPA-96 and the potential loss of older insecticides (for instance, EPA restrictions on the summer use of Lorsban or export restrictions on Thiodan treated orchards), it was time to revisit this pest complex and address how it will be managed in future years. The presence of lacanobia fruitworm suggests that there may have been some important shifts in the species complex of Noctuidae in Washington. If indeed this has occurred, it is critical to understand why and how this might influence pest management programs. This proposal outlined a study aimed at characterizing the noctuid complex in Washington, determining the phenology of key species and developing monitoring and management tactics to prevent crop loss.

#### **OBJECTIVES**:

- 1. Survey noctuids found in orchards throughout Washington identifying the most common species present and host plants they utilize.
- 2. Develop a predictive model for *L. subjucta* that will predict the presence of specific life stages during the growing season.
- 3. Develop chemical control tactics that will successfully suppress *L. subjucta* and other climbing cutworm species that threaten fruit crops.
- 4. Develop monitoring methods specifically for L. subjuncta based on larval and adult behavior.

# SIGNIFICANT FINDINGS and ACCOMPLISHMENTS:

- The biology of *Lacanobia subjuncta* (lacanobia fruitworm) has been examined at several locations and accurately characterized for Washington conditions. This provides a solid basis for developing sound pest management strategies for dealing with this new pest.
- Mark Hitchcox was awarded his MS in Entomology for research on lacanobia fruitworm biology and phenology. Mark is now an employee of the Washington State Department of Agriculture working out of the Yakima office.
- A degree-day model has been developed that accurately predicts the development of lacanobia fruitworm. The lower threshold is 7°C (44°F) and an upper threshold is 31°C (88°F). Degree-day requirements based on constant temperature data for eggs, larvae and pupae were 137.1, 874.0 and 535.8 degree-days, respectively. Eggs, larvae and pupae required field temperatures of 130.7, 946.9 and 492.3 degree-days, respectively. The pre-oviposition period was estimated at 280 degree-days. This model will be very useful in timing control tactics and sampling activities.
- Following a fairly long pre-oviposition period, lacanobia fruitworm females lay eggs over a long period. At constant temperature, 22.5°C, lacanobia fruitworm lay eggs for 22 days. Fifty percent of eggs were deposited in 8 days. Field sampling of egg masses indicated the average mass contained approximately 150 eggs. Based on laboratory observations, a reasonable estimate for average female fecundity is 1000 eggs.
- Lacanobia fruitworm feeds on several broadleaf weeds (see report by Landolt) but is only a pest on apple.
- We hypothesize that lacanobia fruitworm became a pest because it developed resistance to selected organophosphate and carbamate insecticides. Lorsban, Lannate and Thiodan provided superior control of summer larvae. Success and Confirm were effective against young larvae. Surround is effective timed at the early hatch period but requires multiple applications. Ecozin and Avaunt\* have utility as potential controls, but more research is needed on these products. Bt products and pyrethrums do not provide acceptable levels of control.
- The distinguishing features of various noctuid larvae found in orchards was described along with feeding behaviors. These will be published as a guide for growers and crop consultants.
- Various monitoring methods were used to measure densities of different lacanobia fruitworm life stages or feeding activities of larvae. The best predictor of fruit injury was obtained with visual observation of larval feeding activity on foliage. Percent shoot feeding of 30-35% correlated with a 1% fruit injury rating after the first lacanobia fruitworm generation. Peak pheromone trap captures of less than 150 per week did not appear to justify control applications against the first generation.
- There is promise that a food-bait trap developed by Dr. Peter Landolt will provide a method of monitoring lacanobia fruitworm adults, providing a better prediction of this pest's density in orchards and possibly forming the basis for a lure and kill technology.

(A more detailed report can be obtained by requesting it from Jay Brunner [jfb@wsu.edu] or Mike Doerr [mdoerr@wsu.edu], WSU-TFREC, 1100 North Western Avenue, Wenatchee WA 98801.)

# **PROGRESS**:

**Biology, phenology and survey** - The initial phase of any good scientific study is to "know the enemy." In this study cutworm larvae were collected from orchards throughout the state. As part of an effort to learn more about the life cycle and other aspects of cutworm biology, we examined its seasonal phenology and host plants utilized. We especially hoped to gain a preliminary insight into the population dynamics of the lacanobia fruitworm on a local scale by looking at a variety of plants as potential hosts and by sampling fruit trees throughout the season. Larvae were associated with the plants on which they were feeding at the time of collection. Because many cutworm species only feed at night, collections were conducted when necessary to make correct plant host associations. Larvae were reared on host plants or artificial diets as deemed appropriate. National experts identified adults to species.

Sampling for adult moths was accomplished with general purpose bucket-style traps (Unitrap, Pherotech, Inc.) baited with the sex pheromone of female lacanobia fruitworm. Traps were operated from late April through early October and serviced 1 to 3 times per week. Lures were replaced monthly. Traps were placed in 27 locations from Milton-Freewater, Oregon, through Yakima, Washington and Othello to Bridgeport, Washington.

Sampling for larvae was accomplished by tapping tree limbs with a sturdy rubber hose, dislodging lacanobia fruitworm larvae onto a standard beating tray used to sample pear psylla. Larvae collected on beating trays were brought into the laboratory for positive identification. All larvae in question were reared to the adult stage for identification. During peak periods of larval activity similar sampling was conducted on other trees and on weeds within apple orchards. Sampling on alternate hosts was conducted comprehensively twice, first in late June into early July, and again in September.

Season-long sampling for male lacanobia fruitworm moths indicated two major periods of flight. The **first flight** occurred roughly from **late April through June**, **with peak moth capture in early June**. The **second flight** began in **late July and lasted through September**. Lacanobia fruitworm moths were captured at each of the 27 locations monitored. Densities varied dramatically among locations from peak catches of 10 to over 850 per week.

Season-long sampling for **lacanobia fruitworm larva**e indicated two periods of larval activity. The **first generation of larvae occurred from early June through July**. The **second generation of larvae began in mid-August and was present through October**. Season-long larval sampling was conducted in apple orchards only. Although lacanobia fruitworm moths were captured at each location, larvae were not always detected.

**Lacanobia fruitworm larvae were collected** on a number of tree and weed species. Larvae were found **primarily on apple**, and only small numbers were found on pear, cherry, apricot and prune. In orchard ground cover larvae were most often collected from mallow, curly dock and dandelion.

Small numbers of parasites were obtained from field-collected lacanobia fruitworm larvae. Although not yet identified to species, these included several hymenopterous parasites and at least 3 species of Tachinidae.

**Predictive degree-day model**: The lacanobia fruitworm has proven to be the most damaging of the noctuid pests surveyed in this project. Insecticides are the primary means to control the lacanobia fruitworm. While Lorsban (organophosphate) and Thiodan (organochlorine) control all larval stages, selective or "softer" chemicals [e.g. Success (Naturalyte<sup>®</sup> fermentation product)] are most effective against young larvae. Optimizing the application timing of "softer" insecticides is critical to maintaining control of the lacanobia fruitworm while promoting integrated pest management during the summer.

Temperature-dependent development data were developed for eggs, larvae and pupae of the lacanobia fruitworm at constant temperatures ranging from  $10.0^{\circ}$ C- $37.5^{\circ}$ C. Constant temperature development was also compared to development under the fluctuating temperatures of field rearing. Minimum development temperature thresholds for eggs (Fig. 1), larvae and pupae of 6.6, 5.9 and 4.9°C, respectively, were established by extrapolating the linear regression equation for development to the *x*-axis. Optimal temperatures of development as described by McDonald (1990) for eggs, larvae and pupae were 28.8, 30.0 and 27.5°C, respectively.

Degree-day requirements were estimated from constant and fluctuating (field) temperature tests. Using a base temperature of 7°C (44 °F) and upper threshold of 31°C (88°F), average degree-day requirements based on constant temperature data for eggs, larvae and pupae were 137.1, 874.0 and 535.8 degree-days, respectively. Eggs, larvae and pupae required an average of 130.7, 946.9 and 492.3 degree-days, respectively, in the fluctuating temperatures. Preoviposition period data were collected from only one constant temperature, 22.2°C, so the estimate for degree-day requirements was less robust. At 22.2°C, the preoviposition period was 240 degree-days. This was adjusted to a higher value, 280 degree-days for model development, based on our experience with rearing lacanobia fruitworm at other temperatures. It should be noted that the degree-day requirements for the sixth instar are much more than the other instars. The lacanobia fruitworm sixth instar larvae entered a prepupal phase. This period likely accounted for the extended period noted for sixth instar larvae.

Figure 1. Lacanobia fruitworm egg development rates (1/days) under constant temperature regimes. Degree-day models were bound by the following parameters:  $T_1$  lower threshold of development,  $T_o$  optimal rearing temperature and  $T_u$  upper threshold for development.

Oviposition and hatch were monitored by searching for and flagging egg masses in the field. Data were presented as cumulative percentage of activity at degree-days from biofix. Oviposition began at 280 degree-days past biofix and lasted until 1000 degree-days. Hatch started at 400 degree-days past biofix and lasted until 1150 degree-days. These data are closely associated with model predictions developed from laboratory data.

The relative abundance of larval stages was measured by collections from each of the sample orchards during the first generation. Larvae were more difficult to collect, as they matured and distributed themselves throughout the tree canopy and into the ground cover. First instar larvae were initially collected at 400 degree-days after biofix and continued to be collected in samples through 1400 degree-days. These data were consistent with the long oviposition and hatch periods. The first occurrence of sixth instar larvae was at 1000 degree-days. Larvae were collected through 2200 degree-days past biofix during the first generation. First generation degree-day estimates and field validations are summarized in Fig. 2.

Figure 2. Degree day estimates for important phenological developments during the first generation of lacanobia fruitworm.

A stated purpose for developing a predictive model was to aid in the timing of insecticide applications. For example, an ideal timing for the application of a "soft" pesticide such as Success would be when the majority of eggs had hatched and while most larvae were still of a susceptible stage. Compiling the first generation information presented above, 80% egg hatch occurred at approximately 700 degree-days from biofix. At 700 degree-days the majority of larvae were in instars 1 through 3. The presence of more mature larvae was not noted until about 1000 degree-days. Therefore, the ideal timing for a Success application would be between 700 and 850 degree-days after biofix. The 150 degree-day window should provide sufficient opportunity to apply the insecticide during good weather conditions.

**Chemical controls**: Chemical control tactics to suppress the lacanobia fruitworm below damaging levels were evaluated through a series of laboratory bioassays and large plot field trials. Our ability to test experimental insecticides was limited because measurable lacanobia fruitworm populations generally occurred in fully mature apple orchards. Lorsban (chlorpyrifos) and Thiodan (endosulfan) controlled all larval stages. However, summer use of Lorsban is now banned, and use of Thiodan is restricted because of export concerns. Success (spinosad) and Confirm (tebufenozide) had good activity against young larvae. Confirm will likely be replaced by Intrepid (methoxyfenozide) which appears to have greater activity, at least in laboratory tests. Surround provided good suppression of lacanobia fruitworm with the best timing during the early egg hatch period. Ecozin appeared to be effective although multiple applications appear necessary. Ecozin did not result in immediate kill, but delayed mortality was noted and more testing is required before recommendations can be made. Avaunt, an experimental insecticide, demonstrated good activity against lacanobia fruitworm and may soon be registered for use on apple. Pyrethrum had some effect suppressing lacanobia fruitworm populations, but its short residual activity probably limits utility in all but organic orchards. Lannate (methomyl) was very effective but because it disrupts integrated mite control its use should be restricted to emergency situations only. Field observations of Bacillus thuringiensis products suggested that their use had little effect on lacanobia fruitworm.

In the fall of 1999 a laboratory colony of lacanobia fruitworm was established at the Tree Fruit Research and Extension Center. From this colony we began a series of dose-response bioassays on neonate larvae. These assays should help to determine which insecticides show promise as controls in the future. From bioassay evaluations conducted to date, products that show promise as controls for lacanobia fruitworm are Confirm, Intrepid, Lorsban, Malathion, Thiodan, Asana, Larvin\*, Lannate, Avaunt\*, Success and Proclaim\* (those products followed by \* are not yet registered for use on tree fruit). Preliminary studies with our laboratory colony indicate that they become much more susceptible to organophosphate and carbamate insecticides after only 3 generations, suggesting that resistance may not be strongly fixed in field populations.

**Behavior:** An understanding of the life cycle and behavior of noctuid pests aided in the diagnosis of foliage or fruit injury. The spotted cutworm overwinters in the larval stage and is present in apple orchards during the spring. Spotted cutworm larvae cause damage at a time when the lacanobia fruitworm is still in the overwintering pupal stage. Bertha armyworm is most often associated with injury to pear, a host on which lacanobia fruitworm is rare. Bertha armyworm requires a primary host other than tree fruits to complete life cycle. Thus, bertha armyworm was most often associated with orchards having poor broadleaf weed control. There were many orchards where the lacanobia fruitworm and the bertha armyworm coexisted. Arctiid larvae were sufficiently different in appearance from the lacanobia fruitworm that distinguishing the two was not difficult. Although arctiids laid eggs on apple at the same time as the lacanobia fruitworm, their larvae probably do not feed very long on apple. Feeding behavior of redhumped and yellownecked caterpillars (family Notodontidae) was sufficiently different from the lacanobia fruitworm that distinguishing between them was relatively easy. In our studies care was always taken to record fruit and foliage injury that was consistent with lacanobia fruitworm behavior. A photo key of the distinguishing characteristics of key noctuids is being developed to aid in species identification.

**Monitoring methods:** Three sampling methods have proven effective in monitoring lacanobia fruitworm populations. A general purpose bucket-style trap baited with a sex pheromone was highly attractive to males, a limb tap/beating tray technique was useful as a direct sampling method for larvae, and visual examination of foliage for signs of feeding activity provided a good indicator of larval presence. In addition, a food-bait type lure developed by Dr. Peter Landolt was useful for monitoring all noctuid adults.

Sex pheromone traps were used to monitor lacanobia fruitworm males in 27 locations over three years. The pheromone was highly attractive and high captures of moths occurred (600 moths/week) in orchards containing few larvae. In order to limit the effect of outside populations biasing trap data, pheromone traps should be placed in the center of large orchard blocks (10 acres or larger). Traps should be in place by full bloom to establish biofix in order to use the degree-day model to time spray applications.

Direct sampling of larvae using a limb tap/beating tray technique required a large number of samples to accurately measure populations. At least 50 beating tray samples spread through a large block were necessary to obtain an accurate measure lacanobia fruitworm density. Larvae were more likely to be dislodged where foliage was most dense. Therefore, sampling should be concentrated in the lower interior of the trees. Greater numbers of small (instars 1-4) larvae were dislodged with beat tray sampling. This method should be used when 80% hatch is predicted or just prior to a pesticide application.

The most reliable measure of lacanobia fruitworm density was a visual inspection of foliage feeding. It was best to conduct this sample after egg hatch was complete and before larvae became mature. In general, this occurred near the end of June. While this sample timing may be too late to make a decision to apply selective insecticides, it can be used to determine the need for insecticides that will protect the crop from losses due to feeding of large larvae. Visual inspection also requires that a large number of trees be examined because lacanobia fruitworm larvae are not uniformly distributed throughout the orchard. Twenty shoots from 50 trees should be examined in a large block, noting the percentage of shoots that show feeding damage.

**Thresholds**: In an effort to establish treatment thresholds the relationship between pheromone trap catch, shoot injury, beating tray samples and fruit injury was examined. Data were taken from orchards that had limited insecticide inputs such as from untreated portions of chemical trials. Our ultimate goal was to predict fruit injury from various measures of lacanobia fruitworm populations or activities. However, it turns out that there is not a good correlation between any measure of lacanobia fruitworm density or activity and fruit injury. This is most likely because lacanobia fruitworm only incidentally feeds on fruit, and horticultural effects like crop load, clustering of fruit, shoot growth, weed management and fruit maturity can all affect the amount of fruit damage that occurs.

The relationship between foliage feeding and fruit injury is presented in Fig. 3. A 30-35% level of shoot feeding was correlated with 1% fruit injury. It appears that apple orchards can tolerate a high lacanobia fruitworm density before significant fruit injury occurs. However, this threshold is based on foliage feeding at 100% egg hatch and was thus too late to apply insecticides that must be targeted at young larvae. Therefore, treatment thresholds based on trap catch or beating trays were needed to predict either fruit injury or shoot infestations.

Figure 3. Correlation of lacanobia fruitworm larva infested shoots in late June and fruit injury noted at the end of the first generation (early August).

Peak trap catch provides an indicator of population level in an area and can serve as a presence/absence indicator. Orchards with peak trap catch less than 150 moths/week did not have shoot injury in the critical 30-35% range. The problem was that some orchards had very high peak trap catches but had low levels of foliage feeding. Therefore, a peak trap catch that exceeds 150/week should trigger a search for egg masses in late May or early June and/or use of beating tray samples and foliage inspection in mid-June to further assess lacanobia fruitworm densities. If egg masses or larvae are not detected in the orchard, then controls are most likely not required. A sample of neighboring blocks or areas containing weeds such as mallow, curly dock, dandelion, pigweed or lambs quarter may reveal the external source of moths. What constitutes a treatable population is the most important decision for managing lacanobia fruitworm. It is apparent that, after three years of intensive sampling, no single measure of lacanobia fruitworm density provided an accurate and reliable treatment threshold.

<b>PROJECT NO:</b>	4093		
TITLE:	Evaluation of sprayable pheromone and attract & kill for codling moth and leafroller control.		
YEAR INITIATED: TERMINATING YEAR:	1998-99         CURRENT YEAR:         2001-02		
PERSONNEL:	Jay F. Brunner, Entomologist, Wenatchee Tom Fruit, Ag Research Tech II, Wenatchee Tomislav Curkovic, GRA, Pullman Peter Landolt, USDA-ARS, Wapato		
<b>FUNDING HISTORY:</b> Allocated FY 2000-01 Request for 2001-02	\$ 35,330 \$ 16,280 (+\$41,300 from IFAFS/RAMP)		

# **JUSTIFICATION:**

See New 4093 proposal submitted in 1998.

# **OBJECTIVE**:

Develop a pheromone-based management program for codling moth and leafrollers by:

- 1. determining if sprayable pheromone technology (3M Corporation) will provide control of codling moth and leafrollers in commercial orchards.
- 2. developing a leafroller attract & kill product for PLR and OBLR and determining if these will provide commercial level control of these pests.
- 3. determining if the efficacy of new hand-applied dispenser systems (Pacific Biocontrol) will provide suppression of codling moth and leafrollers equal to current technology.

# SIGNIFICANT FINDINGS:

- Sprayable leafroller pheromone showed variability in suppression of moth capture in traps. Larval densities and fruit injury were reduced by pheromone applications at one location.
- Sprayable leafroller pheromone showed a rate response in moth capture suppression from 5 to 40 grams AI/acre. Method of application, conventional versus Proptec, did not seem to have an effect on suppression of moth captures.
- Hand-applied leafroller dispensers reduced moth captures >90% and reduced larval densities, as well as fruit injury, in some locations. There is an accumulative effect on leafroller populations over time.
- Consep's puffer technology was evaluated at two sites. Reduction of moth captures in traps was <80%.</p>
- Last Call-OBLR (attract & kill) suppressed moth captures in large blocks treated with 600 or 1200 drops by >90% in the second flight. However, Last Call-OBLR and PLR formulations were not competitive in attracting moths to pheromone traps.
- A new paraffin emulsion pheromone formulation was tested against leafroller and codling moth. It is easy to apply, and suppression of trap capture was sufficient to warrant further research.

# **PROGRESS:**

**Sprayable leafroller pheromone - Methods:** 3M sprayable pheromone was applied twice at 20 or 40 grams active ingredient per acre at leafroller biofix and three weeks later for each flight. Each study site consisted of approximately 30 acres per location divided into three 10-acre blocks. The control block (non-pheromone treated) in each case was upwind from those treated with pheromone. All blocks were

treated the same for pests other than leafrollers as grower or consultant saw fit. Each block was monitored with Delta traps baited with either a high load (10 mg) or standard load (1 mg) lure. Bait traps were checked weekly, adults collected and recorded, and mating success determined by dissecting females. At petal fall, larvae were sampled by examination of at least 1000 shoots (10 shoots from 100 trees) per block (treatment). In summer (mid- to late July), larvae were sampled again using the same method. A preharvest fruit injury assessment was made in mid- to late August. Twenty fruits from 50 trees (1000 fruits total per block) were visually inspected for leafroller fruit injury.

**Results**: Spring larval populations in all orchards and treatment areas were undetectable based on samples taken. At Stemilt and Saddleback locations the sprayable pheromone treatment did not reduce moth captures during the first generation. At the Allred location OBLR moth captures were high, and the spayable pheromone treatments reduced moth captures in the first generation. In the second flight PLR captures were not suppressed by sprayable pheromone treatments at the Stemilt location. Moth captures at the Allred and Saddleback locations were suppressed by the sprayable pheromone treatments in the second flight. Summer leafroller larval populations somewhat reflected moth captures in pheromone traps. No larvae were detected at the Stemilt location in any treatment. Summer OBLR larval populations were higher in the non-pheromone treated plots at Allred and Saddleback than either of the sprayable pheromone treated plots. Fruit injury was detected only at the Allred location but was lower in the pheromone treated plots than the non-pheromone treated plot. Few moths were captured in the bait-pans. Most moths were captured at the Allred location, and the percent mating was similar in all treatments.

**Methods**: A sprayable leafroller pheromone was applied to large plots of apple orchard at four rates, 0, 5, 10, 20 and 40 g AI/acre. Moth capture in the treatments was assessed using Delta pheromone traps.

**Results**: There was a rate response noted with the sprayable pheromone treatments, with 40 g AI/acre having the greatest effect.

Hand-applied dispensers: Test 1 - Methods: This study was conducted at six locations. Each location consisted of approximately 30 acres divided into three 10-acre blocks. There were two pheromone treated plots along with a non-pheromone treated control. All blocks were treated the same for pests other than leafrollers as grower or consultant saw fit. Pheromone treatments were either 400 Isomate-LR (80 mg) dispensers per acre or 200 Isomate-LR+ (250 mg) dispensers per acre. Dispensers were applied prior to biofix (first moth flight in the spring) and placed approximately 3 feet from top of the trees. The 400 Isomate-LR dispensers were applied again at the beginning of the second flight. Each block was monitored with Delta traps baited with either a high load (10 mg) or standard load (1 mg) lure. Traps were checked weekly, the position rotated at each examination and number of moths counted and recorded. Bait traps were paired with a Delta-style trap to assess adult flight (male and female) and mating success of females trapped. These were checked weekly and adults were collected and recorded. Female leafrollers were dissected to determine mating status. Blocks were extensively sampled approximately one week after the application of delayed dormant treatments. Four hundred fruiting buds per block were collected (five buds from 20 trees in each quadrant per treatment) and dissected to assess overwintering LR larval populations. At petal fall larvae were sampled by examination of at least 1000 shoots (10 shoots from 100 trees) per block (treatment). In summer (mid- to late July) larvae were sampled again by examining at least 1000 shoots (10 shoots from 100 trees) per block (treatment). A preharvest fruit injury assessment was made in mid- to late August at two locations. Twenty fruits of 50 trees (1000 fruits total per block) were visually inspected for leafroller fruit injury.

**Hand-applied dispensers: Test 2 - Methods**: Similar tests were conducted in three locations incorporating a fourth plot, (10-acre addition) treated with 200 Isomate-LR (80mg) dispensers per acre. This experiment satisfied all the conditions of the first.

**Results**: Overall, after the second year of leafroller mating disruption studies at most trial locations leafroller populations had declined to very low levels. All of the leafroller pheromone dispensers suppressed overall trap catch between 94% and 98% in the first flight for the obliquebanded and pandemis leafrollers at all rates tested. In some blocks, second flight trap reductions were not as consistent as in the first flight. At some, though not all locations, trap capture reductions were reflected in low summer larval densities and reduced fruit damage at harvest. There seems to be an accumulated effect of mating disruption on leafroller populations over time. There were no significant differences between treated and untreated blocks in mating success of females.

**Puffer Technology - Methods**: Consep's aerosol puffer units loaded with single leafroller pheromone components were placed in two orchards. Each unit was loaded with 230 ml that released a 10 mg puff every 30 minutes. These units were placed on the perimeter of a 20-acre block every 210 feet (approximately one per acre) with additional units on interior of the block. Another 20-acre block adjacent and upwind of the pheromone treated block was used for a control. Both blocks were treated the same for pests other than leafrollers as grower or consultant saw fit. Blocks were monitored with Delta traps baited with a high load (10 mg) lure. There was one trap per acre (20 total for each block). Traps were spaced equidistant from one another and the puffer units on the perimeter and interior of the treated block. Traps were spaced equidistant from one another throughout the control block. Traps were checked once per week, the number of leafrollers was recorded, and traps were placed/replaced within the top 3 feet of the tree canopy. Trap bottoms were replaced when excessive wing scales and other debris had accumulated or at lure change.

**Results**: The puffers reduced moth captures in traps in both treated blocks to approximately the same degree. In the orchard with a low leafroller population, suppression of moth capture was 76% in the 1st flight when compared to the untreated orchard. In the orchard with the high leafroller population, suppression of moth capture was 79% in the 1st flight when compared to the untreated orchard. The pheromone canister ran out of pheromone part way through the second flight. Results are interesting and warrant further investigation.

Attract & Kill - Methods: IPM Technologies' Last Call-OBLR was applied once at 600 and 1200 drops/acre at leafroller biofix for summer generation, 2nd flight. Each experiment consisted of approximately 15 acres divided into three five-acre blocks. All blocks were treated the same for pests other than leafrollers as grower or consultant saw fit. Each plot was monitored with Delta traps baited with either a standard load (1 mg) or low load (0.1 mg) lure. There were two traps of each lure-load per block (12 total for each experiment). Traps were spaced equidistant from one another, alternating traps with different lure-loads. Traps were checked and rotated twice per week and the number of leafrollers recorded. Trap bottoms were replaced when excessive wing scales and other debris had accumulated.

**Results**: Last Call-OBLR showed similar results for all experimental blocks. Pheromone traps showed consistent reductions of over 90% in treated plots compared to untreated plots. The higher rate of product (1200 drops/acre) had a 2% decrease in trap catch compared to the low rate (600 drops/acre). Further studies should be done evaluating different rates, effects on larval densities, and fruit damage.

**Methods**: IPM Technologies' Last Call-OBLR and Last Call-PLR were placed as droplets in pheromone traps and compared with standard lures for attraction of both species. Lures (Last Call or septa) were placed inside Delta traps. Four traps of each lure type were used. Traps were checked every 2-3 days, moths counted and removed, and position of traps rotated at each inspection.

**Results**: Monitoring lasted for 45 days. The Last Call-PLR droplet attracted only 6% of the standard lure and Last Call-OBLR droplet attracted only 10% of the standard lure. Improvement in the attractive ability of Last Call leafroller products is recommended to improve product efficacy.

**Confuse LR – Methods**: A paraffin emulsion containing codling moth and leafroller pheromone (Gowan, Inc.) was tested for the first time. Treatments were applied at three rates, 15, 30 and 60 g AI/acre on 15 Aug in an orchard located near Othello, Washington (Royal Slope). Plot size (unreplicated) was approximately 0.5 acres per treatment. The same treatments were also applied to the Wenatchee Valley College orchard on 16 Aug and to the Double Diamond orchard on 22 Aug where in both orchards the treatment plot size was approximately three acres. Four Delta traps were placed in each treatment plot. Two were baited with PLR pheromone lures and two were baited with OBLR pheromone lures at the Royal Slope and Double Diamond orchards. At the Wenatchee Valley College orchards only PLR pheromone baited traps were used. Two were baited with 0.01x lures (low load) and two with 1x lures (normal load). Traps were put in place at the time of application, placed in the mid-canopy near the center of each plot. Trapped moths were counted and removed twice weekly and trap locations rotated.

**Results - leafroller:** Trap catch was reduced relative to the control in all trials. A rate effect was also observed in all trials with the 60 g AI/ acre rate producing the highest level of trap capture suppression. Leafroller populations in the Double Diamond orchard were low when treatments were applied and were suppressed to near zero for the duration of the test. The Royal Slope plot had high populations of leafrollers, especially PLR. Data suggest that the Confuse-OBLR formulation provided better trap capture suppression of OBLR than PLR.

**Results - codling moth:** Confuse-CM reduced trap catch for approximately three weeks until the peak of the second flight. A slight rate effect was observed with the high rate (60 g AI/acre) resulting in the highest level (74%) of trap capture suppression. Higher rates or a second application may be necessary to achieve adequate control

<b>PROJECT NO:</b>	4094			
TITLE:	Monitoring and Control of Stink Bugs			
YEAR INITIATED:	2000-01 CURRENT YEAR: 2001-02 TERMINATING YEAR: 2001-02			
PERSONNEL:	Jay F. Brunner, Entomologist Christian Krupke, Research Associate Mike Doerr, Research Associate <b>Collaborators:</b> Peter Landolt, USDA-ARS, Yakima Mike Bush, WSU Cooperative Extension, Yakima Jocelyn Millar, Dept. of Entomology, UC-Riverside			
FUNDING HISTORY: Allocated FY 2000-01 Request for 2001-02	\$57,330 \$44,780 (+\$16,620 from IFAFS/RAMP)			

JUSTIFICATION: (See NEW 4094 - 1999-2000)

## **OBJECTIVES:**

1. Evaluate new trapping systems for efficiency and correlation with in-orchard populations and/or

fruit injury.

- 2. Evaluate new chemical attractants for *E. conspersus* and *Chlorochroa* spp.
- 3. Determine the effect of orchard border habitat management on stink bug damage in orchards.
- 4. Determine the most effective orchard border and in-orchard chemical control programs that protect fruit from injury.
- 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. Determine the relationship between stink bug densities in orchards and fruit injury at harvest and develop a chemical analysis or staining procedure to separate stink bug injury from bitter pit or other physiological disorders.

#### **SIGNIFICANT FINDINGS:**

- Mark-recapture studies demonstrated that the aggregation pheromone has a fairly short range, with an active space of between 10-25 m.
- Management of stink bug populations on orchard borders using an "attract-and-kill" approach killed large numbers of bugs but was not successful in reducing fruit injury at harvest.
- A series of pheromone release rates was evaluated for attraction of *Euschistus conspersus*. These studies demonstrated that it is possible to attract stink bugs using pheromone release rates much lower than what we are currently using.
- Research conducted in conjunction with Dr. Jocelyn Millar (UC Riverside) in search of a generic lure revealed that there is no inhibition of attraction of either of the two major stink bug species in our area (*Euschistus conspersus* and *Chlorochroa ligata*) when the pheromones of both species are present on the same plant.

- For the first time, surveys were conducted in the Columbia Basin to determine the stink bug species complex in this area. These studies revealed some differences from the complex of the Wenatchee/Chelan areas, most notably far lower numbers of *E. conspersus*.
- Male stink bugs are sexually mature upon emergence from overwintering sites (early April) while females mature approximately two weeks after emergence.
- **5.** Laboratory and field bioassay methods were developed to screen insecticides against stink bugs. Carzol was the best product evaluated. No other insecticides evaluated were promising as controls.

## **PROGRESS:** (see report)

## **PROCEDURES:**

**Aggregate-and-kill studies:** Although we were unable to impact damage in the 2000 study, this idea does merit additional study. We are able to attract large numbers of bugs to baited mullein plants; the challenge before us now is to find a reliable method of killing the insects. One key error made in the year 2000 studies was the use of an insecticide (Provado) that, while soft on beneficial parasites and predators, was also not highly toxic to the target insects. This will be remedied in future studies which will use a more toxic contact insecticide (i.e., Carzol). Another improvement we will make to our previous experimental design is the use of much larger plots, preferably entire orchard blocks. An obvious difficulty of this approach is a lack of the compatibility offered with side-by-side blocks. However, the degree of movement exhibited by the insects may obscure any treatment effects of our spring and summer sprays. Therefore, we will seek out large areas in which to work, attempting to ensure equivalent levels of stink bug injury in each.

**Dose/response studies**: It appears from 2000 data that we have overshot the minimum release rate required for attraction of *E. conspersus* in each of our previous studies. Beginning in spring 2001, and with the assistance of Dr. Landolt's lab at Yakima, we will begin a series of lower-rate studies and hope to resolve this issue by summer 2001. This will enable us to move forward rapidly on the development of a commercial lure that may be used for monitoring or attract-and-kill studies of the future. An optimal release rate may also allow us to develop a lure that will induce bug movement into a trap, a difficult problem that has yet to be addressed and may be partially caused by sub-optimal rates of the attractant in the trap.

**Plant volatiles:** We plan to pursue studies investigating the attraction of bugs to plant odors. In 2000 our efforts focused on known compounds common to alfalfa and other plants. In 2001 we hope to examine volatile compounds common to succulent, drought-resistant plants such as mullein. This may enhance attraction to traps, plants or both. With such a compound, bait stations could be made much more attractive. The key benefit of the isolation of such a compound is the ability to move away from our current dependence upon naturally occurring mullein plants. This would allow us to create highly attractive sources for bugs even where mullein is not present.

**Feeding stimulants/arrestants**: Work in the lab has revealed the presence of commercially available products that are attractive to *E. conspersus*, causing bugs that come in contact with these materials to stop moving and begin probing with their stylets. The addition of this compound to current contact insecticide formulations may enhance the kill of these compounds in the field by keeping bugs in contact with the material for longer periods of time. Although in the early stages now, this will be a primary focus of work through the winter months and will be field tested in spring/summer 2001.

**Damage/bug density correlation**: The question of how much damage a single stink bug does is not resolved and remains a difficult problem. In caged-tree studies in 2000 we were unable to stimulate the bugs to feed reliably, and we will attempt to cage more trees in 2001 and cage them earlier in the season (July). This will give us more flexibility with different bug densities and also allow us to avoid the

problems associated with the cold nights of late summer which may have been responsible for the lack of bug feeding in 2000.

**ANTICIPATED BENEFITS AND INFORMATION TRANSFER:** Development of better monitoring techniques for stink bugs will provide an early warning system alerting growers to the invasion of orchard borders, allowing them to better protect crops. Management of habitats on orchard borders may reduce stink bug injury or reduce the need to apply as many control sprays.

Information developed from the project will be reported at grower meetings. New management information will be incorporated into the next revision of *Orchard Pest Management: a Resource Book for the Pacific Northwest*. New chemical control information will be incorporated in EB-0419, Crop Protection Guide for Tree Fruits in Washington.

# **BUDGET:**

Amount allocated by the Commission in 2000-01: 2001-02:	\$57,330 \$44,780	
Salaries (Christian Krupke)	TFREC <u>2001-02</u> \$33,000	RAMP/IFAFS <u>2001-02</u> \$ 0
Time-slip labor (2 - 0.5 FTE labor @ \$10/h) Operations (traps, lures, rearing supplies)	1,550	12,000 0
Travel Employee benefits	0	2,700
Krupke position (31%) Time-slip labor (16%) Totals	$     10,230 \\     0 \\     \overline{\$44,780}   $	0 <u>1,920</u> \$16,620

# **PROGRESS REPORT**

<b>PROJECT NO:</b>	8096			
TITLE:	Control of Apple and Pear Pests with Surround			
YEAR INITIATED: TERMINATING YEAR:	1999-00 2002-03	CURRENT YE	EAR: 2001-02	
PERSONNEL:	Jay F. Brunner, Entomologist and Director Elizabeth H. Beers, Entomologist John E. Dunley, Assistant Entomologist WSU-TFREC, Wenatchee			
FUNDING HISTORY:	2000-01: \$2	2,135	2001-02: \$22,348	

# **OBJECTIVES:**

- 6. Determine the optimum timing, rates and application frequency of Surround® against selected apple pests.
- 7. Determine the optimum timing, rates and application frequency of Surround® against selected pear pests.
- 8. Determine the negative effects of Surround® on natural enemies.
- 9. Determine if use of Surround<sup>®</sup> will increase damage thresholds for spider mites on pear, thus increasing the potential for biological control.

# **SIGNIFICANT FINDINGS:**

- Surround WP was evaluated in small, replicated plots for their ability to control codling moth. Surround WP reduced codling moth injury by approximately 50%. Concerns regarding the effect of Surround on integrated mite control still exist.
- The effect of Surround WP on neonate codling moth larvae was also examined in a laboratory bioassay. In a direct choice test Surround WP deterred codling moth neonates from entering the fruit. Furthermore, in the whole-fruit treatments the number of entries in Surround WP treated fruit were about 60% of the untreated fruit.
- In leaf disk bioassays both Surround WP deterred colonization in choice tests. Only 9% of pandemis leafroller larvae chose the Surround WP treated disk over the untreated disk. In leaf disk bioassays with neonate lacanobia fruitworm larvae only 6% chose the Surround WP treated disk. Mortality was high for both species in Surround WP only arenas.
- Residual activity of Surround WP was evaluated in a field-aged bioassay. Surround WP showed 7 days of activity against pandemis leafroller. Against lacanobia fruitworm, Surround WP residues caused significant mortality through 14 days.
- Surround WP was effective in control of pear psylla when used season-long, as well as prebloom or postbloom only in combination with conventional controls.
- Surround WP was potentially shown to act as a dispersal deterrent to adult overwintering **pear psylla**.

# **PROGRESS:**

Codling moth field trials - Kaolin (Surround, Englehard Corp.), tebufenozide (Confirm 2SC, Rohm and Haas Co.), and azinphos methyl (Guthion 50WP, Bayer Corp., Ag Div.) were evaluated for their ability to control CM when used full season for both first and second generation larvae. The trees were Gala on dwarfing rootstock. Treatments were applied to single-tree plots replicated four times in a randomized complete block. All treatments were applied with a handgun sprayer at 400 psi to the point of drip, simulating a dilute spray of approximately 200 gal/acre. Different treatment regimes were set up with Surround with treatments 1 and 3 receiving three applications on a 14-day schedule starting at pre-oviposition (58°D) on 26 Apr for the first generation. Treatments 2 and 4 were applied pre-hatch (197°D) for three application on a 14-day schedule starting on 16 May. Guthion and Confirm were applied at 250°D on 20 May, with Confirm applied three times on a 14-day schedule and Guthion applied two times on a 21-day schedule for the first generation. For the 2nd CM generation, Surround (Trt 1) was first applied on 10 Jul (1000°D) with repeat applications on a 14-day schedule. Surround (Trt 2), Confirm and Guthion were first applied on 20 Jul (1250°D) with Surround and Confirm receiving repeat applications at 14-day intervals and Guthion receiving a second application 21 days after the first. CM injury evaluations were made on 5 Jul (1st generation) and 31 Aug (1st and 2nd generation CM injury). Mite counts were taken on 30 Jun by picking a composite sample of 50 leaves from each treatment.

CM pressure was moderate in this trial with 5.5% of the fruit having either CM stings or entries at the first generation evaluation and 29.3% at the final (harvest) evaluation in the untreated plots (stings and entries, both generations). At both evaluation dates all treatments had significantly less CM injury than the untreated control. Confirm gave the best control of CM. There was no difference in CM control with Surround WP using different timing regimes. No conclusions could be reached about effects of treatments on spider mite or predatory mite densities. There were no predatory mites in the Surround treatments which brings up concerns about its effects on integrated mite management.

	_	Avg % CM injury <sup>1</sup>					
	Rate		5 Jul 31 Aug				
Treatment	(form. /A)	Stings	Entries	Total	Stings	Entries	Total
Surround WP	50 lb.	1.0 ab	1.5 b	2.5 b	3.3 abc	13.0 b	16.3 b
Surround WP	50 lb.	0.8 abc	0.3 b	1.0 bc	4.3 a	10.5 b	14.8 b
Guthion 50WP	2.0 lb.	0.8 abc	1.0 b	1.8 bc	3.5 abc	8.5 bc	12.0 bc
Confirm 2SC +							
Orchex 796	21 fl oz						
	0.25% v/v	0.0 c	0.0 b	0.0c	2.0 bc	1.3 d	3.3 d
Untreated		1.3 a	4.3 a	5.5 a	3.8 ab	25.5 a	29.3 a

<sup>1</sup>Means in the same column followed by the same letter are not significantly different (p=0.05, Student-Newman-Keuls).

#### Apple dip bioassay:

Kaolin (Surround® WP, Engelhard Corp.) was evaluated in direct choice tests for its ability to deter codling moth neonate larvae from penetrating a treated apple. Treatments were prepared by diluting the appropriate amount of product (equivalent of 50 lb Surround per 200 gal water) in 500 ml water in a glass beaker. Treatment concentrations were equivalent to recommended label rates for dilute applications. The use of an organosilicone surfactant (Sylwet L-77, Helena Chem. Co.) at 4 oz/100 as a spreader was required to ensure even coverage of the particle film over the waxy surface of the apples. An untreated control was prepared using water plus the surfactant only. Apples treated with Surround were dipped, allowed to dry, then dipped again for a total of two applications. Treatment regimes consisted of a direct choice test (10 apples with one Surround treated half and one untreated half), 10 positive controls (whole apples treated with Surround) and 10 negative controls (whole apples left untreated). This entire test was replicated three times. Codling moth eggs were received on waxed paper sheets from the USDA-ARS laboratory in Wapato, WA. A section of waxed paper containing 10 CM eggs was cut and placed on the shoulder of a treated apple and secured with an insect pin. The apples with the attached eggs were placed individually in clear plastic deli container arenas. CM entries were evaluated at 10 days after treatment. The number of CM entries in apples was recorded. In the choice test the number of entries on the treated half and untreated half was recorded separately.

Surround film technology significantly deterred neonate codling moth from entering the treated half of the fruit. However, CM larvae find "holes" in the residues and are able to enter at these locations. The reduction in entries is about what is observed in field trials where multiple applications are made. The CM larvae are able to detect Surround residues and avoid them as shown in the choice tests. Nearly 80% of entries occurred on the untreated half of the apples.

#### Pandemis leafroller and lacanobia fruitworm

### Apple leaf disk bioassay:

Kaolin (Surround<sup>®</sup> WP, Engelhard Corp.) was evaluated in direct choice tests for its ability to deter lacanobia fruitworm and pandemis leafroller neonate larvae from colonizing treated apple leaf disks. Treatments were prepared by diluting the appropriate amount of product (equivalent of 50 lb Surround and 1 pt of a spreader, M-03, per 200 gal water) in 500 ml water in a glass beaker. A small amount (ca.  $2 \Box l$ ) of wetting agent, Latron B-1956 (Rohm and Haas Co.), was added to the treatment. The treatment concentration was equivalent to recommended label rates for dilute applications. An untreated control was prepared using water plus the wetting agent only. Leaves to be treated with Surround were dipped, allowed to dry, then dipped again for a total of two applications. One leaf disk (2.3 cm diameter) was taken from each leaf. Two disks were placed in a petri dish. Care was taken in the choice test arenas so that the leaf disks did not contact the other. Treatment regimes consisted of 50 arenas with a direct choice test (one treated and one untreated leaf disk), 10 positive controls (2 treated disks) and 10 negative controls (2 untreated disks). Petri dishes were chosen randomly, and two 2- to 5-d-old pandemis leafroller larvae were placed on the leaf disks. Choice test arenas were examined after 1 day and 5 days and the number of larvae colonizing each leaf disk was recorded. The controls were examined after 10 days and larval survival recorded.

Surround deterred colonization in choice tests. Only 9% of pandemis leafroller larvae were found on the Surround WP treated disk over the untreated disk. For lacanobia fruitworm neonates, only 6% were found on the Surround WP treated disk. Mortality was higher for both species in Surround WP arenas.

#### Field-aged residues

Using a leaf-disk bioassay, Surround® WP, Engelhard Corp. was evaluated for residue effects on lacanobia fruitworm and pandemis leafroller neonate larvae. The test was designed as a direct choice test between a treated and untreated leaf disks. The treatments were applied on 15 Sep with a handgun sprayer at 300 psi to the point of drip, simulating a dilute spray of approximately 300 gal/acre. The treatment concentration was equivalent to recommended label rates for dilute applications (equivalent of 50 lbs. Surround/200 gal). Surround was applied twice, allowing a drying time between successive applications. There were three single-tree replicates per treatment, Surround and untreated. Five choice test arenas per replicate consisting of one treated and one untreated leaf disk were established on each sample date. In addition, five no-choice arenas were also set up from each of the treatment replicates. These no-choice arenas consisted of either two Surround treated or two untreated leaf disks per arena. Leaves were collected from the interior canopy of each replicate at 1, 7 and 14 d after treatment (DAT). Two disks (2.3 cm diameter) were taken from each leaf. Two disks were placed in a petri dish following the experimental method described above. In the direct choice arenas the untreated disk was marked with permanent ink to distinguish from the treated disks. Care was taken to keep the leaf disks separate to ensure a distinct choice was made between disks. Petri dishes were chosen randomly, and two 2- to 5-dold leafroller larvae were placed on the leaves (30 larvae/treatment at each sample date). The choice arenas were examined after 5 d exposure, and the number of larvae colonizing each disk was recorded. The no-choice arenas were evaluated after 10 d exposure and larval survival was recorded. Weather of note: A rain event occurred at 10 and 11 DAT.

**Results** were similar to those discovered in the leaf disk bioassays. Surround deterred lacanobia and pandemis leafroller from colonizing treated leaf disks to about the same degree, and the effect lasted at least 14 d. Surround also caused mortality significantly higher than the untreated control through 7 d for pandemis leafroller and 14 d for lacanobia fruitworm.

**Leafminer:** Surround was tested in both laboratory and field formats against western tentiform leafminer. In a caged potted tree experiment, Surround deposits on the leaves did not significantly deter leafminer oviposition in relation to the untreated check. In a field test, several strategies were tested to try to minimize the possible impact of Surround on the primary leafminer parasitoid, *Pnigalio flavipes*. There was significantly higher parasitism of the first generation in the late season and delayed early season Surround treatments, both of which avoided the period of parasitoid activity of the first generation. No treatment differences were apparent by the second generation.

**Leafhopper:** As in previous years, Surround gave good suppression of leafhopper nymphs during both the first and second generations. A second application during the second generation improved control of later-hatching nymphs, but only slightly.

**Mites:** Unlike past tests, this material also gave reasonable mite suppression in a test with a high initial mite population (e.g. 20 to 60 mites/leaf). While the activity was slower than conventional miticides, populations were below 1 mite/leaf by 9 days after application. In this case, a second application did not improve control, primarily because mite numbers were already very low. The predatory mite population in this test was high initially (0.2 to 0.9 mites/leaf). The Surround application significantly reduced the predatory mite numbers in relation to the check (48 h posttreatment); however, the phytophagous mite was also lower in the Surround treatments by this time. In behavioral bioassays on detached leaves, mites (*Tetranychus urticae*) tended to avoid Surround deposits where there was a choice of treated and untreated leaf sections.

**Pear psylla:** Kaolin (Surround) treatments were combined with conventional psylla management programs to determine if kaolin is better used prebloom or postbloom. Five treatments were established: kaolin only, throughout the season (nine applications); kaolin only, in the prebloom period (three applications), with conventional psylla treatments postbloom (two Agri-Mek applications); conventional prebloom psylla controls (Thiodan and Esteem), followed by only kaolin in the postbloom period (six applications); a conventional psylla control program (Thiodan, Esteem, two Agri-Mek applications); and an untreated control treatment.

**Methods:** Abamectin (Agri-Mek 0.15 EC, Novartis Crop Protection), Endosulfan (Thiodan 3EC, FMC Corp.), Kaolin (Surround WP, Engelhard Corp.), Raynox (Raynox SL, Solutec, Inc.), and Pyriproxyfen (Esteem 0.88EC, Valent USA Corp.) were used at different timings and combinations to give season-long control of pear psylla on pears. This trial was conducted on Bartlett pears at the WSU Tree Fruit Research and Extension Center Smith Tract block 7 near Orondo, WA. Treatments were applied to plots that were three rows wide by three trees long replicated four times in a randomized complete block design. Treatments were applied with an airblast sprayer calibrated to deliver 200 gal of spray per acre on prebloom applications and 100 gal per acre on postbloom applications.

**Results:** In general, the Surround treatments were as effective as conventional insecticide treatments in season-long programs of pear psylla control. Surround in the prebloom period was more effective than the conventional treatments (Thiodan and Esteem). In the postbloom period, Surround also performed well and reduced the average psylla nymphs/leaf more than the conventional treatment. However, as the residual activity of Agri-Mek was exceeded in the conventional treatment, this result should be considered with caution.

#### **PROGRESS REPORT FOR 2000-2001**

**TITLE:** Maintenance of Guthion Registrations on Pome Fruits: Fruit Residue Reduction through Spray Timing Optimization

Principal Investigator: Allan S. Felsot, Dept. of Entomology and Food & Environmental Quality Lab, Washington State University, 2710 University Drive, Richland, WA 99352 (509) 372-7365; (509) 372-7460 Fax; afelsot@tricity.wsu.edu

**Collaborator:** Doug Walsh, Dept. of Entomology and Food & Environmental Quality Lab, Washington State University

**Funding History:** Commitment to funding during 2000, total \$39,393 (\$15,193 for field phase; \$24,200 for lab phase)

#### **Objectives:**

Six objectives were delineated in the original proposal.

- 1. Measure decline of AZM apple residues in an orchard when applied one, two, or three times during the growing season.
- 2. Measure AZM apple residues in composited and single-serving fruit samples at harvest following one, two, or three applications.
- 3. Measure carbaryl residues in composited and single-serving fruit samples at harvest.
- 4. Develop an empirical phenological (degree-day) based fruit residue decline model that predicts the best application timing window to give the lowest possible residue concentration.
- 5. Validate the fruit residue decline model by measuring AZM residues in fruit harvested from commercial orchards. Carbaryl residues would also be measured.
- 6. Analytical Methods/GLPs/Quality Assurance development.

# Justification:

The goal of the project is to help pome fruit growers optimize timing of AZM use so that residues on the harvested commodity will be close to or below acceptably sensitive limits of detection. We proposed to develop a phenological (or degree-day) based model to predict residues at harvest from any time of application throughout the growing season. We hypothesized that an optimal window of time exists for spraying that will result in very low residues without jeopardizing pest control. Knowing this optimal window may allow up to two applications of AZM to be used without incurring residues that raise the dietary exposure component of the risk characterization. In addition to producing a tool useful for improving AZM's compatibility with IPM in apples, the project should generate AZM and carbaryl residue distribution data on composited and single-serving apples that can be used to further refine dietary risk assessments.

### **Accomplishments:**

Objectives 1-4 and 6 were proposed for year one, and they were designed to produce enough residue data to build an empirical kinetic model of residue decline. The objectives were also designed to generate residue data on composite and individual apples. Such data would be useful for elucidating the variability in residues among individual apples. Knowledge of residues variability serves to further refine acute dietary exposure estimates, whether they are generated probabilistically by the EPA in the U.S. or deterministically by the Codex Alimentarius Committee on Pesticide Residues (CCPR), the international body setting maximum residue limits (MRLs) for international trade.

Activities during year one of the project were divided into field protocols (phase one) and laboratory protocols (phase two). During the first year, a protocol for the field work was written which described the conduct of the study consistent with GLPs. One, two or three applications of Guthion 50W were made to a block of trees on the Klingele Orchard (near Whitstran). Sevin was applied by the grower. The experimental design was a random block with three replications consisting of 15 trees in each replicate. Apples from each of three randomly selected trees within a replicate plot were sampled frequently after each Guthion application. The apples from individual trees were bulked but will be analyzed separately from apples collected on any other tree. The intention of this sampling technique is to determine the variability in residues between trees as well as to reduce the variability in residues between replicate units. To simulate harvest, five apples were collected from each of nine trees in a replicate plot. Twenty apples will be selected for individual analysis and the rest of the apples will be extracted as a composite (with at least five composite samples analyzed per replicate treatment).

In addition to application of Guthion and sampling of apples, canopy temperatures were measured in six trees (two per block) in at least two different locations within the tree. Surface temperatures of fruit and leaves and light penetration were also recorded periodically from three different canopy locations. A significant finding during phase one of this study was that fruit surface temperatures could reach 110-115 °C, but canopy temperatures would remain from 20-30 °C cooler than fruit temperatures. These elevated temperatures could significantly increase dissipation of insecticide residues, perhaps by volatilization.

All field work was accomplished under a protocol consistent with GLPs. A total of 363 apple samples, including composite and individual apples at harvest, were collected and have been stored at –20 °C. The field study and storage protocol were inspected by an outside QA consultant. Phase two of the first year of the project involves analysis of apples for residues of AZM and carbaryl. Current work involves method validation and development of a suitable analytical protocol. The residue analysis will also be done under GLP and subjected to audit. By late spring 2001, the data will be used to build the model for predicting fruit residue dissipation.

The magnitude of apple surface temperature measurements in year one of this project suggested a new hypothesis regarding the influence of multiple applications on AZM residues occurring at harvest. Given that fruit surface temperatures could approach 110-115 °C, volatilization may be a significant dissipation factor. Surface temperatures did not cool down until after the third Guthion spray but remained high after the first two sprays. Thus, one or two sprays may not affect the final residues and thus label changes will be unnecessary to achieve goals of reduced residues at harvest. Thus the year-two project objective will be designed to test this latter hypothesis.

## FINAL REPORT FOR 1999-2000

TITLE:	Leafroller Biology and Potential for Control with Insect Growth Regulators			
PRINCIPAL	David R. Horton, Yakima Agricultural Research			
INVESTIGATORS:	Laboratory, ARS			
<b>CO-INVESTIGATORS:</b>	Alan L. Knight, Yakima Agricultural Research Laboratory, ARS			
FUNDING HISTORY:	Funding in 1999-2000 (Year Initiated): \$21,014			

## **SIGNIFICANT FINDINGS:**

Newly hatched larvae of oblique-banded leafroller were more susceptible to field-weathered residues of CONFIRM than late instar larvae.

Mortality of small larvae occurred on CONFIRM residues field-aged for up to 5 weeks; large larvae were killed by field-residues of 3 weeks in age.

Egg hatch was reduced on 1-week old CONFIRM residues early in the season but not later in the season; spray coverage may have been better early in the season.

## **OBJECTIVES:**

Determine whether moths collected from CONFIRM-treated orchards show reduced fecundity and egg fertility (as suggested by earlier laboratory trials by Knight and Horton).

Quantitatively describe field-persistence of CONFIRM against oblique-banded leafroller larvae and eggmasses.

# **PROCEDURES:**

Light traps equipped with mesh bags were placed in CONFIRM-treated and untreated orchards to collect live female moths. As with last year, captured moths were entirely male, thus the project was discontinued.

CONFIRM was applied with a standard airblast sprayer to a 10 acre block of 'Gala' apples on May 1, 2000 and again on June 6, 2000. The application rate was 17 oz of product per 100 gallons of water per acre. Foliage was collected from this orchard and a neighboring unsprayed orchard 24 hours following application, 1 week following application, and 3 weeks following application. Foliage samples were placed in large glass vials to which a single OBLR larva per vial was added. Two larval ages were compared: newly hatched larvae and 4<sup>th</sup> - 5<sup>th</sup> instar instar larvae. There were 25 replicates per treatment combination. Survival of larvae was determined at 4 days and 7 days.

We allowed laboratory-reared moths (OBLR) to deposit eggs on field-collected foliage. Egg masses were collected and allowed to hatch; number of larvae hatching was determined for each egg mass. After hatch had been completed, area of the egg mass was determined using a LI-COR area-meter. Hatch is expressed as number of larvae per cm<sup>2</sup> of egg mass. Sample sizes were 5 to 20 egg masses per treatment combination (adults were highly variable in egg-laying capacities, which explains the variable sample sizes).

The experiment was repeated in early August at the experimental orchard located at Moxee. Two rows of Golden Delicious apples were sprayed with CONFIRM using a small airblast sprayer pulled by an ATV. Two control rows were sprayed with water. Foliage was collected at 24 hours, 1 week, 3 weeks, and 5 weeks, and assays were conducted as above. Rates were the same as at the commercial site.

## **RESULTS AND DISCUSSION:**

Light-traps equipped with mesh bags collected only 10 moths; all were male, and the project to determine effects of field residues on field-collected, female moths was discontinued.

Figure 1 shows larval survival at 4 days (panels 1 and 2) and 7 days (panels 3 and 4) for small and large larvae fed foliage collected in early May. The grower applied Guthion between the week 1 and week 3 foliage collections, so the survival data for week 3 reflect the combined effects of Guthion and CONFIRM residues. Mortality of small larvae on the 1-week residues was 100%; mortality approached 100% for the large larvae.

Figure 2 shows larval survival for the June experiment. Again, the grower applied Guthion before the week 3 foliage sample could be taken. Mortality of larvae fed 1-week old residues was again high, irrespective of larval age.

Figure 3 shows results for the Moxee orchard, and indicate that small larvae suffered high mortality rates up to 5 weeks following the CONFIRM application (panel 3), and that large larvae suffered high mortality rates up to 3 weeks following the spray (panel 4).

Figure 4 shows that egg hatch rates were severely reduced on CONFIRM-treated foliage for the two trials conducted in the commercial orchard. However, there was no significant reduction in egg-hatch for the trial conducted in August at the experimental orchard in Moxee (Figure 5). Our results for the larval survival suggests that we had better spray coverage at the commercial sites than at the Moxee site (compare Figures 1-2 vs Figure 3), which may explain the poor egg-kill for the Moxee experiment.

# **CONCLUSIONS:**

CONFIRM residues provided kill of newly-hatched and large larvae of oblique-banded leafroller up to and exceeding 3 weeks following the application. Results for egg-kill were less clear, due to poor results for the August trials; however, results at the commercial orchard for applications made in May and June suggest that residues caused substantial egg mortality at least up to one week following the spray application.

## PROJECT NO.: AAFC (New 1998) Progress Report

**TITLE:**Understanding Mechanisms of Communication Disruption to Improve Delivery and<br/>Development of Multiple-Species Mating Disruption Systems

PERSONNEL: Gary Judd, AAFC, Pacific Agri-Food Research Centre, Summerland, BC

YEAR INITIATED: 1998 CURRENT YEAR: 2000 TERMINATING YEAR: 2002

## **OBJECTIVES THIS PERIOD:**

- 1. Complete construction of laboratory and field pheromone permeation wind tunnels for studying mating disruption mechanisms.
- 2. Develop bioassays suitable for evaluating different pheromone delivery systems in permeation wind tunnels.
- 3. Examine the disruptive affects of microencapsulated (MEC) pheromones on leafrollers in field and laboratory wind tunnels.
- 4. Develop techniques for measuring airborne concentrations of sprayable pheromones in wind tunnels.

## **ACCOMPLISHMENTS THIS PERIOD:**

- 10. One laboratory and 2, first of their kind pheromone permeation field wind tunnels capable of housing 24, high density apple trees were constructed and tested. Behaviour and response of moths to synthetic and natural sources of pheromone in these flight tunnels was tested and found to be identical to their behaviour in other wind tunnels and in orchards.
- 11. As part of developing a bioassay for testing efficacy of MEC formulations we demonstrated that hexane extracts of female pheromone glands at 5 and 10 female gland equivalents (FGE) produced upwind flight from males that was equivalent to that elicited by calling females when compared in clean air. This was important as it is necessary to have a standardized attractant for testing disruptants.
- 12. Application of MEC pheromone (Z11-14:OAc) at field rates of 100 gm a.i. /ha, disrupted orientation of male oblique-banded and *Pandemis* leafrollers to calling females for 40 and 26 h respectively. Response to female extracts was disrupted longer than response to calling females, suggesting that live calling females would be the most sensitive measure of disruption and most relevant to field conditions. There was no effect of the solvent carrier used to produce MEC formulations on the flight behaviour of male moths.
- 13. When tested in field wind tunnels a MEC formulation of *Z*11-14:OAc applied to apple trees (100 gm a.i. / ha) disrupted response of *Pandemis* males for at least 28 days and oblique-banded males for at least 16 days.

**RESULTS:** *Laboratory Wind Tunnel Studies.* A pulling-type stainless steel and plastic wind tunnel was designed and constructed for testing MEC formulations of pheromone. Pheromone was applied at field equivalent rates (100 gm a.i. / ha) to 3M Filtrete material at the upwind end of the tunnel which produced a uniform cloud of pheromone. This tunnel was housed in a stainless steel fumigation room which allowed evacuation of pheromone-treated air to the outside of the building. In an effort to obtain a repeatable attractant for bioassaying MEC pheromones we compared attraction of hexane extracts of female leafroller pheromone glands and tested these extracts at various concentrations against live calling

females. In clean air 5 to 10 female gland equivalents (FGE) were as attractive as a live female *Pandemis*.

Using extracts and live females we showed that in pheromone-treated air males began responding to calling females within 26 h after treatment but response to a 5 FGE extract did not occur until 120 h. The short activity times seen in the laboratory suggest that much of the MEC pheromone being applied is lost through absorption on Filtrete. Response to females and extracts returned in a linear time-dependent manner. While longer disruption times had been expected, the short activity times are actually a benefit for achieving the goal of correlating changes in behavioural response with changes in airborne concentration. Pheromone trapping experiments with SPME and Porapak-Q failed to collect pheromone in amounts measurable by GC analysis even though behavioural activity was obvious. EAG analysis could detect pheromone in the Porapak samples and means of using these EAG measure to quantify pheromone is being tested.

*Field Wind Tunnel Studies*. Two field wind tunnels, 3 m cross sectional diameter and 8 m long, were designed and constructed from PVC pipe and polyethylene. Three fans delivered air at about 3 km / hr through 18 potted apple trees (2 - 3 m tall) that were arranged in three rows of 6 trees buried in the ground and set up on drip irrigation. Female leafrollers reared on artificial diet were placed in traps hung in the upwind trees. Fifty to 60 male leafrollers of each species were released from the downwind trees and left overnight. Catches of males after 24 h revealed recapture rates of 70 - 80% and no significant differences between the tunnels in several replications. Catches in synthetic pheromone traps produced similar results.

A 3M, MEC formulation of Z11-14:OAc was applied, as a single application, at a rate of 100 gm a.i. /ha, from a backpack hand sprayer to the 18 trees in the treatment tunnel and a MEC solvent control was applied at the same rate to trees in the control tunnel. Differentially marked moths were introduced into each tunnel every evening for 28 days and female-baited traps were changed each day. No *Pandemis* males were caught during the 28-day test period and no significant numbers of oblique-banded males were caught for 26 days, although a few male OBLR were caught on day 16 and 22.

# **PROJECT MILESTONES:**

1998

- developed protocols for measuring pheromone environments with an EAG
- evaluated the effects of pre-exposure to pheromone on the EAG responses of codling moths
- evaluated effects of pre-exposing male codling moths to the codlemone pheromone and its isomers on the flight to synthetic lures and females
- evaluated effects of pre-exposing oblique-banded and *Pandemis* leafrollers to their complete pheromone and individual components on subsequent flight to synthetic lures and females

1999

- project postponed for 1 year due to equipment construction and collaborator problems 2000
  - evaluated effects of sprayable pheromone clouds on flight of leafrollers in lab and field wind tunnels
  - made measurements of airborne concentrations of pheromone using a new solid phase micro extraction system (SPME) and Porapak-Q trapping techniques and developed protocols for using EAG analysis to quantify the amounts of pheromone trapped.

### **PROGRESS REPORT FOR 1999-2000**

TITLE:	Bisexual Monitoring of Codling moth: Establishing a Female BIOFIX for Management Decisions
PRINCIPAL	Alan L. Knight, Yakima Agricultural Research Lab., ARS
INVESTIGATORS: CO-INVESTIGATORS:	Doug Light, ARS
FUNDING HISTORY:	Funding in 1999-2000 (Year Initiated): \$31,000

### SIGNIFICANT FINDINGS:

DA2313 lures effectively monitored both generations of codling moth all season in 45 apple orchards.

Early-season moth captures in DA2313-baited traps were effective in predicting codling moth egg hatch.

Cumulative first generation moth catches in DA2313-baited traps were more effective than sex pheromone-baited traps in eliminating unnecessary first generation cover sprays based on the use of a catch threshold.

Cumulative moth captures in DA2313-baited traps were correlated with levels of codling moth fruit injury prior to harvest.

#### **OBJECTIVES:**

Evaluate the use of DA2313 lures (Trece Inc.) to monitor the emergence and population density of both sexes of codling moth in apple and pear orchards.

## **PROCEDURES:**

Four orchards were monitored intensively to evaluate the use of DA2313 lures to establish a female mothbased 'BIOFIX' to predict codling moth egg hatch. Each orchard was monitored with two delta traps baited with DA2313 lures and two similar traps with sex pheromone lures. Traps were rotated at each checking period. Orchards were also monitored with 36 plastic interception traps placed as two groups of 18 traps. All traps were checked daily for the first 42 days of the season from early April to late May and biweekly subsequently. Foliage and fruit were inspected several times each week to detect first egg hatch.

In two orchards, replicated one-tree plots were established to evaluate spray timings of azinphosmethyl based on the establishment of 'BIOFIX'. Azinphosmethyl was applied with an air-blast sprayed at a rate of 1 lb active ingredient per acre in 100 gallons of water. Fruit injury was assessed two weeks following the last spray application date.

Thirty-five orchards were monitored by seven consultants with both DA2313 and sex pheromone lures during first generation. Fruit injury was assessed in each block in early July.

Twenty-five orchards in the Brewster area were monitored all season with both DA2313 and sex pheromone lures. Fruit injury was measured at mid-season and prior to harvest.

Fifteen apple and pear orchards were monitored in the Yakima area with DA2313 and sex pheromone lures all season and fruit injury was assessed prior to harvest.

## **RESULTS AND DISCUSSION:**

Six potential 'BIOFIX's were established based on either 250 degree-days after male catch in a sex pheromone-baited trap or 155 degree-days after female catch in a DA2313-baited trap. With both lures, the first capture of moths was the worst predictor of egg hatch. Determination of the beginning of a sustained catch by both types of traps which coincided with suitable temperatures (maximum temperatures >  $70^{\circ}$ F) for sexual activity were the best predictors.

Levels of July fruit injury based on these six potential 'BIOFIX's varied significantly but were similar for the best timing with males in sex pheromone traps and females in DA2313 traps.

Moth catch in either type of baited trap in the consultant study were low and nearly all blocks were sprayed with supplemental organophosphate insecticides. Few orchards had detectable fruit injury in July and the study was discontinued.

The 25 Brewster orchards were all treated with sex pheromones and left unsprayed through first generation. This allowed us to evaluate the currently established moth catch threshold (> 4 moths per trap signals the need for a supplemental spray). Choosing a subset of these orchards which had no injury at mid-season we found that nearly 50% of these blocks exceeded this moth threshold while only 24% monitored with DA2313 exceeded the threshold. Perhaps most alarming was that 25% of the blocks exceeded the 10 moths per sex pheromone-baited trap threshold while only 5% had similar catches in DA2313-baited traps.

The correlation coefficients of trap cumulative moth catch per trap with fruit injury within a 50 m of each trap were 0.71 for the sex pheromone trap and 0.86 for the DA2313 trap. However, false negative catch occurred with both types of lures in a small number of orchards. Further evaluation of these traps and orchards is currently in progress. This will include examining the residual content of lures, and examining various characteristics associated with trap placement and maintenance.

The Yakima orchards monitored with the DA2313 and sex pheromone lures varied tremendously in their levels of codling moth. Catches of codling moth were particularly low in the DA2313-baited traps placed in the six conventional pear orchards. However, no fruit injury was detected in these orchards. The DA2313-baited traps performed well in the six apple orchards with high levels of codling moth. In contrast, the sex pheromone-baited traps often did poorly during the second generation. This pattern was also noted in some high pressure apple orchards in Brewster.

## **CONCLUSIONS:**

The DA2313 lure (Trece Inc.) is a powerful new tool for monitoring both sexes of codling moth. Substantial new information was gathered during this first year of wide scale field testing. Unlike 1999, the DA2313 lure performed equally well during both flights of codling moth and outperformed the sex pheromone lure in some high pressure sites during the second generation. In particular, it appears to provide a better estimate of the codling moth population density surrounding each trap. Also, it maybe less susceptible in creating 'false negative' trap catch.

The DA2313 lure was effective in detecting codling moth emergence in the spring though it tends to catch later than the first catches of males in a sex pheromone-baited trap. Predicting codling moth egg hatch from moth catches in DA2313-baited traps using 155 DD (requirement for egg hatch) appears to be an effective method, especially when the occurrence of suitable temperatures is included in the calculation.

Extensive testing of the DA2313 lure will be required by a range of users to sufficiently validate the robustness of this new tool.

## FINAL REPORT FOR 1999-2000

TITLE:	ers in Apple			
PRINCIPAL	Alan L. Knight, Yakima Agricultural Research Laboratory, ARS			
INVESTIGATORS:				
<b>CO-INVESTIGATORS:</b>	Steve Cockfield, Scientific Methods			
FUNDING HISTORY:	Funding in 1996-1997 (2 <sup>nd</sup> Year): Funding in 1997-1998 (3 <sup>rd</sup> Year): Funding in 1998-1999 (4 <sup>th</sup> Year): Funding in 1999-2000 (5 <sup>th</sup> Year): Total Project Funding:	Funding in 1995-1996 (Year Initiated): \$ 22,998 \$ 29,000 \$ 29,500 \$ 46,000 <u>\$ 31,000</u>		
		\$158,498		

#### **SIGNIFICANT FINDINGS:**

Sublethal exposure to Bt endotoxins causes severe retardation in larval development. Inclusion of this delay into a predictive phenology model improved the prediction of pupation and adult emergence.

Sex pheromones can be used effectively in combination with the use of insecticides to manage OBLR populations. Current sprayable formulations are short-lived. The Isomate CM/LR dispenser is effective but runs out of pheromone before the end of the season. New dispensers being developed are effective.

Aerosol puffers when placed internally in the orchard at 1 per hectare and integrated with border treatments of hand-applied dispensers are effective.

A three year area-wide program effectively managed both codling moth and obliquebanded leafrollers using the Isomate CM/LR dispenser plus reduced use of insecticides.

### **OBJECTIVES:**

Evaluate the effects of Bt endotoxin ingestion on leafroller larval development and develop and then validate a predictive phenology model for leafroller emergence and egg hatch following spring Bt spray applications.

Evaluate the effectiveness of various hand-applied sex pheromone dispensers, sprayables, and puffers for mating disruption of leafrollers.

Evaluate the adoption of a sex pheromone-based area-wide management program for both codling moth and the obliquebanded leafroller.

#### **PROCEDURES:**

Field studies were typically conducted in replicated 20-40 acre orchards in the Brewster region. Orchards were treated with various mating disruption systems including hand-applied dispensers, sprayables, and aerosol devices. Orchards were monitored with traps and both larval sampling and fruit inspections. Emission rates of the various hand-applied dispensers were monitored throughout the season.

Laboratory studies with either Bt or sex pheromone components were conducted with the Yakima OBLR strain under controlled temperature and light conditions.

## **RESULTS AND DISCUSSION:**

The first year of the project focused on both the use of *Bacillus thuringiensis* sprays and both handapplied dispensers (Hamaki-con and Isomate CM/LR dispensers) and sprayables to manage leafrollers. Various field and laboratory studies were conducted that evaluated the influence of larval ingestion of Bt endotoxins on subsequent rate of development and pupal weight. A predictive phenology model for leafrollers was developed which included the delay in larval development following sublethal ingestion of Bt endotoxins. We also surveyed eight endotoxins and reported that the most active toxins are the ones currently being used in the Bt products available to growers. Other studies demonstrated the importance of temperature on the effectiveness of Bt sprays and led to the development of a set of recommendations on how growers can optimize their use of these products. Studies with the pheromone dispensers and sprayable formulations were conducted in 10 - 40 acre replicated blocks and the results were encouraging. Moth catches were reduced > 90% and orchards treated with leafroller pheromone had similar levels of fruit injury as untreated blocks but were treated with \$80 per acre less insecticide. The CM/LR dispenser performed well but was found to run out of pheromone late in the season. The sprayable formulation was found to work best at a rate of 24 g per acre and lasted only three weeks.

Studies conducted during the second year of the project again focused on the Bt-phenology model and the use of sex pheromones to disrupt leafrollers. The Bt-model was validated for OBLR and was found to significantly improve the prediction of pupation and adult emergence in orchards treated with Bt sprays in the spring. The CM/LR dispenser was evaluated in three 20 acre orchards and fruit injury was reduced 64% compared with orchards treated with similar numbers of insecticide sprays but without leafroller pheromone. Also during 1997 six 40 acre orchards were split and treated either with 200 or 400 Isomate CM/LR dispensers per acre for codling moth and obliquebanded leafroller. Moth catches and fruit injury did not differ between treatments. The evaluation of the sprayable formulation in 20 acre orchards demonstrated that multiple applications of this formulation were not effective in managing seasonal leafroller populations.

From 1998 to 2000 a 450 areawide project was conducted with 12 growers who treated their orchards with the Isomate CM/LR dispenser for disruption of both codling moth and obliquebanded leafrollers (OBLR) for three years. Orchards were treated with 200 dispensers per acre and supplemental sprays of *Bacillus thuringiensis* or spinosid were applied for leafrollers. Results were compared with 150 orchards in the Brewster Areawide Management program treated with Isomate-C+ plus insecticides only for leafrollers. Moth catches of codling moth and obliquebanded leafroller were reduced > 95% during the study. Fruit injury for both codling moth and leafroller were reduced > 60% and the number of insecticide sprays for codling moth and leafrollers was decreased by nearly two sprays per season.

Aerosol puffers were evaluated in four 40 acre orchards for OBLR in 1998, three orchards in 1999 and nine orchards were treated with dual canister in 2000. In all cases orchards were treated with 16 puffers placed one per hectare and the perimeter of the orchards were treated with the hand-applied dispensers for leafrollers and/or codling moth. Moth catches and fruit injury varied widely among blocks yet mean moth catches and percent fruit injury did not differ from surrounding orchards treated with Isomate products during all three years. Also during 1999 we evaluated several operational settings of the aerosol puffers to determine how best to release the pheromone. The use of 15 minute intervals for sprays releasing 7.5 mg of pheromone for 12 hours each day (3 pm to 3 am) was found to work the best.

Laboratory studies showed that the levels of E11-14:AC present as a contaminant with the pheromone component, Z11-14:AC does not significantly reduce disruption of mating for *Pandemis pyrusana* at levels from 0.25 - 6.0% in the formulation.

## **CONCLUSIONS:**

Effective use of Bt insecticides is dependent upon minimizing sublethal larval exposures. Factors such as, the formulation's array of endotoxins, field temperature, larval age, spray rate, and time to resumption of larval feeding should be considered in optimizing the use of Bt insecticides.

The use of various methods to disperse leafroller sex pheromone can be an effective tool to manage populations especially when integrated with a reduced- use of insecticides.

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Abstracts for 10 posters presented at the annual Washington Horticultural Association meeting are published in their Proceedings.

# **PROGRESS REPORT FOR 1999-2000**

TITLE:	Attraction Inhibitors f	Combination of sex Pheromones and for Mating Disruption of Codling moth
PRINCIPAL		Alan L. Knight, Yakima Agricultural
INVESTIGATORS:	Research Lab., ARS	
FUNDING HISTORY:		
		Fundin
		g in 1997-1998
		(Year Initiated):
	E 1' ' 1000 1000	\$38,000
	Funding in 1998-1999	
	F 1: : 1000 0000	\$38,000
	Funding in 1999-2000	
		\$36,600

#### **SIGNIFICANT FINDINGS:**

The use of sixteen aerosol puffers (one per hectare) plus a 10 meter border treatment of hand-applied Isomate dispensers was an effective program to manage codling moth in sixteen 40 acre apple orchards situated near Brewster, Washington.

A comparison of these puffer programs with nearby orchards treated with 200 Isomate dispensers per acre found no difference in the effectiveness of the two programs.

### **OBJECTIVES:**

Development of an integrated program using aerosol puffers combined with either hand-applied, sprayables, or attract and kill formulations.

Evaluation of a blend of codlemone plus isomers for mating disruption of codling moth.

#### **PROCEDURES:**

Sixteen Brewster apple orchards (40 acres) were selected to be treated with aerosol puffers (one puffer per hectare) and border applications of Isomate dispensers. Orchards were either treated with codlemone alone or a blend of codlemone and leafroller sex pheromone (Dual). Orchards treated with the dual were either treated with 5.0 mg (low) or 7.5 mg (high) sprays of codlemone. The leafroller pheromone was applied at 7.5 mg per spray. Ten of these orchards were compared with nearby 40 acre orchards of similar cultivars treated only with Isomate-C+. Orchards were monitored all season with eight border traps and fruit injury by codling moth and obliquebanded leafroller was assessed prior to harvest. Spray records are still being collected from each grower.

Flight tunnel studies are in progress to evaluate the importance of sensory fatigue as an important mechanism for disruption of codling moth. Studies are comparing the effect of codlemone versus the codlemone isomers.

#### **RESULTS AND DISCUSSION:**

The results presented in Table 1 show that puffers performed well in most orchards. Unexpected high levels of codling moth occurred in three orchards and some growers did not supplement pheromones with organophosphate insecticide sprays despite high moth catches (> 4 moths per

trap). Complete spray records are still being gathered to further assess each grower's program. At harvest, levels of fruit injury in three blocks were high > 0.5% but these levels were actually lower than in the surrounding blocks treated with Isomate-C+ (Table 2). All growers were very pleased with their use of puffers and appreciated their ease of application and reliability.

Flight tunnel studies have demonstrated that moths placed in close proximity to Isomate dispensers for 5 seconds fail to subsequently respond to a pheromone source for 1 hour. Because the sexual flight period is approximately 3 hours in duration this may be inadequate to substantially reduce mating. Also we do not know what percentage of moths approach the Isomate dispensers under field conditions. Moths had to be exposed to a high rate of pheromone for at least 1 minute to turn-off their response to a pheromone source for 24 hours. No differences were found in this response to either codlemone or the codlemone isomers. Further studies are in progress to study the mechanisms of codling moth mating disruption, but no additional funding is requested for this work.

Puffer type	Orchard	1st Gen. Moth Catch2nd Gen. Moth CatchCMOBLRCM			% Fruit Injury CM OBLR		
Dual Low	1	0.0	0.0	0.1	0.4	0.0	0.0
	2	5.5	0.0	3.3	11.4	0.7	0.4
	3	2.9	2.4	0.8	6.8	0.0	0.0
	4	18.0	0.0	4.1	2.0	2.1	0.1
Dual High	5	0.0	0.0	0.0	14.1	0.0	0.04
	6	4.8	17.3	6.3	27.8	0.1	0.3
	7	0.5	1.8	0.0	9.0	0.0	0.4
	8	0.5	0.0	0.0	0.3	0.1	0.0
	9	4.6	2.9	3.4	24.4	3.1	0.9
CM - only	10	0.1	-	0.5	-	0.0	
	11	1.6	-	0.7	-	0.0	
	12	0.3	-	0.0	-	0.0	
	13	5.4	-	2.0	-	0.7	
	14	0.0	-	0.0	-	0.0	
	15	0.0	-	0.3	-	0.2	
	16	0.4	-	0.1	-	0.0	

Table 1. Seasonal Results of Paramount Puffers for CM and OBLR, Brewster, 2000

,	<b>Fable 2. Comparison of Paramou</b>	nt Puffer-treated with nearby	y Iso	omate-C+-treated orchards

Puffer Treatment	% CM Injury	% OBLR Injury
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# of orchards paired		Puffer	Isomate	Puffer	Isomate
Dual Low	n = 4	0.70	1.73	0.11	0.56
Dual High	n = 2	1.55	0.95	0.32	0.70
CM only	n = 4	0.05	0.0	-	-

# **CONCLUSIONS:**

Codling moth populations in apple orchards treated with aerosol puffers internally at one per hectare and with border applications of the Isomate dispenser were effectively controlled. The importance of supplementing the use of any sex pheromone delivery system with insecticides when populations exceed the action threshold was clearly demonstrated..

We have shown repeatedly that a high proportion of female moths are mated in treated orchards. Current hypothesis suggest that delay of mating may be a major mechanism accounting for population reductions. Further efforts to improve the use of sex pheromones to manage codling moth should focus on the potential differences achieved with variable densities of hand-applied, dispensers, the low point densities of high emission rate sources, and the uniformity of emission achieved with sprayables. This grant will continue to develop the use of aerosol puffers and perhaps other low density, high emission pheromone systems.

## **PROGRESS REPORT FOR 1999-2000**

TITLE:	Determination of Environmental Parameters that Limit or Enhance the Efficacy of Viruses for Control of Codling moth and Fruit tree Leafrollers and <i>Bacillus thuringiensis</i> for Control of Fruit tree Leafrollers
PRINCIPAL	Lawrence A. Lacey, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	Laboratory, AKS
<b>CO-INVESTIGATORS:</b>	Robert Pfannenstiel, ARS
FUNDING HISTORY:	Funding in 1998-1999 (Year Initiated): \$27,500 Funding in 1999-2000: \$22,500

### **SIGNIFICANT FINDINGS:**

Bt toxins that are not currently used in commercial formulations are excellent larvicides of *Pandemis* and obliquebanded leafrollers and oriental fruit moth.

The *Pandemis* granulovirus is virulent for old as well as young instars and has the advantage of being sheltered from UV degradation in larval retreats. Despite the lag time from exposure to low dosages to development of disease symptoms and death, infected larvae consume significantly less foliage than control larvae.

#### **OBJECTIVES:**

Continuation of efficacy studies of new formulations and toxin varieties of *Bacillus thuringiensis* against leafroller species in apple orchards.

Determine the efficacy of Pandemis GV in apple.

Determine the effect of UV radiation, rain simulation, fruit tree variety, temperature and larval age on efficacy and persistence of PpGV virus.

Determine other factors that effect activity *Pp*GV.

#### **PROCEDURES:**

Virus used in assays was produced in *Pandemis* or obliquebanded leafrollers (OBLR) using procedures outlined by Evans and Shapiro (1997). Bacterial toxins (Cry 1Ac, Cry 1Bb, Cry 1C, Cry 2B and Cry 9C) were obtained from a commercial source.

Bioassays of bacterial toxins against *Pandemis* leafroller, OBLR and oriental fruit moth (OFM) and viruses against *Pandemis* leafroller and OBLR were conducted on artificial media according to procedures outlined by Lacey et al. (2000): Larvae used in bioassays were obtained from colonies maintained on artificial diet at the USDA-ARS Yakima Agricultural Research Laboratory. Bioassays against neonate larvae of OBLR, *Pandemis* leafroller and OFM were conducted on artificial diet in two ml plastic conical autosampler vials. A two mm diameter hole in the cap of each vial covered with stainless steel screen (150 mesh) eliminated condensation. The codling moth diet described by Brinton *et al.* (1969) (BioServ, Frenchtown, NJ, USA) was used for OFM neonate larvae. A pinto bean based diet (Shorey and Hale, 1965) was used for assays with the two leafroller species. One ml of molten diet was added to each vial and allowed to cool before application of bacterial toxin suspension. Bioassays of

bacterial toxins against each species were performed with neonates using five concentrations of each toxin that produced mortality ranging from 20 to 90%. The only exception was the Cry 1Ca toxin, in which case only the dosage of 5000 ng/cm<sup>2</sup> was used due to its low toxicity to larvae. Bacterial powders were weighed and diluted just prior to bioassay. A 10  $\mu$ l suspension of toxin was added to each vial using a micropipetter. Vials were then tilted and rotated to ensure even coverage of the surface. The treated vials were left uncapped for 2 h before adding a single larva to each vial. Thirty neonates of each species were used for each concentration of virus and control. Larvae were incubated at 25°C for 7 days before determination of mortality. Replicate tests were conducted on each of 4-6 separate dates.

Identical procedures were used for bioassay of viruses except mortality was read at 7, 14 and 20 days after treatment. Additionally, groups of 45 neonate *Pandemis* larvae were exposed to approximate  $LC_{95}$  dosages of the *Pandemis* virus in the manner prescribed above and monitored daily to track the progression of mortality over a 21 day observation period. Forty-five control larvae were monitored for mortality over the same period. The procedure was repeated on three separate dates.

Seedlings were treated with a commercial preparation of Bt (Dipel 2X) in a DeVries spray cabinet using label dosage rates. The trees were placed outdoors for various periods while measuring solar radiation with a pyranometer. Just after the trees were removed from sunlight, 25 neonate larvae were placed on each tree and monitored for 7 days at which time mortality was assessed.

Seedlings were sprayed with a laboratory prepared suspension of the *Pandemis* virus in a DeVries spray cabinet using one infected larval equivalent of virus/35ml water. The trees were then placed in a solar simulator (dosage: 765 watts/m<sup>2</sup>/hr) for various periods of time. Just after the trees were removed from the simulator, 25 neonate larvae were placed on each tree and monitored after 7,10 and 14 days. Mortality was assessed for each period of incubation.

Studies on the impact of *Pandemis* granulovirus on feeding and development of 4<sup>th</sup> instar *Pandemis pyrusana* was started in the laboratory using bioassay methods developed by Lacey et al. (submitted). Thirty 4<sup>th</sup> instar larvae (30/virus concentration and control) were exposed to three conc. virus on artificial media for 48 h, then transferred to apple leaves. Leaf consumption was quantified using a leaf meter every two days. Leaves were be changed daily until the larvae died or pupated or ceased to feed. Leaf consumption, time until pupation and mortality were measured.

# **RESULTS AND DISCUSSION:**

Response of the two leafrollers and OFM to bioassay with the five Bt toxins was very different for each of the species (Table 1). OFM was overall the most sensitive of the three species and was considerably more susceptible to the Cry 1Ac and Cry 1Bb than were the two leafrollers. On the other hand *Pandemis* was the most susceptible of the three species to the Cry 2B and Cry 9C toxins. OBLR was more susceptible to the Cry 1Ac and Cry 1Bb toxins than to the other toxins, but it was almost 10 fold less susceptible to these than was OFM. None of the species were susceptible to Cry 1Ca. Commercial preparations contain a blend of toxins in the Cry 1A and Cry 2 groups (Höfte and Whiteley, 1989). Several other toxins in the Cry 1 group demonstrate potential as larvicides of leafrollers (Knight et al., 1998). Our results indicate that Cry 2B and Cry 9C have excellent larvicidal potential for control of *Pandemis* and moderately high activity against OFM and warrants further investigation. OBLR was also most sensitive to a toxin group (Cry 1B) not currently used in commercial preparations.

The results of tests that investigated the effect of *Pandemis* larval age on susceptibility to PpGV yielded surprising results: fourth instars were as susceptible to the virus as were neonates, although the onset of mortality was slower (Tables 2-4). Second instars were the least susceptible in the short run. The increased mortality at lower dosages observed in the forth instars was probably a function of the large amount virus consumed with diet. The onset of mortality is both a function of larval age and dosage.

Even moderately low dosages result in high mortality when the observation period is extended to 3 weeks. The effect of 5 and 50 virus granules/mm<sup>2</sup> on onset of mortality is shown in Figure 1. This lingering may cause some concern due to prolonged survival, however there is a significant reduction in feeding by larvae that slowly develop disease symptoms due to consumption of lower amounts of virus (Figure 2).

Despite the rapid reduction of exposed virus on leaf surfaces due to UV-inactivation (Tables 5 and 6), most larvae that die on treated seedlings, do so within larval retreats, a protected niche that would screen harmful radiation. Exposure of Bt treated seedlings to 8 hours of solar radiation has little effect on larvicidal activity. However, additional exposure rapidly denatures Bt activity (Table 7).

## **CONCLUSIONS:**

Research on the use of Bt toxins other than those now commercially available warrants further attention. The *Pandemis* virus offers considerable potential as an intervention for selective control *Pandemis* leafroller. Formulation with adjuvants that promote increased initial consumption of the virus and enhance UV protection should enhance the larvicidal activity and persistence of this microbial control agent as well as Bt.

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## **PROGRESS REPORT FOR 1999-2000**

TITLE:	The Potential of Insect Pathogens for Control of Lacanobia subjuncta (Lepidoptera: Noctuidae)
PRINCIPAL	Lawrence A. Lacey, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	Laboratory, AKS
<b>CO-INVESTIGATORS:</b>	Peter J. Landolt, Yakima Agricultural Research Laboratory, ARS
FUNDING HISTORY:	Funding in 1998-1999 (Year Initiated): \$22,500 Funding in 1999-2000: \$22,500

## **SIGNIFICANT FINDINGS:**

A virus isolated from *Lacanobia* fruitworm in Yakima is effective for control of *L. subjuncta* larvae. Entomopathogenic nematodes have potential for control of *Lacanobia* prepupae, but not pupae.

## **OBJECTIVES:**

Continue bioassay of candidate native nucleopolyhedroviruses and those from *Autographa californica* (*Ac*MNPV) and of *Anagrapha falcifera* (*Af*MNPV) against a range of larval instars of *L. subjuncta*.

Bioassay of candidate *Bacillus thuringiensis* toxins and formulations against a range of larval instars of *L. subjuncta*.

Bioassay of *Steinernema carpocapsae* and *S. feltiae* and other entomopathogenic nematodes for control of *L. subjuncta* pupae in soil.

Investigate the potential of combining the female sex pheromone of *L. subjuncta* or chemical food attractant and entomopathogenic fungi and viruses for the autodissemination of pathogens into populations of *L. subjuncta*.

Survey populations of *L. subjuncta* for naturally occurring entomopathogens.

## **PROCEDURES:**

# Sources of Pathogens

## <u>Virus</u>

A virus collected from *Lacanobia subjuncta*, related to the *Mamestra* virus was isolated at the Yakima Lab. All virus used in our research was produced at the Yakima Lab in cabbage looper larvae (*Trichoplusia ni*) using procedures described by Hostetter and Putler (1991) and Evans and Shapiro (1997). The concentration of occlusion bodies for each virus suspension was determined with a light microscope and a hemacytometer. One ml aliquots of the virus suspensions were stored in 1.5 ml ependorf vials at -80°C until use.

## Nematodes

The nematode species were originally obtained from commercial sources and from Dr. Harry Kaya, Department of Nematology, University of California, Davis, CA. All infective juveniles (IJs) used for laboratory assays and small field plot tests were produced in wax moth larvae following procedures outlined by Kaya and Stock (1997). Tests were conducted with *Steinernema feltiae*, *S. carpocapsae*, *S. kraussei*, *S. riobrave* and *Heterorhabditis bacteriophora*.

## Bacteria

Bacterial toxins (Cry 1Ac, Cry 1Bb, Cry 1C, Cry 2B and Cry 9C) purified from cultures of *Bacillus thuringiensis* were obtained from a commercial source.

# **Bioassay Methods**

Procedures for bioassay of *Bt* toxin and virus against *L. subjuncta* larvae were those we have developed for assay of *Bt* against leafrollers on artificial diet (Lacey et al., submitted). A colony of *L. subjuncta* used in bioassays was established from field collected adults and reared on artificial diet and apple leaves at the USDA-ARS Yakima Agricultural Research Laboratory. Bioassays against neonate and older *Lacanobia* fruitworm larvae were be conducted on artificial diet. Bioassays of the virus were performed using five concentrations of each virus that produced mortality ranging from 20 to 90%. Thirty neonates were used for each concentration of virus and control. After treatment larvae were incubated at 25°C for 7,10 and 14 d before determination of mortality. Replicate tests were conducted on at least 3 separate dates. Bioassay of Bt toxins was conducted in an identical manner to that used for the viruses.

Bioassays of infective juveniles of nematode species against prepupae and pupae were conducted in soil in large Petri dishes using the discriminating dosage of 500,000 IJs/m<sup>2</sup>. After incubation at 25°C for 7 days, mortality was assessed.

Preliminary experiments were conducted in the laboratory using the female sex pheromone isolated by Landolt and Smithhisler (1998) and chemical food attractant (Landolt 1999) to determine if moths attracted to a virus contaminated substrate can contaminate eggs and neonates with the virus.

# **RESULTS AND DISCUSSION:**

The larvicidal activity of the virus isolated from *Lacanobia* fruitworm is dosage and time dependent, i.e. the greater the dosage the higher the mortality in the short term, but relatively low dosages produce near complete mortality 14 days after initial exposure (Table 1). The LT  $_{50}$  of neonate larvae to the low dosage of 9 virus occlusions /mm<sup>2</sup> is approximately 7 days (Figure 1). Second instar larvae are slightly less susceptible to the virus than are neonates (Table 2). Bioassays with 4<sup>th</sup> instars resulted in excessive control mortality. The *Lacanobia* fruitworm is apparently very sensitive to handling.

Results of bioassays of entomopathogenic nematode species against prepupae of *Lacanobia* are reported in Table 3. Although the control mortality was excessive, larvicidal activity is apparent. The three nematode species produced roughly the same mortality in prepupae. *Lacanobia* pupae were almost completely resistant to infection.

Bioassays with Bt toxins and the viruses from are on going and will be reported at the entomological review.

# **CONCLUSIONS:**

The *Lacanobia* virus offers good potential for the control of larvae. If nematodes are present in leaf litter and soil at the time *Lacanobia* prepupae are wandering in search of pupation sites, significant mortality can be expected.

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## **PROGRESS REPORT FOR 1999-2000**

TITLE:	Development of Entomopathogenic Nematodes for Control of Codling moth and Fruit tree Leafrollers in Orchards
PRINCIPAL	Lawrence A. Lacey, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	
<b>CO-INVESTIGATORS:</b>	Thomas R. Unruh, Yakima Agricultural Research Laboratory, ARS
FUNDING HISTORY:	Funding in 1996-1997 (Year Initiated): \$20,000 Funding in 1997-1998: \$22,596 Funding in 1998-1999: \$20,000
	Funding in 1999-2000: \$27,500

### **SIGNIFICANT FINDINGS:**

Cold active nematodes have been identified that will enable effective control in climatic conditions that are below 10°C (50°F).

Evaluation of candidate a cold active species confirms its utility in Spring and Fall conditions under orchard conditions.

Irrigation will be a useful tool for wetting tree trunks before application of nematodes, but will not provide a useful method for post-treatment wetting.

Preliminary results from formulation studies indicates that improvement in efficacy can be expected by formulating nematodes to retard desiccation and speed the penetration of hibernacula.

## **OBJECTIVES:**

Evaluate different methods of application (various configurations of airblast sprayer, hand gun, backpack sprayer) of entomopathogenic nematodes.

Develop irrigation based wetting of orchards after treatment with nematodes.

To compare and evaluate the activity of additional steinernematid and heterorhabditid nematodes against codling moth prepupae under controlled laboratory conditions and under orchard conditions.

To compare and evaluate the activity of steinernematid and heterorhabditid nematodes against overwintering and actively feeding larvae of OBLR and *Pandemis* leafrollers.

Compare the susceptibility of codling moth pupae and larvae to nematode infection.

To assess the potential of formulation adjuvants in preventing desiccation of infective juvenile nematodes in the laboratory and their value under field conditions.

#### **PROCEDURES:**

Nematodes will be purchased from commercial sources and produced *in vivo* using late instar *Galleria mellonella* using techniques described by Kaya and Stock (1997). Laboratory procedures developed by Lacey and Unruh (1998) will be utilized for laboratory studies conducted with *Steinernema feltiae*, and *Steinernema sp.* from apple orchards in Jura, Switzerland (a potentially cold tolerant species), new

isolates of *S. carpocapsae*, and *Heterorhabditis* spp. against codling moth prepupae and overwintering leafroller species on pear and apple stumps under simulated field conditions and in orchards using methods outlined by Lacey et al. (2000) and Unruh and Lacey (2000).

The effect of formulation adjuvants on viability and infectivity of infective juveniles will be conducted at  $25^{\circ}$ C using bioassay procedures prescribed by Lacey and Unruh (1998). For comparison with formulated nematodes, our treated controls consisted of treating infested substrates with aqueous suspensions of *S*. *feltiae* at 10 IJs/cm<sup>2</sup> and then covering half of the substrates to prevent evaporation and leaving the others uncovered. All formulated treatments were left uncovered.

Small plot field applications of *S. carpocapsae* and *S. feltiae* will also be made at the Moxee farm and in a commercial orchard using an airblast sprayer configured for different droplet sizes, a hand gun sprayer and backpack sprayer. Testing various post-treatment wetting strategies (irrigation-based) on the efficacy of the nematodes for control of codling moth larvae will continue. Sentinel larvae in logs will be placed in the orchards just prior to application of nematodes and removed 24 hours after treatment and incubated for an additional 4 days. This provides adequate numbers of target larvae. Incubation does not affect the level of kill, but allows development of patently infected larvae that can be separated from larvae that died from other causes. The effect of application on nematode viability and infectivity will also be conducted in conjunction with field trials.

# **RESULTS AND DISCUSSION:**

One of the limiting factors with some species of entomopathogenic nematodes will be cessation of infection at temperatures much below 15°C. Several species with potential larvicidal activity in cold temperatures were evaluated under laboratory conditions at 5-25°C(Table 1). Two of the species with very good activity at 10°C are *S. feltiae* and *S. kraussei*. *S. feltiae* was also used in orchard trials in fall and spring conditions. In September when temperatures were warm, *S. feltiae* and *S. carpocapsae* performed in a similar manner; both species provided nearly complete control (Table 2). However, when temperatures declined in October and March, *S. feltiae* was clearly the more effective of the two for control of cocooned codling moth larvae. The search for nematode species that are efficacious in low temperatures will continue. Cooperative links with the USDA European Biological Control Lab in Montpellier have already provided several candidate nematodes from apple orchards in cold climate (Vega et al., 2000).

Comparison of hand gun application with airblast sprayer and effect of post treatment irrigation on nematode activity was completed in the 1999 growing season. In one trial in June, orchards were prewetted with ground based irrigation for one hour and then irrigated again for 6 hours following application of nematodes (2 million IJs/tree). The level of control with *S. carpocapsae* was not significantly different from that in control plots. Mortality of codling moth larvae on tree trunks in plots treated with *S. feltiae* was also not acceptable (»50%). We believe sustained post-treatment wetting with ground based sprinklers washed IJs from the trees and served to lower temperatures substantially during the 6 hours following application. Larvae that were in cardboard strips placed in cracks in the soil, however, were completely controlled with *S. feltiae* and significantly reduced with *S. carpocapsae*. The July field trial was conducted without pre and post-treatment irrigation.

Field trials conducted in August combined one hour of pre-treatment irrigation with careful periodic posttreatment moistening of trees by hand and compared the two nematodes applied using a hand gun sprayer and airblast sprayer. Good control was obtained using *S. feltiae* with both sprayers and fair to moderate control was obtained with *S. carpocapsae*. Only moderate control of larvae in the soil was obtained with this method of post-treatment wetting. It is clear that sufficient moisture, but not excessive drenching is necessary for IJs to be active on tree trunks. However, IJs in the soil are most effective against larvae when sufficient water enables their penetration of areas in which the codling moths are located. Formulation with wetting agents and humectants could provide the rapid penetration of hibernacula that is required while helping to sustain the necessary moisture to enable nematode survival.

# **RESULTS AND DISCUSSION:**

Results from initial formulation studies indicate that improvement in larvicidal activity of nematodes in the absence of continual post-treatment wetting will be possible (Table 3). The most promising adjuvants used thus far are the wetting agents Silwet L-77 and Silwet 408 and Freeway. The adjuvants themselves had no effect on codling moth larvae.

Experiments to compare the susceptibility of codling moth pupae and larvae revealed a marked difference in susceptibility to nematode infection. Pupae were significantly less susceptible to infection by *S. carpocapsae* than larvae (Table 4). This will necessitate the application of IJs in the Spring before pupae has taken place.

# **CONCLUSIONS:**

Nematodes can provide control of overwintering codling moth even under early Spring and late Fall conditions, but limitation due moisture requirements will have to be addressed through formulation and application strategy before widespread usage is possible.

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### **PROGRESS REPORT FOR 1999-2000**

TITLE:	Identification of Chemical Attractants from Apple Foliage and Fruit for Codling moth
PRINCIPAL	Peter J. Landolt, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	
FUNDING HISTORY:	Funding in 1998-1999 (Year Initiated): \$29,000 Funding in 1999-2000: \$34,400

### **SIGNIFICANT FINDINGS:**

Female codling moth adults are more strongly attracted to apple fruit infested with codling moth larvae, compared to uninfested fruit.

Female codling moths are weakly attracted in the flight tunnel and irregularly in the field to one of the chemicals found in apple headspace odor.

Larvae of the codling moth are attracted to at least 2 chemicals found in the headspace from infested granny smith apples.

#### **OBJECTIVES:**

The overall project objective is to define and develop chemical attractants for codling moth adults and codling moth larvae based on host plant chemistry. Specific objectives of the current year are to:

Test chemicals for responses of codling moth adult females.

Test chemicals for responses of codling moth larvae

Study moth behavioral responses to host odor.

Repeat GC-EAD work with walnut.

#### **PROCEDURES:**

Flight tunnel assay procedures were developed by Dr. Hal Reed, visiting from Oral Roberts University. Mated female codling moths were tested for attraction responses to thinning apples, infested thinning apples, airflow over apples, and airflow over infested apples, with appropriate experimental controls. Additionally, mated female codling moths were tested for attraction responses to several chemicals found in the headspace from over apples, at a range of release rates overlapping that found from apples.

Olfactometers were used to evaluate the responses of neonate larvae of the codling moth to apple fruit, to volatile collections from apple fruit, and from selected chemicals found in apple odor. These experiments involved both Y-tune and parallel tube assays. Volatile collections from apples were made using filtered, metered cyllinder air passed over apple fruit in a glass jar and then through a small superQ trap. The SuperQ sorbent was extracted with various solvents and applied to filter papers in the olfactometer for assays. Additionally, volatile collection samples from apples were analyzed by GC-MS to track the presence of specific compounds of interest. Active extracts of volatile collection traps were fractionated further using an LC technique to further isolate the attractant, based on solvent polarity. Fractions of the extract were tested in the olfactometer for evidence of activity in comparison to a stock sample of apple

odor. Several synthetic chemical samples were tested in the Y-tube olfactometer for attractiveness to codling moth larvae, over a broad range of release rates, formulated in rubber septa.

Field experiments were conducted to test several chemicals and chemical blends for attractiveness to codling moth. Chemicals were formulated in rubber septa, in glass capillary tubes, and in polypropylene vials, depending on molecular weight and targeted release rates. These dispensers were then used in Pherocon 1C traps to evaluate their attractiveness to codling moth.

## **RESULTS AND DISCUSSION:**

## Flight Tunnel Studies

Mated female codling moths were attracted to thinning apples placed in the flight tunnel, but were more strongly attracted to thinning apples infested with codling moth larvae. Similarly, when apples were placed in a glass jar outside of the flight tunnel and the airflow was piped into the tunnel, female codling moths were attracted to this odor and were more strongly attracted to the odor from infested apples placed in the glass jar.

Mated female cabbage loopers exhibited relatively weak responses to several synthetic chemicals tested in the flight tunnel, in comparison to their responses to apples. One chemical however did elicit complete orientation responses from a small number of tested moths, including zigzagging upwind flights leading to contact with the septum releasing the chemical.

This flight tunnel work continues along two lines: the testing of blends of chemicals and the testing of volatile collection system fractions, using classical holistic isolation techniques. It is not yet evident whether the moth responds to several different chemicals separately or responds optimally to a specific blend when searching for a host. Work conducted in other laboratories suggests that codling moth adults orient to more than one chemical when presented separately (Hern and Dorn 1999; P. Witzgall, personal comm.; Doug Light, unpublished).

## Olfactometer Studies

Previous findings of neonate larval responses to apple fruit infested on the tree were confirmed in 2000. Subsequently, we use a column LC technique to isolate two distinct attractive fractions, demonstrating the presence of at least two different attractive compounds. These two samples have been further fractionated, using reverse phase chromatography. A preliminary GC-MS analysis of those isolates has provided a short list of candidate attractants.

Testing of two different chemicals has been conducted to determine the responses of neonate larvae to a range of release rates of two chemicals. One of these chemicals has been reported by others to be an attractant for codling moth and the other is indicated to be an attractant in our isolation studies with apple odorants. In both cases, significant responses were observed by larvae at several chemical release rates. However, in both cases, these responses were weak in comparison to the larval responses to infested apples and occurred in response to chemical release rates that are high in comparison to apple. We are working now to determine the structure of the attractive chemical in the second LC reverse phase fraction.

## Field Testing

Field testing in the spring failed to indicate any activity of several chemicals thought to be candidate attractants based on previous GC-EAD studies. This included the field testing of several sets of chemical blends. However, during the second flight, one chemical did attract significant numbers of both sexes of codling moths to traps when presented in a blend or when presented separately.

### **CONCLUSIONS:**

Both larvae and adult codling moth are more strongly attracted to apple fruit that are infested. The attractant from infested apples involves two very different chemicals that come out in different solvent fractions.

### **REFERENCES:**

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#### **PROGRESS REPORT FOR 1999-2000**

TITLE:	Development of Feeding Attractants as Monitoring Lures for moth Pests of Apple
PRINCIPAL	Peter J. Landolt, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	Laboratory, AKS
FUNDING HISTORY:	Funding in 1998-1999 (Year Initiated): \$22,750 Funding in 1999-2000: \$23,700

#### **SIGNIFICANT FINDINGS:**

Superior trap designs were indicated for *Lacanobia subjuncta*, bertha armyworm, and spotted cutworm moths attracted to acetic acid with 3-methyl-1-butanol, for codling moth attracted to acetic acid and for *Pandemis* leafroller attracted to acetic acid. For the *Lacanobia* fruitworm, bertha armyworm, and spotted cutworm, the best trap was the Universal moth trap, which we call the bucket trap or Unitrap. For codling moth, the best trap was the Pherocon 1C trap, also known as the wing trap. For *Pandemis* leafroller, the best trap was the Agrisense Trappit trap, also known as the dome trap.

The optimum release rates of acetic acid for capturing codling moth and *Pandemis* leafroller are quite different, with codling moth attracted best at a much lower release rate (0.5 mg per hr) compared to *Pandemis* at 3.5 mg/hr.

### **OBJECTIVES:**

The overall project objectives are to develop chemical attractants, lures, and trapping systems for using feeding attractants for monitoring and sampling of moth pests of apple and pear. Specific objectives of the current year are to:

Determine the most efficient dry trap design for capturing *Lacanobia* fruitworm, bertha armyworm, and spotted cutworm, *Pandemis* leafroller, and codling moth, attracted to feeding attractants.

Determine the most efficacious trap placement for a feeding attractant trap for Lacanobia fruitworm.

Determine capture efficiency of an optimized lure and trap.

Determine the optimum release rate for the codling moth 2 component blend.

Discover additional chemical attractants or co-attractants for the codling moth.

Compare feeding attractants and sex pheromone lures in relation to female oviposition and population density.

#### **PROCEDURES:**

#### Objective 1

Several experiments were conducted to compare different trap designs baited with feeding attractants. These tests were conducted separately for the noctuid moths, for codling moth and for *Pandemis* leafroller. Five trap designs were tested and all were baited with feeding attractant in a polypropylene dispenser, permitting the use of both wet and dry traps. Chemicals used and vial hole sizes governing chemical release rates were determined in previous studies as optimum for those moth species targeted.

# Objective 2

Trap height was evaluated by placing traps in the lower third of the apple tree canopy, near the center of the canopy and in the upper third of the canopy. Agrisense dome traps were used, baited with feeding attractant in a polypropylene vial.

## Objective 3

Moth responses to synthetic feeding attractant dispensed from polypropylene vials were evaluated in a flight tunnel to determine the percentage of moths that are attracted and the fraction of responding moths that enter into a Unitrap placed in the tunnel.

# Objective 4

Optimum release rate of codling moth attractant. More field assays were conducted comparing dispensers releasing different amounts of attractant to determine the optimum release rate range. Determination of release rates for dispensers was done using both gravimetric methods and volatile collection or aeration techniques.

# Objective 5

Trapping experiments were conducted to evaluate several additional chemicals. These included experiments comparing the attractiveness of acetic acid with the attractiveness of two component blends (acetic acid and phenethyl carbinol,terpin-4-ol, alpha toluic acid, and anethole).

# Objective 6

Experiments to compare numbers of moths in traps with oviposition and with population levels were not conducted.

## Other

A series of concentrations of acetic acid were compared in wet traps for attractiveness to *Pandemis* leafroller moths. These were 0.5%, 1%, 2%, 5%, and 10%. In a previous test in 1999, testing a lower range of percentages of acetic acid in water, greatest numbers of moths were captured with the highest concentration tested (2%). A higher range of concentrations were tested here, to determine the optimum.

## **RESULTS AND DISCUSSION:**

## Objective 1

For *Lacanobia* fruitworm, bertha armyworm, and spotted cutworm, greatest numbers of moths were captured in Universal moth traps, confirming results of 1999. Lesser numbers of these noctuid moths were captured in dome traps, in Multipher traps, in Nadel style traps, and in glass McPhail traps. For codling moth, greatest numbers of moths were captured in Pherocon 1C traps, with lesser numbers captured in Trappitt dome traps, in Universal moth traps, and in Delta traps. For *Pandemis* leafroller moths, greatest numbers of moths were captured in dome traps, with fewer numbers of moths captured in Delta traps, Pherocon 1C traps, Multipher traps, Unitraps.

## Objective 2

Greatest numbers of *Lacanobia* fruitworm moths were captured in dome traps placed in apple orchards when they were placed in the upper one third of the tree canopy, compared to the center or bottom thirds of the tree canopy.

## Objective 3

These experiments are not finished. We have not been able to rear suitable numbers of moths for proceeding more rapidly with assays. Studies have been conducted to determine optimum flight tunnel conditions for assaying *Lacanobia* moths and to determine appropriate pretreatment conditions for

obtaining moths that will be attracted to the lures. There should be no problem continuing this work through the winter using lab reared moths and laboratory flight tunnels.

## Objective 4

Optimum numbers of codling moths are trapped with feeding attractant vials with holes that are 0.04 inches in diameter. This vial provides a release rate of 0.5 mg per hr under laboratory conditions. Traps with vials releasing 0.2 mg per hr and 1.0 mg per hr captured significantly fewer moths.

# Objective 5

There were no chemicals or combinations of food based chemicals tested that provided a trap catch dramatically better than an optimized release rate of acetic acid. We still have one alcohol that provides an increase in codling moth captures when presented in a trap together with acetic acid. However, that increase in codling moth trap captures was weak.

# Other

Greatest numbers of *Pandemis* leafroller moths were captured in dome traps baited with 2% solutions of acetic acid in water, compared to higher (5%) and lower (1%) concentrations.

# **CONCLUSIONS:**

The different moth pests of tree fruits studied not only are optimally attractive to different lures, but are optimally captured with different trap designs. For *Lacanobia* fruitworm, it is best to use a combination of acetic acid and 3-methyl-1-butanol loaded in vials with 0.12 inch diameter holes used to bait Universal moth traps. For *Pandemis* leafroller, it is best to use acetic acid loaded in vials with 0.12 inch holes, used in Agrisense dome traps. For codling moth, it is best to use acetic acid loaded in vials with 0.04 inch holes, used in Pherocon 1C traps.

Despite repeated testing of additional chemicals, there are no combinations of compounds tested yet that are dramatically better feeding attractants for *Pandemis* leafroller and codling moth than is acetic acid alone.

# **REFERENCES:**

Landolt, P. J. 2000. New chemical attractants for trapping *Lacanobia subjuncta, Mamestra configurata*, and *Xestia c-nigrum* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 101-106.

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### **PROGRESS REPORT FOR 1999-2000**

TITLE:	Development of Feeding Attractants for Control of moth Pests of Apple
PRINCIPAL	Peter J. Landolt, Yakima Agricultural Research
INVESTIGATORS:	Laboratory, ARS
FUNDING HISTORY:	Funding in 1999-2000 (Year Initiated): \$32,213

### **SIGNIFICANT FINDINGS:**

Male *Lacanobia* fruitworm moths attracted to pheromone lures contact lures with their legs and display their hairpencils but do not try to copulate directly with the lure.

Some females attracted to feeding attractant dispensers directly contact the dispensers, which should result in their mortality when dispensers are combined with a pesticide.

### **OBJECTIVES:**

Objective 1. Develop formulations

Objective 2. Verify attractant release rates

Objective 3. Develop bait stations

Objective 4. Conduct assays to assess models.

#### **PROCEDURES:**

*Lacanobia* fruitworm larvae were reared in the laboratory in individual cups containing a bean based diet developed for the cabbage looper. Eggs were obtained from females captured in a light trap at the Yakima Agricultural Research Laboratory, and from females paired with males in 16 oz plastic cups with water, sugar water, and apple foliage.

Bioassays were conducted in a flight tunnel on a reversed light cycle to facilitate testing during normal working hours. Moths were tested singly and were given 2 minutes to respond before they were removed. Because most assays involved moth responses to feeding attractants, moths were not provided sugar water but were provided water.

A series of tests were conducted using the flight tunnel, to assess moth attraction and short range responses to chemical lures, and to assess the responses of attracted moths to visual stimuli. For each evaluation, a series of 10 to 20 moths were tested to a given lure, bait or station and this series was then replicated over time.

Aeration measurements were made on some formulations, by placing those formulations in a glass jar and passing filtered metered air through the jar and then through a trap. Traps were then extracted and analyzed by gas chromatography with appropriate standards to quantify the amounts of chemicals emitted. Additionally, release of chemicals were quantified from some formulations by a gravimetric analysis. That is, the formulations were weighed over intervals of time to determine weight loss due to the release of active ingredients.

#### **RESULTS AND DISCUSSION:**

To facilitate the rearing of larger numbers of *Lacanobia* fruitworm moths for flight tunnel bioassays, several improvements were made in rearing techniques. These included the shift from rearing batches of larvae to rearing larvae individually (which greatly improved the percent survival to pupation), the use of cabbage looper diet instead of leafroller diet (which speeded up the rate of development) and several changes in procedures for obtaining eggs from moths. Related work underway on the suitability of plants as hosts for *Lacanobia* fruitworm larvae has shown that larvae are cannibalistic when held together in groups and might best be reared in individual containers.

Flight tunnel assays revealed close range behavior of moths attracted to pheromone lures comprised of Z-11-16:Ac, Z-11-16:Ald, and Z-11-16:Alc in rubber septa (Landolt and Smithhisler 1998). Moth responses to pheromone lures are of interest as a means of spreading viral pathogens if males can be induced to make genital contact with a virus source. This technique was demonstrated by Jackson (1992). However, males of *Lacanobia subjuncta* did not directly contact rubber septa lures with the genitalia, but did touch septa with the forelegs while displaying hairpencils within one cm of the pheromone septum. It may be possible to manipulate the position of the septum to promote contact between the males and virus on a substrate. These studies will be continued through the winter season and are being conducted in collaboration with Dr. Lerry Lacey.

Flight tunnel assays also revealed close range behavior of female moths attracted to feeding attractant lures. These lures are the combination of acetic acid and 3-methyl-1-butanol (Landolt 2000) dispensed from polypropylene vials (Landolt and Alfaro 2001). What is sought is a behavioral response that brings a high proportion of moths in contact with a bait station. Moths attracted to the chemicals did directly contact the dispensers releasing the feeding attractant, but the response rate was not high. It is anticipated that the selection of a combination of chemical cues and visual stimuli will be required to achieve this objective, as was previously accomplished with the cabbage looper moth attracted to chemical lures and bright white targets laced with sugar and methomyl (Landolt et al. 1991). Studies will be continued through the winter to devise a combination of chemical attractant and visual targets that should provide better rates of contact between attracted moths and dispensers or targets.

Measurements of amounts of attractant chemicals released from different formulations has resulted in the selection of methods to devise both wet and dry bait stations with optimum release rates of acetic acid and 3-methyl-1-butanol.

Other laboratory studies continue under this project, in order to make adequate progress for beginning field evaluations of bait stations during the first flight of *Lacanobia* in May 2001. These studies include assays of selected pesticides for mortality of moths contact those pesticides and the testing of sugar substitutes as feeding stimulants in bait stations for *Lacanobia* fruitworm moths. Studies of feeding stimulants are conducted in cooperation with Drs. John Brown and Maciej Pszczolkowski of WSU Department of Entomology.

## **CONCLUSIONS:**

Male moths attracted to pheromone contact lures but do not copulate with them. Because contact between male genitalia and virus might promote transfer of virus to females and then to eggs, we must design lures to promote attempted copulations by males.

Female moths attracted to feeding attractants contact dispensers but at an inadequate rate. Changes in associated visual cues might be necessary to increase this rate of contact to adequate levels for use in bait stations.

Rearing of *Lacanobia* fruitworm in the laboratory is enhanced with the substitution of cabbage looper diet for leafroller diet and the use of individual cups to separate larvae and prevent cannibalism.

### **REFERENCES:**

Jackson, D. M. 1992. Autodissemination of a baculovirus for management of tobacco budworms (Lepidoptera: Noctuidae) on tobacco. J. Econ. Entomol. 85: 710-719.

Landolt, P. J. 2000. New chemical attractants for trapping *Lacanobia subjuncta*, *Mamestra configurata*, and *Xestia c-nigrum* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 101-106.

Landolt, P. J. and J. Alfaro. 2001. Trapping *Lacanobia subjuncta*, *Mamestra configurata*, and *Xestia c-nigrum* (Lepidoptera: Noctuidae) with acetic acid and 3-methyl-1-butanol in controlled-release dispensers. Environ. Entomol. (In press).

Landolt, P. J. and C. L. Smithhisler. 1998. Isolation and identification of female sex pheromone and development of a sex pheromone attractant for *Lacanobia subjuncta*. J. Chem. Ecol. 24: 1369-1379.

Landolt, P. J., B. Lenczewski, and R. R. Heath. 1991. Lure and toxicant system for the cabbage looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 84: 1344-1347.

# FINAL REPORT FOR 1999-2000

TITLE:	Plant Based Repellents for Codling moth
PRINCIPAL	Peter J. Landolt, Yakima Agricultural Research Laboratory, ARS
INVESTIGATORS:	Laboratory, ARS
FUNDING HISTORY:	Funding in 1999-2000 (Year Initiated): \$13,400

## SIGNIFICANT FINDINGS:

Methods of formulating lavender essential oil were inadequate to protect apple fruit from codling moths.

# **OBJECTIVES:**

The overall project objective is to define and develop chemical attractants for codling moth adults and codling moth larvae based on host plant chemistry. Specific objectives of the current year are to:

Develop formulation methods for essential oils

Evaluate 2 plant essential oils to protect individual apples

Characterize the odors emitted by essential oils.

# **PROCEDURES:**

Two approaches were tried for formulating essential oils of interest for testing as repellents against codling moth. The objective of the formulating was to slow down the release of repellent chemicals from the essential oil because this was found to be short-lived in laboratory assays. That is, the oil soon lost its repellency, due to evaporation of repellent chemicals from the oil. One approach was to dilute the plant essential oil in a heavier mineral oil. The mineral oil was then loaded onto cotton dispensers for use in trials on apple trees. A second approach was to load a plant essential oil into slow release dispensers used presently for feeding attractant chemicals. These also were then used on apple trees in an attempt to repel codling moths. Field cage assays lasted one week, at which point the fruit was removed and replaced with new fruit for additional assay replicates.

The objective of assays conducted on apple trees was to prevent codling moths from infesting apples by repelling them with lavender essential oil. This should occur both through the repelling of ovipositing moths and the repelling of newly hatched larvae searching for fruit. Mated female codling moths were released into field cages containing small apple trees with 20 fruit per tree. The two types of formulation or dispenser were evaluated using this assay design, with comparisons made of the percentages of fruit infested on treated versus untreated trees. Formulations were evaluated at 20 units per tree.

The major components of the odor of lavender oil were determined through a combination of available scientific literature and air collections made on oil used in tests. These air collections were made using solvent extractions of SuperQ filters and extracts were analyzed by GC-MS to make structural assignments.

## **RESULTS AND DISCUSSION:**

There were no significant effects of the repellent treatments on the rate of infestation of apple by codling moth and most apples were infested by released codling moths on both treated and control trees. These negative results were similar with both methods of formulation.

It is assumed that the discrepancy between these results and those obtained in laboratory bioassays is due to insufficient quantities of the repellents around fruit for a long enough time to prevent moths from laying eggs near fruit and for larvae to find fruit. In previous testing of essential oil repellency of codling moth larvae, it was determined that this effect was short lived (Landolt et al. 1998) indicating that the active ingredient (s) in the oil are very volatile and leave the oil in a short space of time. The disadvantage of extending the repellency of the material in time is that reduces the release rate and we run the risk of losing the repellent effect. If this is the problem, it might be corrected either by greatly increasing the numbers of dispensers per tree or by identifying the active chemicals in the essential oil and then using highly concentrated synthetic chemicals as repellents.

The most abundant compounds volatilized from the essential oil of lavender are identified. Most are commercially available and these will be purchased for use in additional assays. If one or a small number of compounds is found to deter or repel larvae or adults at a low dose, the use of a synthetic chemical in place of the oil may give new hope to this approach.

## **CONCLUSIONS:**

Attempts to protect fruit on trees with dispensers loaded with essential oil of lavender were unsuccessful. It is assumed that the lack of protection was due to the volatilization of too little material for too short of a space of time to be effective. This might be overcome by determining which volatiles from the essential oil repels codling moth and then using the synthetic repellent chemical instead of the oil in larger scale tests.

## **REFERENCES:**

Landolt, P. J., R. W. Hofstetter, and L. L. Biddick. 1999. Plant essential oils as arrestants and repellents for neonate larvae of the codling moth (Lepidoptera: Tortricidae). Environ. Entomol. 28: 954-960.

# **PROGRESS REPORT**

TITLE: Biorational Materials for Orchard Pest Control

**PERSONNEL:** Gary Puterka

**REPORTING PERIOD:** 1999-2000

# **ACCOMPLISHMENTS AND RESULTS:**

A new project was initiated to further the development of biorational materials for orchard pest control. The two materials of interest include particle film and sugar esters. The objectives this season were to:

1) Compare the efficacy of particle film formulations amended to increase activity against arthropod pests not typically controlled (leafminer, mites)

2) Compare the efficacy of particle films used at different AI/acre to determine if rate affected efficacy against insects.

3) Determine if objectives 1 and 2 had effects on apple quality.

**I.** Comparison of New Particle Film Formulations and Surround WP with Sulfur Amendments. Season-long particle film application program was applied to 'Dixie Red' Delicious apple to compare formulations and Surround WP with different sulfur amendments (Fig. 1). Particle film formulations were applied at 50/lbs/acre. Two-spotted spider mite populations were low but showed that Surround WP alone did not disrupt mite populations compared to the untreated control. The Particle film #2 formulation and the Surround WP (50 lbs) + 4 lbs elemental sulfur showed elevated two-spotted spider mite levels at the beginning of July. After this time, mite levels came down to levels near the untreated control for the rest of the season. The Particle film #2 formulations also was the only formulation to show elevated European mite levels only on July 31, but these levels subsided afterward. Results indicate that Surround WP had little effect on mite populations. New formulations or Surround WP with the sulfur amendments showed no improvements over Surround WP.

**II. Comparison of Surround WP Used at Different AI/acre and Different Application Timings.** Season-long Surround WP applications were applied to 'Dixie Red' Delicious apple at two different rates (25 lbs. versus 50 lbs./acre) and applications began at two different timings (pre-bloom versus petal-fall) (Fig. 2). Application timings had little affect on the population development of two-spotted spider mite and European red mites. However, reducing rates to 25 lbs./A produced elevated mite levels, particularly for applications made at prebloom. These results suggest that reducing particle film rates could lead to elevated mite levels.

**III.** Affects on Apple Scab and Fruit Quality. New particle film formulations and sulfur amendments had no affect on apple scab. This was a particularly wet season and apple scab levels were very high. The fruit was essentially destroyed by apple scab so fruit quality could not be assessed.

**Conclusion.** Results showed that altering particle film formulation or adding sulfur amendments to Surround WP could affect the performance of Surround WP in a negative manner with regard to European red mites and two-spotted spider mite. Continuing research in new particle film formulations and new amendments appears worth pursuing and could lead to better formulations for control of mites and leafminer.

# **PROGRESS REPORT**

**PROJECT NO:** 8244

TITLE: Tien A new pollinator from the homeland of apples: The

Shan Mountain Gray Bee

**YEAR INITIATED: 2000/2001 CURRENT YEAR: 2000/2001** 

**TERMINATING YEAR: 2001/2002** 

PERSONNEL: W. Steve Sheppard, Entomologist, WSU, Pullman

## **OBJECTIVES FOR PROJECT PERIOD 2000-2001**

- to characterize the pollinating behavior of Tien Shan Mountain bees within regions of the wild apple forest and on cultivated apples.
- > to establish collaborators within this region and apiaries for queen rearing and importation
- Initiate the importation, quarantine and field testing of stock. Develop protocol for the dissemination of honey bee stocks to queen producers and beekeepers that serve the tree fruit growers of Washington State.

## **PROCEDURE – TIMELINE:**

Funding year 2000: Conduct field measurements of honey bee pollinating activity and general survey of pollinating insects in area of wild apple forests and cultivated apples. Evaluate Tien Shan stocks in apiaries. Evaluation of *Varroa* mite tolerance and disease prevalence in Tien Shan bees. Select queen rearing apiaries-collaborators.

## **ACCOMPLISHMENTS:**

#### Characterization of honey bee stocks

Field and apiary studies of a newly discovered subspecies of honey bee were conducted in the Oxul-Jabagly region of the western Tien Shan Mountains. Both wild and managed apple trees occur in this location. Field observations indicated loaded pollen foragers returning to hives at temperatures of 51F. Extensive pollen foraging activity occurred at temperatures of 52.5 - 53 F. Swarming activity and feral colonies were detected at elevations of over 6000 in June 2000, with nightly low temperatures of 32-34 F during the observation period.

Evaluation of an apiary of managed hives of this subspecies maintained in the village of Jabagly included behavioral, disease and parasite assessments. The honey bees were found to exhibit behavioral characteristics highly suitable for managed beekeeping. Interviews with local beekeepers provided anecdotal support for the observations that the bees were quite gentle and easily managed. Beekeepers stated that the overwintering and cold weather flight characteristics of the local bees were superior to subspecies imported from Russia and elsewhere, including honey bees from the Caucasus region, Ukraine, Austria and Italy. Samples of honey bees collected from throughout the Tien Shan in 1999 and from this region in 2000 were subjected to morphometric discriminant analysis and compared to all neighboring subspecies from eastern Europe and all known imported subspecies. The bees were clearly a genetically distinct group and a taxonomic publication describing the new subspecies is in preparation. DNA analysis of the samples from the Tien Shan indicates that they belong to the "C" lineage of honey bees. This lineage includes the most important races of use to beekeeping and pollination, including Caucasian, Carniolan and Italian honeybees.

## Collaborative arrangements

Mr. Urazajev Zufar, a local beekeeper from the village of Jabagly agreed to collaborate on the queen rearing and stock selection aspects of the project scheduled for 2001. Mr. Zufar had assisted in the initial "discovery" of this subspecies during a WSU-USDA expedition (Unruh and Sheppard, 1999) and also during the 2000 WSTFRC-funded stock assessment research. In addition, Dr. Roman Jashenko of the Institute of Zoology in Almaty agreed to assist with all exportation permits and "red tape" that may be encountered in completing the second year of the project. As President of the NGO Tethys, Dr. Jashenko is highly familiar with the regulations and procedures needed to export scientific material from Kazakhstan.

Dr. Tom Rinderer of the USDA Honey Bee Breeding and Genetics Laboratory in Baton Rouge, LA will collaborate on the importation and quarantine assessment of the new subspecies. His laboratory is experienced in the procedures needed for the release of new honey bee stocks under the regulations imposed by the US Honey Bee Act.

# ANTICIPATED BENEFITS AND INFORMATION TRANSFER:

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

# **PROGRESS REPORT**

## PROJECT NO.: AG CANADA

TITLE: Mechanisms of resistance to tebufenozide in leafroller populations from British Columbia

**PERSONNEL**: Michael Smirle, AAFC - PARC, Summerland, B.C.

# **REPORTING PERIOD: 2000**

### **ACCOMPLISHMENTS:**

- 16. Demonstrated that including synergists in toxicity bioassays using tebufenozide (Confirm<sup>®</sup>) against resistant obliquebanded leafrollers (OBLR) made the insecticide much more effective.
- 17. Documented resistance to methoxyfenozide (Intrepid<sup>®</sup>) and indoxacarb (Avante<sup>®</sup>) in three leafroller species in the Okanagan Valley of B.C.
- 18. Generated preliminary data on the activities of detoxifying enzymes in susceptible and resistant strains of OBLR; these assays are currently ongoing.

## **RESULTS:**

**Synergists.** Apple leaf disks were dipped in solutions of Confirm 2F (23 % tebufenozide), either alone or in combination with insecticide synergists PBO (mixed-function oxidase inhibitor) or DEF (esterase inhibitor). Leaf disks were then placed in 1 oz. plastic solo cups containing 5 ml agar to provide adequate moisture. Four doses of insecticide, plus a control (water + 0.25% Agral 90 surfactant + appropriate synergist), were used for each bioassay. The concentration of both synergist compounds was 60 ppm. Five newly-emerged OBLR leafroller larvae from previously-identified resistant populations were placed in each cup and allowed to feed on the treated disks for seven days. Cups were held in an incubator at  $20^{\circ}$ C; approximately 50 larvae were treated at each dose.

7-day toxicity values (LC 50) for Confirm alone were 12.8 ppm for leafrollers from Orchard #1, and 12.6 ppm from Orchard #2. When PBO was included in the test, LC 50 values were 6.8 ppm from Orchard #1 and 1.4 ppm from Orchard #2. Inclusion of DEF had less effect, actually increasing the LC 50 in Orchard #1 (17.7 ppm). From these data, it seems likely that resistance to Confirm is associated with higher activities of mixed-function oxidase enzymes, at least in some populations of OBLR.

**Resistance to Intrepid® (methoxyfenozide) and Avante® (indoxacarb).** 2000 was the third year that leafroller populations were sampled from orchards in the Okanagan Valley. Resistance had been detected to both azinphosmethyl and Confirm in 1999, and was present in three species (single-generation Fruittree and European leafrollers, and OBLR). At the request of the WTFRC Apple Entomology Committee two other new materials, Intrepid and Avante, were included in resistance testing in 2000.

The existence of leafroller populations resistant to azinphosmethyl and Confirm was reported in last year's Progress Report. More orchards were sampled in 2000, giving a total of 24 populations tested. The highest level of resistance to azinphosmethyl was found in one population of OBLR, 23-fold higher than the most susceptible population from an organic orchard. For Confirm, the highest resistance was found in a population of single-generation European leafrollers (*Archips rosanus*) that were 41-fold more

resistant than a population of the same species from an organic site. For OBLR, the highest resistance ratio (most resistant/most susceptible) was 17.6-fold.

In 2000, 12 orchards were tested for possible leafroller resistance to Intrepid® (methoxyfenozide = RH2485; Rohm and Haas, Inc.). This new IGR is much more acutely toxic to leafrollers than Confirm, with LC 50 values ranging from 0.030 ppm to 1.79 ppm. The resistance ratio (highest/lowest) is almost 60-fold, although there is no direct evidence of field failure because Intrepid has never been used in the "resistant" orchard (not registered in Canada). The highest resistance was observed in a population of OBLR; this population also had one of the highest levels of resistance to Confirm.

15 orchards were also tested for resistance to Avante (indoxacarb = DPX MP062; DuPont, Inc.). Some orchards were, by conservative estimate, 300-fold higher than the most susceptible sites. The introduction of Avante into the Canadian market must therefore proceed cautiously, with pre-screening of resistance levels desirable in some locations.

**Enzyme activities and metabolism studies.** Studies on the metabolic capacities of leafroller populations are continuing. Preliminary results indicate that esterases are higher in resistant populations, although this is not always the case. Mixed-function oxidases are also likely involved, as shown by our synergist studies. We have recently obtained radiolabelled (i.e. C-14) Confirm and Intrepid from Rohm and Haas, and will be starting detailed metabolism studies shortly.

**Conclusions.** The presence of resistance in leafrollers to new chemistries appears to be more widespread than previously thought. Resistance levels are high enough in some populations that field failure would be predicted, and pre-screening may be necessary before some materials are used in some locations. Biochemical screening may be extremely useful as a resistance management tool. The use of chemical synergists such as PBO may help to overcome resistance in leafroller populations.