Page	Time	Name	Title	Duration
			Tuesday, 22 January 2002	
	9:00	McFerson	Introduction	
1	9:15	Brunner	Survey of IPM practices in Washington tree fruit crops	01-01
7	9:35	Brunner	New pest management programs for apples and pears	01-03
12	9:40	Brunner	Bait-based monitoring systems for lepidopteran pests	00-02
18	9:45	Knight	Bisexual monitoring of codling moth and establishing a female biofix	00-02
23	9:50	Knight	Pheromones and attraction inhibitors for codling moth mating disruption	98-02
27	9:55	Knight	Managing codling moth with bisexual attract and kill formulations	00-02
30	10:00	Brunner	Sprayable pheromone and attract & kill for codling moth and leafroller	99-01
	10:15		**Break**	
38	10:30	Lacey	Strategies to control codling moth using granulovirus and nematodes	01-03
44	10:35	Pszczolkowski	Feeding enhancements for insecticides targeting lepidopteran larvae	00-02
50	10:40	Landolt	Development of feeding attractants for Lacanobia	00-02
53	10:45	Landolt	Insect responses to induced defensive chemistry of apple and pear	01-03
56	10:50	Jones	Developing sampling plans for leafrollers and their natural enemies	01-03
62	10:55	Unruh	Leafroller biological control	99-02
68	11:00	Smirle	Leafroller resistance to Confirm, Intrepid, and Avaunt	00-02
73	11:05	Unruh	Effects of new insecticides on natural enemies of apple pests	01-03
78	11:10	Miliczky	Biology and behavior of natural enemies of apple pests	01-02
83	11:15	Schrader	RAYNOX for suppression of insects in apple and pear	00-02
89	11:20	Brunner	Control of apple and pear pests with Surround	99-01
95	11:35	Beers	Mites and water stress effects on photosynthesis and productivity	00-01
99	11:50	Walsh	Lygus bug and thrips ecology	01-03
105	11:55	Yee	Trapping apple maggot using ammonia and sugar-derived baits	01-03
111	12:00	Moreno	Development of an attractive toxic bait for apple maggot	01-03
	12:00-1:30		**Lunch**	
116	1:30	Neven	Autocidal biological control of codling moth	98-02
120	1:45	Brunner	Monitoring and control of stink bugs	00-01
130	2:00	Brunner	Enhancing biocontrol of leafroller with ground covers	99-00
136	2:15	Landolt	Feeding attractants to monitor apple pests	99-01
139	2:30	Landolt	Chemical attractants from apple foliage and fruit for codling moth	98-02
142	2:45	Felsot	Guthion and Carbaryl residue decline	00-01
148	3:00	Lowery	Identification and insecticide resistance of green apple and spirea aphids	99-01
158	3:15	Sheppard	Tien Shan mountain gray bee -- a new apple pollinator	00-01

FINAL REPORT
WTFRC Project #AE-01-52 **WSU Project # 7089**

Project title: Survey of IPM practices in Washington tree fruit crops

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA
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Objectives:

1. Develop and implement an IPM practices survey, including pesticide use on apple, pear and cherry in Washington.
2. Produce a report to the industry comparing changes since the 1989-90 surveys and comparing data with the NASS surveys in 1991, 1993, 1995, 1997 and 1999.
3. Evaluate the processes used to assess crop losses due to different causes and develop guidelines for standardizing these assessments.

Significant findings:

- IPM practices in apple increased in several areas compared to the 1989 survey. For example, the use of pheromone traps as monitoring tools increased from 66% in 1989 to 87% in 2000.
- For the first time, a solid figure for the implementation of pheromones as a pest control tactic has been documented at 56% of apple acres in this survey.
- The survey identified by pesticide the average date of last pesticide application which is valuable in countering assumptions often used by the EPA when making regulatory decisions.
- Regionalized data are available to compare differences in IPM practices and pesticide use, providing greater detail in future assessments of change in these parameters.
- The cost of IPM programs in 2000 is provided and compared with that from 1989.
- Pear - - -
- For the first time, a detailed cherry IPM practices survey has been conducted. The data characterize existing practices and provide a valuable baseline for evaluating changes in these practices.

Methods:

Survey: A pesticide use survey patterned after those conducted in 1989 (apple) and 1990 (pear) was developed in both written and electronic form (web access). An industry mailing list was used to randomly select apple, pear and cherry growers for the survey. Balance between growing regions was achieved by selecting proportional numbers of growers from different areas to respond to the survey. The survey document was reviewed by industry representatives and by the WSU survey center. Complete confidentiality of survey respondents was maintained. The time table was to develop the survey tool by December of 2000, mail and have a web version of the survey form available by early January of 2001, with the goal of receiving returned surveys by March 1. Survey information has been entered into a computer, double checked for accuracy and summarized statistically, and a report will be written and circulated back to the industry by the end of December of 2001.

Report: The report will be a completed by the end of December 2001 and summarized at the next WSHA meeting (poster). The report will provide a measure of changes that have taken place in pesticide use and IPM practices over the last decade. Sufficient details will provide comparisons of IPM practices in different growing regions, pointing out the importance of local implementation differences that occur in pest management relative to pests and environmental conditions. The report will contrast information from a detailed survey with that collected every other year by the NASS. This should bolster the value of national data collection and provide an encouragement for NASS to change some aspects of its survey where it does not reflect adoption of new IPM practices.

Crop loss estimates: The ability of an agricultural industry to document crop losses due to different causes is important when seeking support from external sources (government, granting agencies, etc.) for research or disaster relief. There is no consistent method in Washington for evaluating crop losses. This project proposes to evaluate different methods currently used to estimate crop losses, gather preliminary data on losses affecting the 2000 crop, and develop guidelines to standardize the process in the future. This activity should result in the development of an educational program to train people making crop loss assessments, including materials to help them more accurately categorize damage or defects. These latter objectives would be developed through a separate grant request in 2002-03.

Results:

Apple

Survey returns: Out of nearly 1000 apple surveys mailed, 17% were returned. Of those returned, 57.6% (98) were complete and usable. Several respondents did not participate in the survey for a variety of reasons including that they disagreed that the survey should be conducted, had retired, had gone out of business, had sold or leased the orchard, or were currently not growing apple.

Characterization of farming operations: Seventy percent (70%) of the survey respondents classified themselves as full-time growers. The remaining group classified themselves as part time, deriving most of their income from off-farm activities. The average farm size of full-time growers was 131 acres compared to 28 acres for part-time farmers. The majority of the respondents, 89.5%, characterized themselves as using conventional pest control practices, with 10.5% involved in organic production. These are compared with results from the 1989 survey in Table 1. The increased involvement in organic production is clear.

Table 1. A comparison of the relative proportion of farmers utilizing different farming practices in 1989 and 2000 growing seasons.

Farming practice	1989	2000
Conventional	98.6%	89.5%
Mixed	0.5%	3.2%
Transitional	0.3%	1.0%
Organic	0.6%	6.3%

Growers were also asked to identify the different apple varieties grown in their operations, along with the amount of acreage. In the 1989 survey Red Delicious comprised 68.3% of all varieties while in 2000 this variety comprised only 41.2%.

Pest management advice: In Washington, several different sources of information and advice are available to growers to help them make pest management decisions. Each potential source of advice was listed in the survey for growers to select and rate its relative usefulness in providing advice for making pest control decisions. Grower responses to the question regarding the importance of different information sources in making pest management decisions are summarized in Table 2.

Table 2. Percentage of survey respondents who rated the value of information from different sources in helping them arrive at pest management decisions, 2000.

Information source	Very important	Somewhat important	Not important
Private consultant (PC)	39.2	17.6	38.2
Agricultural chemical industry fieldman (ACF)	40.2	40.2	14.7
Cooperative Extension (CE)	28.4	42.2	24.5
Warehouse fieldman (WF)	37.3	36.3	21.6
Other growers (OG)	14.7	54.9	40.2
WSU Crop Protection Guide (CPG)	51.0	34.3	10.8
Orchard Pest Management (OPM)	21.6	32.3	40.2
Outside management	1.0	0.0	0.0
Own experience	2.9	3.9	0.0

Pest management activities: Orchard monitoring was by far the most frequent pest management activity employed, with 95% of respondents indicating its use. Fifty-nine percent of the growers said they used alternate row spraying, an increase of nearly 30% from the 1989 survey. The percentage of growers using reduced pesticide rates increased from 54% in 1989 to 77% in 2000. In 1989 two-thirds of the growers said they used pheromone traps while 87% of the respondents in 2000 used this monitoring method. In 2000, 66% of growers reported using biological control practices, compared to 34% in 1989. There was a significant increase in the number of growers incorporating use of economic thresholds, from 37% during 1989 to 77% in 2000.

Reporting block information: The average size of the reporting block was 23.2 acres, the smallest being under one acre and the largest being 489 acres. Tree density has increased over time with the introduction of dwarfing rootstocks and new planting systems. This is clearly seen by comparing the average number of trees per acre for the reporting blocks for this survey, 301 (62 to 1340) vs. an average of 194 (64 to 800) in 1989. The average percentage of Red Delicious in the reporting blocks was 72.8%, with Gala, Fuji and Golden Delicious being the next most common varieties represented. Seventy-three percent of respondents indicated use of under-tree irrigation and 18% used over-tree irrigation. There was an even distribution of tree ages represented in the reporting blocks. Airblast sprayers were the dominant pesticide application method, 98.5%; and most growers used spray volumes of between 100 and 300 gallons per acre, 64.4%.

Pesticide use data: An average of 2.56 applications of azinphosmethyl was applied per acre in 2000, a significant increase from data reported by the 1999 NASS survey. Mating disruption was reported to have been used on 56% of acres in the 2000 survey. This is much higher than reported by the 1999 NASS survey for Washington. Bacterial pesticide formulations (Bt products) were used an average of 2.85 times on 34% of the acres in 2000. The most used fungicide was sulfur (57% acres treated) followed by mycobutanil (33%), triflumizole (26%), and oxytetracycline (24%). Carbaryl was the most used plant growth regulator, followed by NAD. Additional specific information on use of pesticides will be provided as a handout at the research review.

Timing of pesticide applications: The time of a last pesticide application is important because regulatory agencies often use default values of the preharvest interval as the last spray date. The average last application, based on the 2000 survey, for each pesticide is shown in Table 3.

Table 3. Average date of last application of a pesticide class during 2000.

Pesticide class	Avg. date of last spray
Insecticide	10 July
Fungicide	10 June
Nutrients	29 June
PGR	13 June

Pear

Eight hundred sixty-six surveys were sent to pear growers and 22% were returned. Of those surveys returned, 67% were complete and usable. Almost 90% of the respondents came from the four main pear growing areas of the state, upper and lower Yakima valley, Wenatchee and Okanogan.

Characterization of farming operations: The percentage of respondents reporting as full-time growers decreased from 84.1% in 1990 to 72.4% in 2000. There was a corresponding increase in respondents reporting as part-time growers, 27.6%, compared with the 1990 survey, 15.9%. The majority of the respondents, 86.1%, characterized themselves as using conventional pest control practices (primarily using synthetic pesticides), 5.4% as using both conventional and organic means (mixed acres), 3.9% as being transitional to organic, and 4.7% as using only organic farming practices. The most significant change from 1990 to 2000 was that only 4.5% of the growers reported being involved in any organic production in 1999, while 14% reported activity in organic production, full time or transition, in 2000.

Pest management advice: As for Washington apple production, several different sources of information and advice are available to pear growers to help them make pest management decisions. Each potential source of advice was listed in the survey for growers to select and rate its relative usefulness in providing advice for making pest control decisions. Grower responses to the question regarding the importance of different information sources in making pest management decisions are summarized in Table 4.

Pest management activities: Whether conducted by the grower, fieldmen or private consultants, orchard monitoring was by far the most frequent pest management activity employed, with 93% of the respondents indicating its use and only one individual reportedly never employing monitoring. The next most frequently used technique was pheromone trapping, with the portion of respondents reporting use as ‘often’ or ‘sometimes’ at 78%. Only about 38% of the growers reported using alternate row spraying as a control practice. This was a slight decrease from 1990 in which nearly 44% said they either ‘often’ or ‘sometimes’ used this practice. The percentage of growers using reduced pesticide rates remained the same at 68%. Seventy-seven respondents reported the use of degree-day models for timing sprays. There was no change in the number of growers reporting the incorporation of economic thresholds for their management practices, with 68% reporting its use during both survey years. In 2000 there was a slight increase in the use of biological control practices, with 64% reporting their use compared to 50% in 1990. Sixty four percent of the growers reported using integrated mite management.

Table 4. Percentage of survey respondents who rated the value of information from different sources in helping them arrive at pest management decisions, 2000.

Information source	Very important	Somewhat important	Not important
Private consultant (PC)	44.2	13.0	31.9
Agricultural chemical industry fieldman (ACF)	47.8	31.1	14.5
Cooperative Extension (CE)	28.3	45.7	15.2
Packinghouse Reports (PR)	32.6	40.6	18.1
Other growers (OG)	10.9	63.0	17.4
WSU Crop Protection Guide (CPG)	43.5	34.1	12.3
Orchard Pest Management (OPM)	19.6	41.3	29.0
Outside management	0.7	0	0
Own experience	5.8	0	0
Other	0.7	0.7	0

Details on pesticides used are still being summarized. A more complete report will be provided at the research review in January.

Cherry

Five hundred surveys were sent to cherry growers and 22% were returned. Of the surveys returned, 65% were complete and usable. This represents the first detailed IPM practices survey from Washington for cherry.

Only 1.45% of cherry growers indicated that they use organic practices. However, another 1.45% were transitional organic, and 2.9% indicated they had mixed conventional and organic production. The dominant cherry variety produced was Bing (62%) followed by Lambert (14%).

Pest management advice: As for Washington apple and pear production, several different sources of information and advice are available to cherry growers to help them make pest management decisions. Each potential source of advice was listed in the survey for growers to select and rate their relative usefulness in providing advice for making pest control decisions. Grower responses to the question regarding the importance of different information sources in making pest management decisions are summarized in Table 5.

Table 5. Percentage of survey respondents who rated the value of information from different sources in helping them arrive at pest management decisions, 2000.

Information source	Very important	Somewhat important	Not important
Private consultant (PC)	27.40	16.44	39.73
Agricultural chemical industry fieldman (ACF)	43.84	31.51	13.70
Cooperative Extension (CE)	27.40	35.62	21.92
Packinghouse fieldmen (PF)	43.84	36.99	10.96
Other growers (OG)	6.85	64.38	16.44
WSU Crop Protection Guide (CPG)	41.10	34.25	13.70
Orchard Pest Management (OPM)	15.70	24.66	45.21

Pest management activities: Whether conducted by the grower, fieldmen or private consultants, orchard monitoring was by far the most frequent pest management activity employed, with about 90% of respondents indicating its use, either often or sometimes. The use of economic thresholds and reduced pesticide rates ranked next in importance, with both employed by nearly 75% of the growers. The least popular was alternate row spraying, with nearly 65% of the growers seldom or never practicing this activity.

Details on pesticides used are still being summarized. A more complete report will be provided at the research review in January.

Budget:
Survey of IPM practices in Washington tree fruit crops
Jay F. Brunner
Request for 2002: $0

Year	**Year 1 (2001)**	**Total cost**
Total	$10,000	$10,000

Current year breakdown

Item	**Year 1 (2001)**	**Total cost**
Salaries[1]	0	0
Benefits (30%)	0	0
Wages[1]	2,500	2,500
Benefits (16%)	400	400
Equipment	0	0
Supplies[2]	6,600	6,600
Travel[3]	500	500
Miscellaneous	0	0
Total	10,000	10,000

CONTINUING REPORT **YEAR 2/3**
WTFRC Project # AE-01-53 **WSU Project # 6089**

Project title: New pest management programs for apple and pear

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave.,

Co-PIs Elizabeth Beers, Entomologist, John Dunley, Associate Entomologist, and Vince Jones, Assistant Entomologist,

Cooperator: Ted Alway, Associate in Research, WSU-TFREC, Wenatchee, WA

Objectives:
1. Compare pest control at several sites using codling moth (CM) mating disruption as a base program supplemented with either "conventional" insecticides or newly registered selective insecticides.
2. Monitor CM and other pests using newest technology available to supplement monitoring by crop consultants.
3. Assess the impact of selected natural enemies on pests at each site under different pest control programs.
4. Evaluate the impact of different programs based on crop loss due to pests and costs of pest controls.
5. Use the demonstration sites as opportunities to educate growers and crop consultants on how different selective pest control programs work.

Significant findings:
- There was no difference in fruit damage by CM, San Jose scale, bugs or lacanobia fruitworm in apple sites between organophosphate (OP) or non-OP supplements to mating disruption-based programs.
- There was significantly less fruit damage in programs using non-OP supplements compared to those using OPs.
- There was significantly less feeding by larvae of leafrollers and lacanobia fruitworm in programs using non-OP supplements compared to those using OPs.
- There was no significant difference in the cost of the pheromone-based pest management programs in apple whether supplemented by OP or non-OP products.
- Monitoring with non-pheromone lures for CM, leafrollers and lacanobia fruitworm has established a database from which greater understanding of the value and utility of these tools will arise.
- There were no differences in populations of secondary pests or their natural enemies in programs using OP or non-OP supplements.
- In pear, there appeared to be no differences in psylla populations in the four treatment regimes imposed over a pheromone-based pest management program.

Methods:
The 15 apple and six pear sites established in Washington will continue using the same methods established in year 1 (2001). Each site consists of a grower and crop consultants willing to participate in the project. The entire site will be treated with CM mating disruption. Within each site, supplements to CM mating disruption will be either conventional insecticides (OP supplements) or selective insecticides (non-OP supplements, e.g., oil, Intrepid, Esteem, granulosis virus). Controls for

other pests will again follow a "traditional conventional" approach or a "selective alternatives" approach. These programs have been established with growers and crop consultants but will be more rigorously segregated in year 2. Close adherence to control programs will be expected unless crop injury exceeds acceptable levels. Management of sites will be through a combination of a local on-site manager, grower committee, extension agent and technical advice from researchers. Crop consultants contracted to work on the project will perform assigned pest monitoring activities as in year 1. Standardized record keeping has been established for all monitoring activities and will be continued in years 2 and 3. Pest control costs will be borne by the grower with the exception of certain non-OP products that might be donated by the registant.

***Monitoring activities*:** Standardized monitoring activities developed under CAMP will be used to assess pest insects in cooperation with crop consultants working at the sites. Pheromone trapping will be used for CM and leafroller. New monitoring systems under development for leafrollers, CM and lacanobia fruitworm will also be incorporated into the areas to obtain data on efficiency relative to pheromone monitoring systems and correlation with pest density and crop injury measurements.

***Assessment of biological control*:** Orchards at each site will be monitored to compare effects of different programs on the kinds and abundance of natural enemies. Pest/natural enemy systems to be monitored will be spider mites/predatory mites, aphids/parasitoids-predators and leafminer/parasitoids in apple, and pear psylla/general predators-parasitoids in pear.

***Program evaluation*:** The efficacy of different control programs will be evaluated from several different perspectives. The level of pest density in each pest control program will be determined throughout the season based on monitoring activities and compared to arrive at a relative value for pest suppression. The level of natural enemy density will also be monitored as described above and their abundance compared in the different pest control programs to arrive at a relative value for natural enemy conservation. Crop losses will be determined from in-field inspection of fruit in orchards using the different pest control programs and as much as possible from packout records obtained from warehouses. The level of crop loss by pest will be determined for each pest control program and compared using statistical inference across all sites. Cost of pest control will be compared for each pest control program.

***Education*:** The demonstration study sites will be used as field locations for tours and workshops showing growers the results of the different pest control programs. These educational events will be held in each region where the demonstration study sites are located to provide a local value to the experiences of growers and crop consultants participating in the project. Workshops and demonstrations will be held each year of the project to update the industry on progress and new products being integrated into the pest control programs.

Results and discussion:
Fifteen apple (20-40 acres each) and six pear sites (15-20 acres each) were established in the spring of 2001 in major fruit growing districts of Washington. Seven tree fruit pest management consultants, with assistance by personnel of the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC) and the United States Department of Agriculture Yakima Agricultural Research Laboratory (USDA-YARL), collected the data on pests and natural enemies at all sites. The consultants recorded the data in the field with a PDA (hand-held computer) and sent it electronically to the WSU-TFREC where it was entered in the database from which weekly reports were generated.

***Codling moth*:** A wide range of CM populations was found within the 15 AWII apple sites, as shown by the catch in pheromone and DA lure-baited traps. CM captures were higher in the first compared to the second generation at most sites. There seemed to be some correlation between CM captures in the pheromone-baited traps and those baited with the DA-lure. The latter traps seemed to be better indicators of orchard risk (fruit damage at harvest) in the second generation compared to pheromone-baited traps. At harvest and based on bin samples (Table 1) or field observations, there was no difference between OP or non-OP supplements to mating disruption based programs.

Table 1. Percentage of fruit injury from bin samples in AWII apple sites at harvest by different insects, 2001.

	Codling moth		Leafroller		Cutworm	
Grower	OP	No OP	OP	No OP	OP	No OP
1	0.08%	0.04%	0.00%	0.00%	0.00%	0.00%
2	0.04%	0.00%	0.04%	0.00%	0.00%	0.00%
3	0.04%	0.16%	1.38%	0.24%	0.13%	0.12%
4	0.60%	0.24%	0.72%	0.48%	0.20%	0.16%
5	0.08%	0.08%	0.04%	0.04%	0.00%	0.04%
6	0.68%	0.16%	0.04%	0.12%	0.04%	0.00%
7	0.00%	0.00%	0.40%	0.08%	0.04%	0.12%
8	0.00%	0.00%	0.68%	0.16%	0.08%	0.12%
9	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
10	0.16%	0.00%	0.00%	0.00%	0.12%	0.12%
11	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
12	0.00%	0.00%	0.00%	0.08%	0.28%	0.08%
13	0.00%	0.00%	0.08%	0.00%	0.04%	0.00%
14	0.00%	0.00%	0.80%	0.08%	0.00%	0.00%
15	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Average	0.11%	0.05%	0.28%	0.09%	0.07%	0.05%
SEM	0.061%	0.022%	0.110%	0.034%	0.023%	0.017%

***Leafroller*:** A wide range of leafroller populations was noted in the AWII blocks. The pandemis leafroller (PLR) tended to be the dominant species in north-central Washington and the Yakima Valley, being trapped in 11 of 15 orchards. The obliquebanded leafroller (OBLR) predominated in the Columbia Basin and Brewster areas but was found at all sites. Capture of OBLR moths tended to increase in the second generation while PLR did not. The number of orchards in which OBLR predominated increased from eight in the first generation to 11 in the second, while PLR declined from dominance in four orchards in the first generation to two in the second. Two Brewster area orchards used mating disruption as a tactic for leafrollers in their non-OP blocks. There was no significant difference in estimates of leafroller larval populations in designated OP or non-OP blocks based on spring samples, and there was no difference in the capture of leafrollers in any trapping systems in either generation throughout the first year of the project. However, there was significantly less feeding on shoots by leafroller larvae in the summer samples in non-OP (0.17 ± 0.07%) compared to OP-treated blocks (0.40 ± 0.08%), and this was reflected in lower fruit injury at harvest (Table 1).

***Lacanobia fruitworm*:** This relatively new pest was monitored with both a pheromone and a food-based attractant (AAOH) lure. There was a wide range of populations, as reflected in the pheromone lure-baited trap captures among the 15 orchards, although all orchards captured some moths. Eleven of the 30 AWII blocks had seasonal catches exceeding 1000 per trap, while 11 others caught less than

500. Catches in pheromone lure-baited traps tended to be lower in the second generation. There was no significant difference in the average capture of lacanobia fruitworm moths between treatments in either generation. There was significantly less feeding on shoots by lacanobia fruitworm larvae in the summer samples in non-OP compared to OP-treated blocks, but this was NOT reflected in lower fruit injury at harvest (Table 1).

***Pesticide use*:** Foliar applications of pesticides are summarized in Table 2. All AWII apple blocks used CM mating disruption, generally at rates close to 200 dispensers/acre. For the summary, CM mating disruption was counted as one pesticide application with cost based on the number of dispensers per acre. The number and cost of pesticide applications were not significantly different between the OP and non-OP pheromone-based programs, with far greater variability between sites than within sites. The total number of sprays varied with the cultivar (e.g. mildew-susceptible varieties received more mildewcide applications) and the pest pressure at the site. For example, from zero to four codling moth sprays were applied depending upon trap counts and history of pressure from this pest.

The main OP insecticides used in the OP treatment blocks were chlorpyrifos (Lorsban) [13 of 15 blocks] and azinphosmethyl (Guthion) [eight blocks], with a limited amount of phosmet (Imidan) [three blocks] also used. For the control of lepidopteran pests, the non-OP blocks relied upon methoxyfenozide (Intrepid) [14 of 15 blocks] and spinosad (Success) [six blocks], with lesser use of pyriproxifen (Esteem) [two blocks], indoxacarb (Avaunt) [two blocks], and *Bacillus thuringiensis* [one block]. The use of the more selective "soft" insecticides was not limited to the non-OP blocks; five OP blocks also received methoxyfenozide and eight were treated with spinosad.

Table 2. The number and class of pesticides and cost of programs applied at each site in the AWII project, 2001.

	Codling moth		Leafrollers		Lacanobia		CM+LR		Other		TOTAL		Cost	
Site	OP	***Non-OP***	OP	Non-OP	OP	Non-OP	OP	Non-OP	OP	Non-OP	OP	Non-OP	OP	Non-OP
1	3	4	1	1	0	0	0	1	8	8	12	14	$285	$307
2	2	2	1	1	0	0	0	0	8.4	6	11.4	9	$278	$281
3	4.7	5	1	0	0	0	0	3	4	8	9.7	16	$281	$404
4	1.4	1.6	1	1	0	0	0	0	6.5	6.5	8.9	9.1	$323	$331
5	2.5	2.2	1.1	1	0	0	0	0	1.4	1.4	5	4.6	$108	$113
6	2	1	2	2	0	0	0	0	4.9	6.3	8.9	9.3	$128	$126
7	2.1	3	1.9	0	0	0	0	0	6.4	6.4	10.4	9.4	$250	$247
8	1.1	2	2	0	0	0	0	0	3	2	6.1	4	$133	$89
9	1	2	2	0	1	1	0	0	11	11	15	14	$350	$322
10	2	2	1	0	0	0	0	0	3	4	6	6	$136	$144
11	2	2	1	0	0	0	0	0	3	2	6	4	$125	$112
12	2	1	2	2	0	0	0	1	7.3	13.3	11.3	17.3	$272	$398
13	2	2	0	1	0	0	0	0	6	6	8	9	$214	$258
14	1	1	2	2	0	1	0	0	9	8	12	12	$226	$268
15	3.2	3	1	1	0	0	0	2	3	3	7.2	9	$192	$336
mean	2.1	2.3	1.3	0.8	0.1	0.1	0	0.5	5.7	6.1	9.2	9.8	$220	$249
SEM	0.25	0.29	0.16	0.2	0.07	0.09	0	0.24	0.71	0.86	0.74	1.08	$20	$28

***Pears*:**
At the time of this report data were still being analyzed and results will be available in December 2001.

***Education*:** Workshops are planned for February of 2002 to review results of year 1 and discuss issues associated with mating disruption, pesticide resistance and new pesticide efficacy and mode of action.

***Summary*:** A high degree of variation was found in the populations of CM, leafrollers and lacanobia fruitworm across the 15 apple orchard sites. Pest monitoring programs seemed to provide good estimates of pest pressure, and growers and consultants responded to these data with appropriate control actions. The same monitoring systems will be used in the next two years to build a base of knowledge on how to use the new methods.

At orchards where there was a significant risk of damage from CM based on monitoring information there were no control failures, and the non-OP programs supplementing mating disruption provided acceptable levels of fruit protection. There was an apparent advantage to the non-OP programs in the suppression of leafroller and lacanobia fruitworm populations as noted in lower levels of feeding damage on foliage. This was reflected in lower levels of leafroller damage to fruit at harvest in the non-OP blocks. It seemed that there was no disadvantage from eliminating the chlorpyrifos application in the non-OP blocks in the first year of the project. However, the long-term effect of such a strategy is yet to be determined.

There were no surprises relative to secondary pests or their natural enemies in any orchards. This is not unexpected in the first year of a project like this. It often takes two to three years for either negative or positive effects of altered programs to be expressed. Continuation of the AWII project at all apple orchard sites is critical in order to determine the full impact, positive or negative, of the non-OP programs.

Budget:
New pest management programs for apple and pear
Jay F. Brunner
Project duration: 3 years **Current year**: Year 2 (2002)

Year	Year 1 (2001)	**Year 2 (2002)**	Year 3 (2003)
Total	$89,760	**106,321**	108,298

Current year breakdown

Item	Year 1 (2001)	**Year 2 (2002)**	Year 3 (2003)
Salaries[1]	24,000	**37,440**	38,938
Benefits (32%)	9,360	**11,981**	12,460
Wages[1]	40,000	**40,000**	40,000
Benefits (16%)	6,400	**6,400**	6,400
Equipment	0	**0**	0
Supplies[2]	8,000	**8,000**	8,000
Travel[3]	2,000	**2,500**	2,500
Miscellaneous	0	**0**	0
Total	89,760	**106,321**	108,298

[1] Ted Alway at 75% appointment years 2 and 3.
[2] Traps, lures, vials, solvents, etc.
[3] Reimbursement for Alway travel; vehicle for six months plus fuel and maintenance.

CONTINUING PROJECT **YEAR 3/3**
WTFRC Project #AE-01-48 **WSU Project # 4095**

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

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center, 1100 N. Western Ave.,

Co-PI and affiliation: Peter Landolt, USDA-ARS, Wapato

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

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

Significant findings:

Noctuids

- A food-bait based lure developed by Dr. Peter Landolt for monitoring noctuid pests was evaluated in side-by-side comparisons with a lacanobia fruitworm pheromone baited trap at 12 orchards from Royal Slope to Brewster. Both lure technologies tracked adult lacanobia fruitworm phenology in a similar way.

- The food-bait lure attracted fewer lacanobia than the pheromone lure, on average about 15% as many as the pheromone lure.

- The food-bait lures attracted lacanobia males and females in a 50:50 ratio over the entire season.

- No shift in sex ratio was noted at either the beginning or end of the flight. The food-bait lure also attracted many noctuid species, including Bertha armyworm and spotted cutworm. Therefore, this monitoring system is able to monitor information on all three of these orchard pests. Because it requires some skill to accurately identify the noctuid species, this trapping method may not prove practical for a grower.

- Because the food-bait lure is highly attractive, Dr. Landolt is proposing to use this technology to develop a "lure and kill" system that could control all noctuid pests in an orchard without insecticide sprays.

Codling moth

- A novel pear-kairomone lure ("DA lure," Trécé, Inc.) attracted about 10-15% as many codling moths as the pheromone lure (1X CM red rubber septum, Trécé, Inc.) during both the first and second generations.
- An acetic acid based attractant developed by Peter Landolt attracted about 1% as many codling moths as the pheromone lure during both the first and second generations.
- Aging studies suggest that the DA lure should last through an entire codling moth generation.
- The DA lure attracted both males and females during each generation. Codling moth males were captured about 80 DD before the first female during the first generation.
- Male flight as monitored by the DA lure was closely correlated to pheromone captures as well as model predictions early in flight, but in 2001 the majority of the first generation actual trap catch accumulations was ahead of model predictions.
- During the second generation, pheromone catch was fairly well correlated with model predictions early in the flight but lagged behind model predictions later in the flight.
- Detailed observations of oviposition and egg hatch reflected the same pattern as moth flight in regards to model predictions for both generations. Eighty percent of the first females captured in the DA lure were mated, and mating remained very high through the entire flight.
- During the first generation, capture from DA lure traps was not a good predictor of fruit injury, especially in high-pressure situations.
- The standard-load pheromone lure was a relatively good predictor of damage in orchards that were not receiving any codling moth controls.
- There was no difference in relative captures between large delta-style traps and standard wing traps that were baited with DA lures.

Leafrollers

- An acetic acid food-based lure developed by Dr. Peter Landolt was evaluated for monitoring OBLR adults. Due to unanticipated low moth captures in Milton-Freewater, OR no consistent correlation between acetic acid release rate and attractancy was noted.
- In non-pheromone treated orchards captures were biased towards females (60:40 F:M).
- Various traps baited with acetic acid lures attracted from 5-35% as many moths as the standard-load pheromone lure in a large delta-style trap. The wing-style trap captured the highest number of moths on average, but there were no significant differences among trap styles.

Methods:
Lacanobia food-bait lures: Dr. Peter Landolt developed a food-bait lure for monitoring noctuid pests in orchards. The food-bait lure was a blend of acetic acid and isoamyl alcohol (AAOH) believed to be attractive 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 system. Because of this development, the food-bait lure could be used in standard monitoring traps, allowing a direct comparison of the food-bait lure and pheromone lure with all other conditions being equal. The AAOH lure was deployed in general purpose bucket-style traps (Unitrap, Pherotech, Inc.) and evaluated in a side-by-side comparison with bucket-style traps baited with a lacanobia fruitworm pheromone lure (Scenturion, Inc.). Twelve orchards from Royal Slope to Brewster were monitored. Orchards were selected for study because of a history of reduced insecticide inputs and were thought to have lacanobia fruitworm populations based on previous studies. This was important in order to limit disruption to normal phenological development. Each of the bucket-style traps had a small piece (2" x 1") of "kill-strip" (Hercon Vaportape, DDVP toxicant strip, Great Lakes, IPM) placed in the collection area. A fresh "kill-strip" was placed at the start of

each of the two generations. The pheromone lures were replaced every six weeks and the food-bait lures every four weeks. The traps were monitored every week, and the total number of lacanobia fruitworm, Bertha armyworm and spotted cutworm males and females was recorded.

Codling moth non-pheromone lures: The relative attractancy of non-pheromone baits ("DA Lure," Trécé, Inc.; Acetic Acid Lure, Dr. Peter Landolt) was compared with a standard-load (1 mg) pheromone lure (CM red rubber septum, Trécé, Inc.) in several orchards using trap rotation experiments during both codling moth generations. Moth captures were recorded two or three times per week. The sex ratio, as well as mating status and relative age of females captured in non-pheromone lure baited traps, was assessed through laboratory dissection. The experimental design was a randomized complete block with six replicates. Tests were conducted in non-pheromone treated blocks at the Tree Fruit Research and Extension Center. To minimize position effects, traps were rotated each time they were inspected. Red septum lures were replaced after each complete rotation (9-10 days) during both generations. There were two DA lure treatments. The first was an "aged" DA lure that was placed at the start of flight and not changed, and the second was a fresh DA lure placed after each complete rotation (9-10 days). The acetic acid lure was replaced every four weeks. The traps used for this test were l g delta-style sticky traps (Scenturion, Inc.). Trap bottoms were replaced after a cumulative catch of 30 moths, more often if dirty.

Detailed observations of oviposition behavior, egg hatch and fruit injury were made during each generation at the highest-pressure sites in the trap rotation experiment. During the first generation, 10 branches each containing 5-10 fruiting clusters were flagged. This design was replicated three times. After adult flight was detected, the flagged branches were intensively sampled every one to two days for oviposition activity. The fruit clusters and surrounding leaves were examined under magnification for egg deposition. When an egg was found it was marked and observed for hatch. Fruit injury was also recorded. During the second generation the same principle was followed although the design differed slightly. One hundred uninjured fruit clusters were selected for monitoring from each of three blocks. The clusters were examined, and eggs deposited during the first generation were either removed or marked. Weather data were collected at each site, and male flight and egg hatch were compared to model predictions.

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

The relative attractancy of large delta-style traps (Trécé, Inc.) and Pherocon 2B traps (Trécé, Inc.) baited with DA lures was evaluated in paired comparisons. The test was replicated four times in high-pressure orchards. The traps were checked every two to three days. To minimize position effects, traps were rotated each time they were inspected. The test was run for four weeks.

Leafroller attractants: Evaluation of acetic acid baits for OBLR moth attraction was conducted in several orchards using trap rotation experiments. During the first generation, the release rate of acetic acid from polypropylene vials (5 ml vial, VWR Scientific) was controlled by varying the hole size in the vial lid. Hole sizes tested were 1.0, 1.6, 3.3 and 6.4 mm. During the second generation a vial with an optimal release rate was used to test various trap designs. Acetic acid lures with a hole size of 3.3 mm were placed in "wet" traps (Agrisense Dome Trap, Pherotech, Inc), wing-style traps (Pherocon II, Trécé, Inc.) and l g delta-style traps (Pherotech, Inc). Further, acetic acid captures were compared to a standard-load red rubber septum pheromone lure (OBLR W, Trécé, Inc.). The pheromone lure was placed in a large delta-style trap.

Moth captures were recorded two or three times per week, and the sex of moths was determined in non-pheromone lure baited traps. The experimental design was a randomized complete block with six replicates per treatment. Tests were conducted in non-pheromone treated orchards in Milton-Freewater, OR. To minimize position effects, traps were rotated each time they were inspected. Lures were placed during peak flight activity, and the test was conducted for four weeks.

Results and discussion:
Lacanobia food-bait lures: Lacanobia fruitworm was 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 development of a "dry" food-bait lure that can be placed into the same monitoring device as a pheromone lure was an important innovation in 2001. These lures appear to be very good at monitoring a variety of noctuid pests under all management practices and should also prove to be valuable tools for both research of male and female behavior and the practical application of developing consistent and reliable treatment thresholds based on trap catch. If similar technologies can be developed for all lepidopteran pests, thresholds could be developed that would not be influenced by mating disruption. It remains important to determine the optimum release rates as well as the area of attractancy for non-pheromone baits to best utilize this new technology. Another possible use of this technology would be to develop an "attract and kill" product or some kind of larger scale kill stations. Dr. Peter Landolt is working in this area, and we will be cooperating with him if his research project is funded.

Codling moth non-pheromone lures: The DA lure attracted 10-15% as many codling moth as a 1X pheromone lure, and the AA lure attracted about 1% as many as the pheromone lure during both the first and second generations. At each evaluation period during both generations the pheromone lure attracted significantly more moths than the DA lure. The difference between the DA lure and the acetic acid lure was statistically significant during peak flight periods of both generations and across the whole test for each flight. Aging studies suggest that the DA lure should last through an entire codling moth generation. The relative attractancy of aged and fresh DA lures did not change over the entire 66 days of the first generation test. However, there is evidence that at the end of the flight the DA lure attracted relatively fewer moths than the pheromone lure, from days 47-66. A majority of the flight was over prior to that time period, and thus the reduced capture may not be of limited concern. During the second generation there was a slight increase in the relative attractancy of the fresh DA lure at 37 days, although this increase was not statistically significant. The same decrease in attractancy of the DA lure relative to the pheromone lure at the end of the flight was not as obvious during the second generation.

Evaluations of the flight patterns for each sex showed that male flight was detected first and female flight was delayed by about 80 degree-days. Male flight as monitored by the DA lure was closely correlated to pheromone captures as well as model predictions early in flight. Pheromone trap catch remained closely correlated with model predictions for the entire flight period, but male captures in the DA lure accumulated faster during the peak flight. This was also observed where captures in DA lure traps decreased relative to the pheromone lure traps late in the flight. During the second generation, pheromone trap catch was fairly well correlated with model predictions early in the flight. However, as the generation progressed model predictions were ahead of observed capture in the pheromone-baited traps. DA lure captures lagged behind model predictions for the entire flight. As noted above, detection of male flight prior to female flight results in captures that are strongly biased toward males early in the flight. During peak flight activity about 60-70% of the moth captures were male. Interestingly, the male bias is stronger again at the end of the flight. A majority of the first females captured in the DA lure baited traps were already mated. At 100 DD from male biofix 80% of the females were mated, and the percent of females mated remained very high through the entire flight, 75-100%. It remains possible that the DA lure does not detect the earliest female emergence and may be biased towards older females. Detailed observations of oviposition and egg hatch reflected the same pattern as moth flight with regards to model predictions for both generations. During the first generation, initial hatch occurred at approximately 230 DD, slightly ahead of model predictions. By 275 DD, model predictions were slightly behind observed cumulative hatch, and the model remained slightly ahead of observed events through approximately 90% of the flight. There was no “clean” break between generations as measured by egg hatch so it was difficult to set the start of second generation hatch. This may account for the relatively high percentage of hatch seen early in the generation. However, observations of egg hatch were fairly well correlated with model predictions early in the second generation. The second generation flight pattern measured by both DA lures and pheromone lures was spread out, and model predictions were ahead of actual events for much of the flight. This was also reflected in oviposition and hatch. That is, for much of the second generation, model predictions were ahead of observed hatch accumulations.

During the first generation, capture from DA lure traps was not a good predictor of fruit injury, especially in high-pressure situations. It appeared that there was a non-linear relationship between DA lure capture and fruit damage. The standard-load pheromone lure was a relatively good predictor of damage in orchards ($r^2 = 0.7$) that were not receiving any codling moth controls.

It continues to be important to monitor the effectiveness of pheromone lures for attracting codling moth, especially with new technologies being developed annually. Each new technology represents a potential for changes in treatment thresholds based on trap catch. A lure that attracts both males and females will also allow researchers to more intensively monitor female behavior under a variety of circumstances including pheromone treated environments. The DA lure should provide researchers a means of assessing efficacy and modes of action of various mating disruption technologies. Further, the DA lure provides a means to validate the codling moth model and monitor any changes in behavior that may affect predictions based on female flight and ovarian development. However, much work is still needed with the DA lure. Ratios of pheromone:DA lure captures, as well as sex ratio changes, should be monitored over a range of codling moth pressures and pheromone levels. There is preliminary evidence that the sex ratio becomes less biased towards males in the presence of mating disruption. The utility of the DA compound in an “attract and kill” formulation should also be studied.

Leafroller attractants: OBLR adult captures in acetic acid baited “wet” traps were low and variable during the first generation. No significant differences in moth captures or sex ratio were noted by manipulating release rates. Moth captures in the non-pheromone treated environment were biased towards females by approximately a 60:40 ratio. Based on previous tests that showed that the 3.3-mm hole size provided optimized release rates of acetic acid to monitor PLR, it was chosen to monitor OBLR in the second generation tests. The pheromone lure/delta-style trap configuration attracted significantly more moths than any acetic acid baited trap. Various traps baited with acetic acid lures attracted from 5-35% as many moths as the standard-load pheromone lure in a large delta-style trap. The wing style trap captured the highest number of moths on average, but there were no significant differences among trap styles.

The leafroller acetic acid bait test suggests that the same release rate for OBLR as that recommended for PLR should be sufficient to monitor OBLR male and female activity. Further, a "dry" trap (wing or delta-style) should be suitable for implemention using this technology. The "dry" trap represents a significant improvement on the "wet" trap protocol previously used, making it easier to count and identify moths. Under the conditions of these tests (i.e. non-pheromone treated orchards), the acetic acid lure attracted far fewer moths than the pheromone-baited traps. This is not necessarily to be considered a negative result. Previous studies have suggested that the OBLR pheromone lure is actually too attractive and may not accurately represent in-orchard leafroller populations. Further studies are needed to verify the active space of the acetic acid lure and measure its relative attractancy under mating disruption conditions. Preliminary evidence from mating disruption trials suggests that different sex ratios may exist under those conditions (i.e. decrease in female bias). This attraction technology may also prove to be a valuable tool in designing an "attract and kill" product or larger scale kill stations.

Budget:
Development of bait-based monitoring systems for codling moth, leafrollers and lacanobia fruitworm
Jay F. Brunner
Project duration: 3 years
Current year: Year 3 - 2002
Original budget request: $30,718

Year	**Year 1 (2000)**	**Year 2 (2001)**	**Year 3 (2002)**
Total	$20,960	21,980	21,980

Current year breakdown

Item	Year 1 (2000)	Year 2 (2001)	**Year 2 (2002)**
Salaries[1]	14,976	0	**7,500**
Benefits (36%)	6,913	0	**2,700**
Wages[1]	5,025	16,000	**8,000**
Benefits (16%)	804	2,560	**1,280**
Equipment	0	0	**0**
Supplies[2]	2,000	1,500	**1,500**
Travel[3]	1,000	900	**1,000**
Miscellaneous		0	**0**
Total	30,718	20,960	**21,980**

[1] 25% of salary for Betsy Valdez, Associate in Research, and temporary summer labor.
[2] Traps, lures, alcohol, gloves, etc.
[3] One vehicle for three months plus fuel and service.

Approximately $8,000 of IFAFS/RAMP funding is dedicated to this project.

CONTINUING PROJECT **YEAR 3/3**

Project Title: Bisexual monitoring of codling moth and establishing a female biofix

PI: Alan L. Knight
USDA, ARS, Wapato
C0-PI and affiliation: Doug M. Light, USDA, ARS, Albany, CA

Objective:

1. Evaluate the use of DA2313 lures to monitor the emergence and population density of both sexes of codling moth in conventional and sex pheromone-treated apple and pear orchards.
2. Evaluate the use of DA2313 lures to monitor mating and egg laying of codling moth.

Ethyl (2E,4Z)-2,4-decadienoate (DA2313) is a characteristic ripe pear odor that attracts male and virgin and mated female codling moth. This compound has a number of attributes that make it a useful tool to monitor and manage codling moth populations. The compound is stable and can be loaded into regular dry lure formulations such as red or gray septa, it is attractive to codling moth at very low rates comparable to the potency of sex pheromone, and it is less expensive than the sex pheromone of codling moth. However, there are a range of factors that affect the behavioral response of both sexes to this compound that will impact its use in monitoring adults. Studies are in progress to evaluate these factors within several geographical areas over multiple years.

Ethyl (2E,4Z)-2,4-decadienoate was also found to be a potent stimulant of egg laying by codling moth. This attribute may allow us to develop a tool to monitor eggs within orchards. A preliminary study conducted in 2001 successfully detected elevated rates of egg laying on foliage adjacent to lures. Further studies are needed to optimize this potential tool.

Significant findings:

- ❖ DA2313 lures effectively monitored both generations of codling moth in > 120 apple orchards during 2000 and 2001.

- ❖ The cumulative catch of codling moth in DA2313-baited traps versus sex pheromone-baited traps was 3-4 times higher in apple orchards under mating disruption during both flights in 2001 and the second flight during 2000. However, the DA2313 lure caught 25% fewer moths than the sex pheromone lure during the first flight in 2000.

- ❖ The DA2313 lure is less effective than the sex pheromone lure in conventional apple orchards – catching ca.75% as many moths during the first flight and 25% during the second flight.

- ❖ The DA2313 lure catches 5 – 95% as many moths as sex pheromone lures in pear orchards.

- ❖ The first moth caught in a DA2313-baited trap is usually a male. During the 2000 season pheromone traps caught their first moth 1-2 weeks before the DA2313-baited traps. However, during 2001 there was no difference in the timing of the first moth caught between lures.

- ❖ The first female caught in a DA2313-baited trap is usually mated. Greater than 85% of females captured in conventional orchards and nearly 75% of females in sex pheromone-treated blocks were mated. However, a comparison of the female mating status of moths caught with a passive interception trap and the DA2313-baited trap suggested that the proportion of virgin females is underestimated by nearly 2-fold with the DA2313 lure.

- The beginning of sustained moth captures in DA2313-baited traps plus 155 degree-days was as effective as the beginning of sustained male captures in sex pheromone traps plus 250 degree-days in predicting codling moth egg hatch. However, timing egg hatch from the first moth caught in either type of trap was a variable predictor of egg hatch. Frequent trap checking plus consideration of dusk temperature and wind speed improves the prediction of egg hatch.

- Cumulative first generation moth catches in DA2313-baited traps were more effective than similar catches in sex pheromone-baited traps in eliminating unnecessary first generation cover sprays. Cumulative moth captures in DA2313-baited traps were more closely correlated than catch in sex pheromone-baited traps with the levels of codling moth fruit injury prior to harvest. Establishing action thresholds of 1 or more female moths or > 3 total moths caught in a DA2313-baited trap during the first flight was useful in avoiding fruit injury. However, false negatives occurred with about 10% of traps baited with either the DA2313 or sex pheromone lure.

- Factors such as trap height and trap position within the canopy can affect the numebrs and the sex ratio of moths caught.

- Apple cultivar appeared to be another factor that influences the performance of the DA2313 lure. Compared with moth catch in a sex pheromone-baited trap the DA2313 lure was most effective in late-season cultivars, such as ‘Granny Smith’ and ‘Fuji’, and least effective in ‘Gala’ and ‘Golden Delicious’.

- A codling moth egg trap was tested during 2001 with several rates of ethyl (2E,4Z)-2,4-decadienoate loaded in septa. Egg deposition was increased 3-fold on foliage surrounding the most attractive lures compared with an unbaited control.

Methods: We will continue to monitor the same three orchards in the Yakima Valley for a third and final year with DA2313 and sex pheromone lures and passive interception traps. All traps will be checked and rotated daily during the first 45 days of the season to establish an accurate picture of the spring emergence of codling moth. Egg density and the detection of egg hatch will be monitored twice weekly in these orchards. Beginning in June these orchards will be monitored twice per week. In addition, five sex pheromone-treated and conventional apple and pear orchards (20 orchards) will be monitored weekly in the Yakima Valley all season with DA2313 and sex pheromone lures. Another forty apple orchards (ten replicates of four cultivars) treated with sex pheromone dispensers will be monitored in the Brewster area. Fruit injury will be assessed prior to harvest in all orchards and spray records will be summarized.

Several factors affecting the performance of the DA2313 lure will be evaluated during 2002. Lure emission rates are determined by both their chemical loading and the ambient temperature. Additional studies will be conducted to optimize the loading of lures to maximize moth catch throughout the season. Preliminary studies have developed improved lures (4x more attractive than DA2313) for use in pear orchards. These studies will be expanded during 2002. A protocol for using DA2313 lures to monitor codling moth in apple orchards under mating disruption will be developed in consultation with Trécé Inc. and validated in conjunction with WSU and California researchers.

Results and discussion: DA2313 lures were effective in detecting the two flights of codling moth during both 2000 and 2001 (Figs. 1 and 2). DA2313-baited traps caught their first moth 1 to 2 weeks later and on average caught fewer moths than sex pheromone-baited traps during the first flight in 2000. In contrast, both types of lures caught their first moths during the same week and DA2313-baited traps caught nearly 3-fold more moths than sex pheromone-baited traps during the first flight in 2001. The DA2313-baited traps outperformed the sex pheromone-baited traps during the second flight in both years.

DA2313-baited traps generally catch their first females several days later than their first catch of males. A high proportion of females captured in DA2313-baited traps are mated in both conventional (85%) and mating disrupted orchards (70%).

Fig. 1. Seasonal captures of codling moth in DA2313- and sex pheromone-baited traps within 22 apple orchards treated with MD in Brewster during 2000.

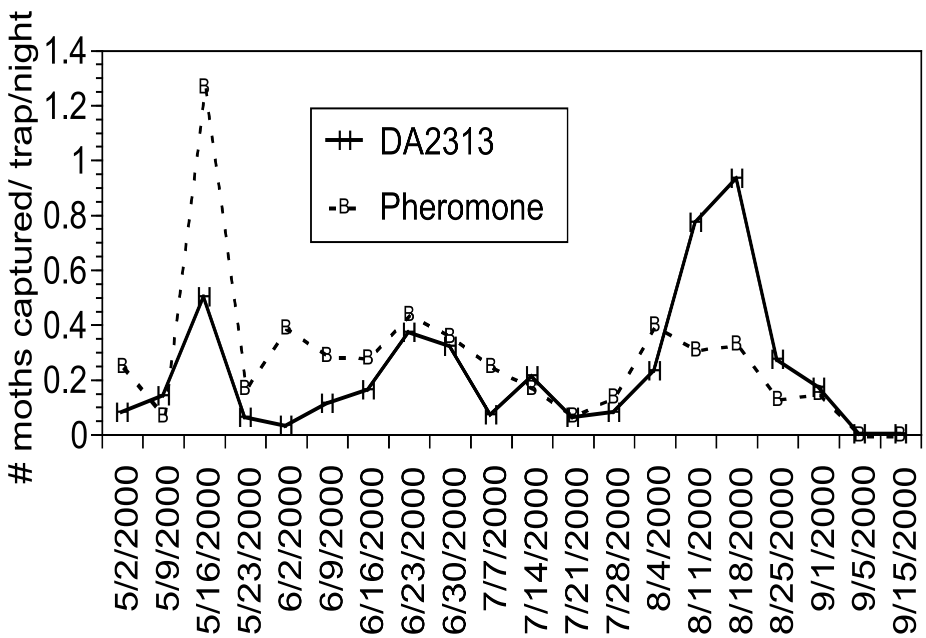
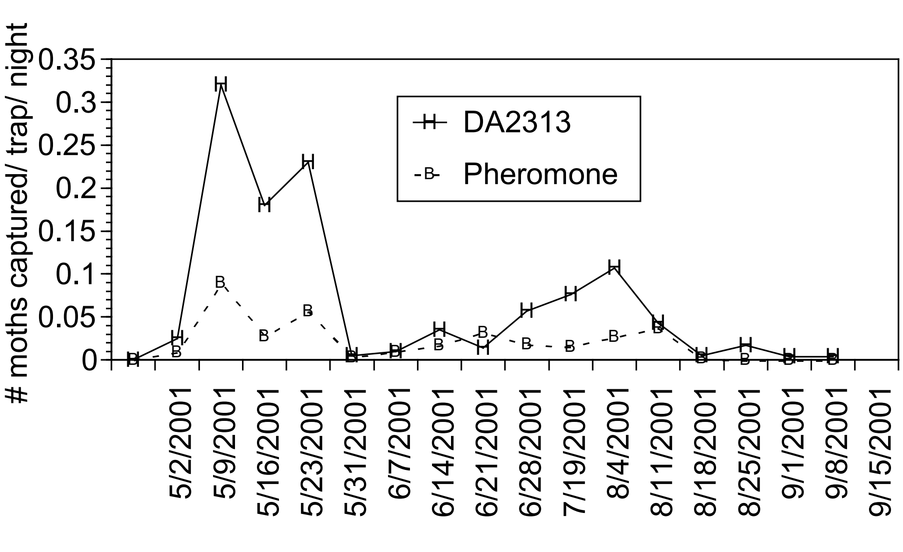


Fig. 2. **Seasonal mean captures of codling moth in DA2313- and sex pheromone-baited traps within 57 apple orchards treated with sex pheromone dispensers in Brewster during 2001**.



The timing of first moth catch in DA2313-baited versus moth catch in sex pheromone-baited traps was evaluated to predict the egg hatch of codling moth. Both the standard technique of establishing a male biofix plus 250 degree-days or the use of a female-based biofix plus 155 DD (thermal requirement for the completion of egg development) were successful in estimating the timing of first egg hatch during both 2000 and 2001. However, our data suggests that using the first moth caught in either a sex pheromone or DA2313-baited trap can have variable results. The relative timing of first male and first female moth caught in a DA2313-baited trap was variable (7 days at Garza, 2 days at Wallace, and 0 days at P-Block) and this variation suggests that moths should be sexed to improve the sensitivity of establishing a female-based biofix with a DA2313-baited trap. Using traps to precisely predict the population emergence of codling moth remains difficult. Establishing the start of a sustained period of moth catch would be difficult without daily trap checking. Similarly, the detection of the *crossover event* where the DA2313 lure becomes as or more attractive than the pheromone lure would also require that traps be checked daily. Regardless, of which method a pest manager uses to time sprays, he needs to consider daily maximum and minimum temperatures, as well as wind speed at dusk, to interpret moth counts and improve their correlation with female mating and egg laying events.

Table 1. Prediction of first egg hatch for codling moth based on moth catches in sex pheromone or DA2313 baited traps.

Orchard	**Key Event:** 1st hatched eggs	1st Male caught in P-traps + 250DD	Sustained male catch in P-traps + 250 DD	1st Female caught in DA traps + 155 DD	Sustained female catch in DA traps + 155 DD	Crossover Event (DA catches > Phero catches) + 155 DD
Wallace	**5/20**	**5/14**	5/19	**5/15**	5/18	5/21
P-block	**5/21**	5/20	5/21	**5/12**	5/23	5/21
Garza	**5/25**	5/24	5/26	5/24	5/24	5/26

During 2000 we showed that the use of DA2313 lures would reduce the over-spraying of uninfested orchards during the first flight. We suggested that sex pheromone traps pull male immigrants into the orchard and are less effective in reflecting the local population density of females than the DA2313 lures. Seasonal cumulative catches of moths in DA2313-baited were more closely correlated with fruit injury at harvest than similar traps baited with sex pheromone lures in both years (correlation coefficients for DA2313 versus Pherocon Mega lures: 0.84 versus 0.71 in 2000 and 0.69 versus 0.36 in 2001, respectively). However, false negative catches of moths occurred with both types of lures in about 10% of orchards.

Seventy-five apple and pear orchards were established to evaluate the performance of the DA2313 lure versus sex pheromone lures in MD and conventional orchards during 2001 (Table 2). The DA2313 lure caught more moths than the sex pheromone lure in MD apple (except Gala) compared with conventional apple orchards. Apple cultivar effects are significant and it appears that the DA2313 lure works better in orchards planted with later versus early cultivars. In general, catch in DA2313 relative to pheromone traps declined in the 2nd generation in conventional orchards and increased in MD orchards.

Table 2. Ratio of moth catches in DA2313 and sex pheromone-baited traps under either MD or conventional practices during both generations.

		MD			Conventional	
		DA:Pher Ratio			DA:Pher Ratio	
Cultivar	n	1st	2nd	n	1st	2nd
Pear	**12**	**0.11**	**0.93**	**3**	**0.16**	**0.05**
Gala	10	0.93	3.25	-	-	-
Golds	12	1.58	2.73	1	0.55	0.13
Reds	12	1.54	1.61	4	0.80	0.22
Fuji	10	2.69	2.45	1	0.75	0.47
Granny	10	7.72	15.86	0	-	-
Total apple	**54**	**2.90**	**4.73**	**6**	**0.75**	**0.25**

About 80% of the females captured in DA2313-baited traps were mated (Table 3). However, it appears the DA2313 lure is biased for mated females because the percentage of virgin females caught by the unbiased, passive interception trap in the same orchards was nearly twice as high (Table 4). These data support our previous work that has shown that levels of mating are very high in sex pheromone-treated orchards and support the idea that a delay in mating may contribute to the reductions usually observed in pheromone-treated orchards.

Table 3. The percentage of virgin females captured in traps during the season.

Location	Crop	n	MD	n	Conventional
Brewster	Apple	48	22.3%	3	23.1%
	Pear	10	25.0%	-	-
Yakima	Apple	3	21.4%	7	13.3%
	Pear	2	13.6%	2	19.0%

Table 4. Percent capture of virgin females in DA2313-baited and interception traps within high-pressure apple orchards.

Orchard	**DA2313 trap**	**Passive trap**
Conventional	12.5%	22.5%
MD	24.3%	42.3%

Budget:

Bisexual monitoring of codling moth and establishing a female biofix
Alan L. Knight
Project duration: 2000-2002. This is the last year of this project.
Current year: 2002

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

Current Year Breakdown

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

CONTINUING PROJECT **YEAR 3/3**

Project Title: Pheromones and Attraction Inhibitors for Codling Moth Mating Disruption

PI: Alan L. Knight, USDA, ARS, Wapato

Objectives:

1. Evaluate the use of CM puffers releasing 5.0 mg of codlemone per puff to achieve a further cost savings with this approach.
2. Develop I.-H.E.L.P. approaches that do not require any hand application of dispensers on the orchard's perimeter to lower the labor cost of applying pheromone.
3. Evaluate the proportion of mated females in orchards treated with hand-applied dispensers, puffers, and sprayable formulations.

New technology is needed to reduce the cost of mating disruption, increase grower=s flexibility in integrating this approach with other tactics, and improving its effectiveness. Within this project I am developing a lower-cost, effective integrated sex pheromone-based management program for both codling moth and leafrollers using the I.-H.E.L.P. approach (Integrated High Emission Low Points) where high emission point sources are combined with either hand-applied, sprayables, or >attract and kill= pheromone formulations.

Significant findings: Twenty-five 40-acre apple orchards (1,000 acres) were treated with one of five integrated, high emission low point density approaches (I.-H.E.L.P.) during the 2001 season in the Brewster, Washington area. Comparison orchards were established for each of the twenty-five I.-H.E.L.P. orchards. These were paired prior to the season based on similar size, location, pest pressures, cultivar, ownership, and expected spray practices. All comparison orchards were treated with Isomate C+ dispensers (Pacific Biocontrol, Vancouver, WA) applied at 200 dispensers per acre.

- All five cases of the I.- H.E.L.P. approach outperformed the standard use of Isomate C+ (200 dispensers per acre) based on lower mean fruit injury levels and insecticide usage (Table 1).
- Growers preferred the use of these I.-H.E.L.P. approaches to the standard hand-applied system in all cases due to ease and lower cost.
- Mechanical and phytotoxic problems associated with puffer use were considered to be minor problems.

Methods: Twenty-five 40-acre apple orchards (800 acres) will be treated with one of four I.-H.E.L.P. approaches during the 2002 season in the Brewster, Washington area. Ten orchards will be treated with the standard I.-H.E.L.P. puffer arrangement (1 per hectare) but each puffer will release only 5.0 mg codlemone every 15 min for 12 h per day (3:00 pm to 3:00 am). The perimeter of all orchards will be treated with hand-applied dispensers. Another fifteen orchards will be treated with the standard M.B.A. approach but will differ in terms of how their perimeters are treated. The perimeters of five orchards each will either be treated with hand-applied dispensers, monthly sprays of microencapsulated formulations (10-20 g pheromone per acre), or DPH mass trapping devices (80 units per 1,600 m). All orchards will be monitored with sixteen Pherocon VI delta traps baited with either a sex pheromone lure or the DA2313 kairomone lure for codling moth. The proportion of mated females will be determined from catch in the DA2313-baited traps. Traps will be checked weekly, liners replaced as needed, and lures replaced after 8 weeks. Comparison orchards will be established for each of the twenty-five I.-H.E.L.P. orchards. These orchards will be paired prior to the season based on similar size, location, pest pressures, cultivar, ownership, and expected spray practices. All comparison orchards will be treated with Isomate C+ dispensers applied at 200 dispensers per acre. Spray records will be obtained from the growers and field managers at the end of the season. Fruit injury will be assessed just prior to harvest by sampling thirty fruit from twenty trees within each quadrant of the orchard (2,400 fruit sampled per orchard). An equal number of fruit will be sampled from the interior and from the edge of each quadrant. An additional five orchards treated with only a sprayable pheromone formulation will be monitored with both pheromone and DA2313 lures. These data will allow us to compare the relative efficacy of the three major approaches used for mating disruption of codling moth.

Results and Discussion: Prior to the start of this project researchers basically agreed that the point source density of pheromone dispensers was a key factor affecting the success of mating disruption. However, a range of studies conducted during the mid 1990's with funding from WTFRC suggested that something else was happening. First, we consistently found that up to 80% of females in MD orchards were mated. We showed that a delay in mating can occur and that this may be a major factor reducing the population growth of codling moth in pheromone-treated orchards. We also showed that codling moths are attractive to individual hand-applied dispensers and that false trail following is likely the key mechanism affecting the level of disruption. Thus point source density should be important. Yet, initial studies with puffers in California suggested that disruption could also be achieved with only 1-2 dispensers per acre. Our subsequent laboratory studies have shown that moth exposure to high rates of pheromone does cause sensory habituation to its pheromone and we can turn off male sexual behavior for 24-48 h. Thus the use of high emission point sources such as puffers may be effective through a different mechanism. My work on this is continuing with support from ARS base funds.

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

Based on these two years of successful results we elevated the I.-H.E.L.P. approach in 2000 to on-farm grower testing. We evaluated both a dual puffer for codling moth and oblique banded leafroller and a puffer for codling moth alone in sixteen 40-acre orchards. Orchards treated with puffers achieved similar levels of pest control as in the paired orchards treated with Isomate C+.

We expanded our study in 2001 to include a passive system of dispensing high rates of pheromone from a low number of point sources due both to continuing mechanical problems with the aerosol puffers and concern that a closer array of high emission point sources would be more effective,.

Coined M.B.A. (Multiunit Box Approach), this program placed 64-screened cages housing 100 Isomate C+ dispensers in a grid beginning 25 m from the edge and spaced 50 m apart (1.6 cages per acre). With M.B.A. in 2001 we again treated the perimeter of the orchard with a 10 m-band of hand-applied dispensers. In addition to M.B.A. we continued to test the CM and the low and high CM/LR Paramount puffer plus the Consep CM puffer. The testing of the low CM/LR puffer explored the use of releasing only 5.0 mg of pheromone for codling moth per puff. Each of these five treatments were evaluated in five 40-acre orchards (1,000 acres treated) and each orchard was paired with a similar orchard treated with Isomate-C+. All five I.-H.E.L.P. treatments outperformed Isomate C+ (Table 1). During 2001 Paramount Farms acquired the Consep Inc. line of products and are now developing only the Paramount puffer system plus both a hand applied dispenser and a microencapsulated sprayable formulation.

The results from this project to date have been very important to the development and adoption by growers of the use of sex pheromones for mating disruption. Originally the manufacturer of the puffers recommended 1-2 puffers per acre and these were to be applied to the perimeter of the orchard. We have shown over several years that a much lower density of puffers can be effective. This will dramatically reduce the cost of this technology. The development of the M.B.A. approach is another significant accomplishment as it is a grower-friendly approach that can also reduce the cost of using sex pheromones by reducing the grower's application costs. Testing during 2002 will focus on approaches that do not require the hand application of dispensers and this will further benefit the grower. Parallel to this study we will continue to test the efficacy of sprayable formulations for codling moth.

Budget:

Project duration: 1998-2002. This is the last year of this project.
Current year: 2002

Original budget requested

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

Current Year Breakdown

Item	Year 1 (1998)	Year 2 (1999)	Year 3 (2000)	Year 4 (2001)	**Year 5 (2002)**
Wages	26,000	25,500	22,000	23,600	**22,500**
Benefits (10%)	2,000	2,500	1,600	2,400	**2,250**
Supplies	8,000	8,000	12,000	7,000	**4,750**
Travel (local)	2,000	2,000	1,000	2,000	**1,500**
Total	38,000	38,000	36,600	35,000	**31,000**

Table 1. Comparison of five I.-H.E.L.P. strategies (5 orchards and 200 acres per treatment) paired against similar orchards treated with Isomate C+ dispensers (200 dispensers per acre) in the Brewster area during 2001.

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

CONTINUING PROJECT **YEAR 3/3**

Project Title: Management of Codling Moth with a Bisexual 'Attract and Kill' Formulation

PI: Alan L. Knight
USDA, ARS, Wapato

C0-PI Doug M. Light, USDA, ARS, Albany, CA

Objectives:

1. Develop and test the use of bait stations using DA2313 for management of codling moth.

DA2313 was identified as a potent bisexual adult attractant for codling moth by Doug Light in 1998 and I identified it as a larval attractant in 1999. Studies have been in progress since 2000 to develop commercial products which could be used to effectively manage codling moth via attract and kill or mass trapping approaches. We believe that the attractiveness of DA2313 for adults can be used to manage codling moth by allowing the removal of both virgin and mated female moths. Female codling moths lay an average of 30 - 100 eggs in the field. Male moths mate frequently, thus their removal does not assure that females are left unmated. Unlike the use of sex pheromones for mating disruption the direct removal of female moths will assure that reductions in the number of eggs laid by the population will occur. Also removal of females that are mated outside but then immigrate into the orchard will be possible. Our first studies conducted during 2000 attempted to develop a paste droplet formulation to be used for attract and kill of adults similar to *Last Call* but effective for both males and females. However, these studies showed that female moths are attracted but do not touch a paste droplet laced with DA2313 and we discontinued this approach. Testing during 2001 and studies proposed for 2002 are to develop bait stations (traps) to kill both sexes of codling moth.

Significant findings: During 2001 we focused on developing an insecticide-laced bait station.

- We developed an effective insecticide-impregnated bait station.
- We conducted a large field trial with this approach where comparing untreated plots with plots treated with mating disruption only, mating disruption + bait stations, mating disruption + oil sprays, and mating disruption + oil + bait stations. .
- Fruit injury mid-season was reduced 95% with the addition of bait stations compared with untreated plots.
- Fruit injury at harvest was only reduced 60% in the bait station plots.

Methods: Further development of insecticide-impregnated bait stations will likely focus on the use of Deltamethrin which is a long-lived synthetic pyrethroid which will soon be registered in apple. This is the insecticide that has been used in mass trapping of the olive fruit fly in Europe and for the apple maggot in the U.S. We will determine the optimal concentration of insecticide and test the longevity of its residual activity. We will also test a variety of formulations that will transfer a toxic dose to the moth.

The major focus of the project for 2002 is the development and testing of a new trap which has been developed under a secrecy agreement with Powdertrap Inc. The goal is to have a non-maintenance trap which can be used in both conventional and certified-organic orchards.
We have tested a number of prototypes and have developed a trap that is equivalent to the most effective sticky trap on the market. This prototype have been sent to three southern Hemisphere countries for further testing this winter. A commercial design is being developed for production of thousands of traps.

We envision testing the use of this trap in several ways. Replicated studies in apple will be established with and without the use of supplemental sex pheromone dispensers for mating disruption. We will continue to optimize the baits used in these traps to increase moth catch. We will be using these traps to supplement the use of the I.-H.E.L.P. pheromone strategy. Traps will be placed on the perimeter of five 40-acre orchards treated with the passive M.B.A. design. We will evaluate the use of mass trapping along with other approaches such as horticultural oil to manage codling moth without the use of organophosphate insecticides.

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

We are unclear on whether these poor late season results are solely due to our failure to maintain a toxic deposit on the traps during August or also due to a reduction in the attractiveness of the lures. We have made some rough calculations as to the impact of this program. Trees averaged 182.6 fruit per tree. In a separate study we created a plot treated with 24 sticky Pherocon IIB traps baited with the DA and L2 lures. These traps averaged 101 males and 19.4 females from 11 July to 21 August. If each female removed by the trap (80% mated 20% virgin) had laid 50 eggs then 969 fewer eggs were laid on the 9 trees serviced by each bait station or 107.6 eggs per tree. This would have reduced the fruit injury by 59% but we observed only a 30% reduction (Table 1). This difference may be explained by the fact that injured fruits in the MD-only plots averaged 2.85 injuries per fruit versus 2.09 injuries in the DA + MD plots. Thus we actually reduced the number of larvae injuring fruit by 65%. If we look at the moth catch by individual DA and L2 baited traps located within these orchards but outside of the plots we see that together these two lures caught 159 males and 46 females in the first generation and 83 males and 56 females in the 2nd flight. This suggests that the lures would have performed similarly during both flights in terms of female removal but did decline in terms of male removal. A final problem with this study was that the 1-2 acre plots were surrounded by trees with > 90% injury at mid-season and the effectiveness of the bait stations in removing this huge number of adults was likely inadequate.

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

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

Budget:

Management of Codling Moth with a Bisexual 'Attract and Kill' Formulation
Alan L. Knight

Project duration: 2000-2002. This is the last year of this project.
Current year: 2002

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

Current Year Breakdown

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

FINAL REPORT
WTFRC Project #AE-01-49 **WSU Project # 4093**

Project title: Evaluation of sprayable pheromone and attract & kill for codling moth and leafroller control

PI: Jay F. Brunner, Entomologist
Organization: WSU Tree Fruit Research and Extension Center

Co-PI and affiliation: Peter Landolt, USDA-ARS, Wapato

Cooperators: Betsy Valdez, Associate in Research, WSU-TFREC, Wenatchee, WA

Objectives:
Develop a pheromone-based management program for codling moth and leafrollers by:
1. determining if sprayable pheromone technology 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:
1999
- Early tests with a sprayable pheromone (3M) for leafrollers showed that the 40 g AI/acre rate was more effective than the 20 g AI/acre rate. One application per flight was not sufficient to reduce moth activity throughout the entire flight period. There was little difference between sprayable pheromone applications applied at a two-week versus a three-week interval. None of the sprayable pheromone treatments was as good as a hand-applied dispenser leafroller pheromone treatment.
- A long-term project was initiated comparing leafroller hand-applied pheromone dispensers (Pacific Biocontrol). In the first year the 250 mg dispenser (season long) suppressed moth activity slightly better than the 80 mg dispenser (90 day). There was indication that the pheromone treatments reduced leafroller larval densities, but results were not consistent at all sites.
-

2000
4. A second year of leafroller sprayable pheromone tests showed fair effect of treatments on moth activity in a three- or four-spray program. Where sufficient populations existed, there was a suppression of leafroller larval densities and fruit damage in the pheromone treatments.
5. In a rate study comparing 5, 10, 20 and 40 g AI/acre of sprayable leafroller pheromone there was a significant direct relationship between rate and suppression of moth activity.
6. Application method, airblast versus Proptec, did not make a difference in activity of the sprayable pheromone.
7. A paraffin emulsion containing codling moth and leafroller pheromone (Gowan, Inc.) was tested in 2000. Treatments of 15, 30 and 60 g AI/acre showed a slight rate effect. The formulation shows some promise for development as a possible mating disruption product, but it needs more development.
8. IPM Technologies' Last Call-OBLR at 600 and 1200 drops/acre suppressed moth captures in traps but did not affect the density of larvae in test plots.

2001

- Two formulations of sprayable codling moth pheromone were tested and showed varying degrees of promise as a technology for managing this pest. Additional large plot trials are required before it can be recommended for growers use.
- A third year trial with a sprayable leafroller pheromone formulation showed some promise as a technology for managing this pest. Trap shut-down is not as great as with hand-applied dispensers.
- Hand-applied leafroller dispensers were evaluated for the third year. The season-long dispenser or single-flight dispenser showed promise in reducing moth activity and in limiting leafroller densities. There was indication of reduction in mating frequency associated with the treatments and in reduction of larval densities.
- An attract & kill (A&K) formulation using the Last Call (IPM Technologies) base formulation and different concentrations of PLR or OBLR pheromone was evaluated. Attraction of moths to the A&K formulation was proportional to pheromone concentration. It appears that higher pheromone concentrations are required in the A&K formulation than currently are being used. Incorporation of permethrin in the A&K formulation did not reduce attraction of leafrollers to the sources.
- Aerial applications of Scentry's fiber pheromone formulation showed promise as a control of leafrollers based on reduction in trap captures throughout the entire first flight (50 days). This formulation has a promise to be developed as an attract & kill product for leafrollers and codling moth.
- Aerial applications of Scentry's fiber pheromone formulation showed some promise as a control of codling moth based on reduction in trap captures and damage at harvest. Additional research is needed on application technology, distribution of fibers and attraction of fibers to moths.
- The evaluation of five different hand-applied codling moth pheromone dispensers showed variable pheromone release behaviors when analyzed using three different methods. This information will be helpful in clarifying some confusing claims made of different dispenser systems by registrants.
-

1999-2001 Summary

- **Sprayable leafroller pheromones** – Three years of studies, some at the same site show that this technology requires at least two applications per flight, probably at 20 g AI/acre. There is interest in this technology, and continued research is proposed working with newer formulations. It is likely that this technology will be integrated with soft pesticides for successful leafroller management.
- **Sprayable codling moth pheromones** – There appears to be promise in this technology as a CM control. Two companies are working on formulations. Continued research is proposed working with this technology.
- **Hand-applied leafroller pheromones** – These products have a strong effect on moth activity. There was also a reduction in mating and larval densities in pheromone treatments compared to conventional control programs. The season-long dispenser (250 mg) could be adapted to CM hand-applied dispenser programs because it could be placed at the same time as the CM dispensers and still last the entire season.
- An older pheromone delivery technology, Scentry fiber pheromone, shows promise as a codling moth and leafroller control. This technology was used in cotton to suppress the pink bollworm. It also has the potential to be developed as an attract & kill product. The advantage of this technology is that it can be applied by air. Additional research is proposed working with this technology.
- **Attract & kill** – A technology based on Last Call formulations shows that the leafroller pheromone is not released from the matrix as efficiently as the codling moth pheromone. At higher concentrations of pheromone in the formulation, attraction to the source increases. Additional development research is required to make this technology more efficient as an attract & kill approach for leafrollers.

Methods - 2001:

3M sprayable codling moth pheromone – Royal Slope: 3M sprayable CM pheromone (3M-CM) was applied to an unreplicated large block (6 acres) at the Royal Slope orchard at a rate of 10 g AI/acre in the first flight. The orchard was monitored with the standard 1X pheromone lures and Trécé DA kairomone lures in delta-type traps at one per acre. A two-acre untreated plot directly adjacent to the pheromone treated plot was monitored in a similar manner. The 3M-CM was applied on April 26. Damage after the first generation was rated by examining 30 fruits per tree from 30 trees in each two-acre section of the pheromone treated and untreated section.

In the second flight period the same orchard was divided into approximately one-acre blocks. Three treatments were assigned to each of three blocks in a Latin square design. The treatments were 3M-CM without a sticker, 3M-CM with a sticker, and Isomate-C+. The 3M-CM treatments were applied at 20 g AI/acre and the Isomate-C+ at 400 dispensers per acre. There was also a two-acre control plot located adjacent to the pheromone treated blocks. Two pheromone traps with a 1X lure were used to monitor moth activity in each block and the untreated control.

3M sprayable codling moth pheromone – Chelan: 3M-CM pheromone was applied to approximately two-acre blocks in an abandoned orchard near Lake Chelan. Treatments consisted of 1 g or 20 g AI/acre of 3M-CM, Isomate-C+ at 400 dispensers/acre and an untreated block. The 3M-CM treatments were applied to two two-acre blocks, the Isomate to only one block, and there was one untreated block. Each block was monitored with two pheromone traps baited with 1X lures and two traps baited with the DA kairomone lure. Traps were checked two times per week. In the second flight the orchard was divided into one-acre blocks. The experimental design and treatments were similar to that described for Royal Slope.

Consep sprayable codling moth pheromone: The Isomate-C+ was applied by hand to all blocks on May 1 at a rate of 200 dispensers per acre. The Consep sprayable pheromone (CSP) was applied through normal orchard airblast spray equipment in 80 gallons of water per acre. The intent was to apply 20 g AI/acre in the first spray and 10 g AI/acre in the subsequent sprays. The actual rate applied based on orchard spray records was 25.6 g AI/acre in the first and 12.8 g Ai/acre in subsequent sprays. Two applications of CSP were applied against each CM flight. A supplemental insecticide was applied to the CSP blocks in the first CM generation in response to captures of moths in traps and to reduce risk of testing a new technology.

Consep sprayable codling moth pheromone:

CM trapping – Delta-type pheromone traps baited with Pherotech CM SuperLures were used to monitor male moth activity. One trap was used for every 2.5 acres. In addition, Delta-type traps baited with the Trécé DA2313 kairomone lure was used to monitor CM male and female activity at a density of one every 2.5 acres. Traps were checked weekly. Pheromone CM SuperLures were changed every six weeks and DA2313 lures every eight weeks.

Fruit injury – At the end of the first generation 40 half-fruits from 50 trees per plot (10 trees in five rows - 1000 whole fruits) were examined for presence of codling moth injury. Just prior to harvest, 50 half-fruits from 40 trees per plot (1000 whole fruits) were examined for presence of codling moth injury. Edges of blocks were specifically monitored for fruit injury so reported damage levels likely overestimate fruit injury in the entire block.

3M sprayable leafroller pheromone – Royal Slope: A 3M sprayable pheromone was applied twice at 20 and 40 g AI/acre at leafroller biofix and four weeks later for each flight. Nu-Film 17 was added as a spreader/sticker at 1 pt/acre with each application of sprayable pheromone. Each experiment consisted of approximately 30 acres divided into three 10-acre blocks. The control block in each case was upwind from those treated with pheromone. There were two pheromone treated plots (20 and 40 g AI/acre). All blocks were treated the same for pests other than leafrollers as grower or consultant saw fit.

Each block was monitored with delta-style traps baited with either a high load (10 mg) or standard load (1 mg) lure. There were four traps of each lure-load per block (24 total for experiment). Traps were spaced equidistant from one another, alternating traps with different lure-loads. Traps were checked and rotated twice weekly, the number of leafrollers was recorded, and traps were placed/replaced within the top 3 feet of the tree canopy. Lures were changed once per generation. Trap bottoms were replaced when excessive wing scales and other debris had accumulated or at each lure change, whichever came first. The delta-style trap was used this year with a bait lure to assess adult flight (male and female) and mating success of females trapped. The bait lure consisted of a 10 ml polypropylene vial with a 0.33 mm-sized hole drilled in the lid and loaded with 5 ml of glacial acetic acid (aa). Ten bait traps per block, (30 per orchard), were spaced evenly throughout each treatment block. The lures were changed every four weeks. Bait traps were checked twice weekly and adults were collected and recorded. Mating success was determined by dissecting females collected.

Bud samples were collected at delayed dormant timing from each orchard to determine leafroller larvae overwintering population. A total of 200 buds per treatment were collected (50 buds per quadrant) and dissected in the lab. At petal fall, larvae were sampled by examination of at least 1000 shoots (10 shoots from 25 trees/quadrant) per block (treatment). In summer (mid- to late July), larvae were sampled again by examining at least 1000 shoots (10 shoots from 25 trees/quadrant) per block (treatment). A preharvest fruit injury assessment was made in mid- to late August. Ten fruits from 25 trees/quadrant (1000 fruits total per block) were visually inspected for leafroller fruit injury.

Leafroller hand-applied pheromone dispensers: Test 1 – This experiment was conducted at six different locations. Each experiment consisted of approximately 30 acres divided into three 10-acre blocks. There were two treatment plots along with an untreated control. All blocks were treated the same for pests other than leafrollers as grower or consultant saw fit. Pheromone block 1 was treated with 400 Isomate OBLR/PLR (80 mg) dispensers per acre. Pheromone block 2 was treated with 200 Isomate OBLR/PLR+ (250 mg) dispensers per acre. Dispensers were applied prior to biofix (first moth flight in the spring) and placed approximately 3 feet from the top of the trees.

Each block was monitored with delta-style traps baited with either a high load (10 mg) or standard load (1 mg) lure. There were four traps of each lure-load per block (24 total for experiment). Traps were placed in the top 3 feet of the canopy, spaced equidistant from one another, alternating traps with different lure-loads. Traps were checked twice weekly, the position rotated at each examination, and the number of moths recorded. Lures were changed once per generation. Trap bottoms were replaced when excessive wing scales and other debris had accumulated or at each lure change, whichever came first. The delta-style trap was used this year with a bait lure to assess adult flight (male and female) and mating success of females trapped. The bait lure consisted of a 10 ml polypropylene vial with a 0.33 mm-sized hole drilled in the lid and loaded with 5 ml of glacial acetic acid, (aa). Ten bait traps per block (30 per orchard) were spaced evenly throughout each treatment block. The lures were changed every four weeks. The bait traps were checked twice weekly, and adults were collected and recorded. Mating success was determined by dissecting females collected.

Bud samples were collected at delayed dormant timing from each orchard to determine leafroller larvae overwintering population. A total of 200 buds per treatment were collected (50 buds per quadrant) and dissected in the lab. At petal fall, larvae were sampled by examination of at least 1000 shoots (10 shoots from 25 trees/quadrant) per block (treatment). In summer (mid- to late July) larvae were sampled again by examining at least 1000 shoots (10 shoots from 25 trees/quadrant) per block (treatment). A preharvest fruit injury assessment was made in mid- to late August at all locations. Ten fruits from 25 trees/quadrant (1000 fruits total per block) were visually inspected for leafroller fruit injury.

Test 2 – Similar tests were conducted in three other locations incorporating a fourth plot, (10-acre addition) treated with 200 Isomate OBLR/PLR (80 mg) dispensers per acre. This experiment satisfied all the conditions of the first.

Attract & Kill – leafroller formulation: 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-style traps. Four traps of each lure type were used. Traps were checked every two to three days, moths counted and removed, and position of traps rotated at each inspection.

Last Call base matrix was used to formulate a leafroller attract & kill formulation with different concentrations (0.012, 0.032, 0.16, 0.8, 2.0 and 4.0%) of PLR and OBLR pheromone. A drop (50 mg) of the formulation was placed inside a mess container and then placed inside a pheromone trap. Leafrollers of the appropriate species were released in a field wind tunnel and the number of moths attracted to the trap determined. Pheromone traps with drops of the attract & kill formulations were placed in the field, and the number of wild moths captured in each concentration was recorded.

Scentry CM fiber pheromone:
Fiber mixing and application – The Scentry NoMate Fiber pheromone was mixed with Bio-Tac in a 5-gallon bucket prior to application via helicopter (Fig. 3). The combined NoMate Fiber and Bio-Tac was placed into a hopper attached to the helicopter. The fiber/Bio-Tac formulation was augured out of the hopper at a constant rate, and individual fibers covered with Bio-Tac were flipped off a high-speed spinning cone. The helicopter flew as low to the orchard canopy as allowed by physical constraints such as power lines or houses (Fig. 4). The objective was to apply fibers individually to the target, foliage or twigs of the orchard.

Codling moth – In the first codling moth generation three locations were established as evaluations sites. One location (Kucera/Alvino) was not used in the second generation because of low pest pressure, and a different location was substituted (Knapp Old Cherry/Organic 20). The first application of NoMate CM Fiber was made on April 28 to Kucera and May 4 to Knapp and Taylor Orchards. The second application was made on July 10 to Knapp and Taylor Orchards. The NoMate LR Fiber application was made on June 1. The distribution of fibers was evaluated following the second NoMate CM Fiber application.

CM trapping – Delta-type pheromone traps baited with Pherotech CM SuperLures or Trécé standard CM lures were used to monitor male moth activity. One trap was used for every 2.5 acres. In addition, delta-type traps baited with the Trécé DA2313 kairomone lure were used to monitor CM male and female activity at a density of one every 2.5 acres. Traps were placed following the application of the Scentry pheromone and checked weekly. CM SuperLures were changed every six weeks, standard CM lures every two weeks, and DA2313 lures every eight weeks.

Leafroller trapping – Delta-type traps baited with pheromone lures were used to monitor OBLR moths. Pheromone lures were supplied by Scenturion, Inc. and contained loads of 10 mg, 1 mg (standard) and 0.1 mg per lure. Two traps of each lure load were used in each block. Traps were placed following the Scentry LR NoMate Fiber application. Traps were checked weekly and the number of moths recorded. Lures were changed every six weeks.

Leafroller larval sampling – After egg hatch of the summer generation, August 7, was complete but prior to pupation, a sample was conducted to assess the density of leafroller larvae or feeding activity. Twenty growing shoots were examined in each of 50 trees per plot. The number of shoots with leafroller larvae present or with feeding damage was recorded.

Fruit injury – At the end of the first generation 30 half-fruits from 50 trees per plot (750 whole fruits) were examined for presence of codling moth injury. Just prior to harvest, 50 half-fruits from 50 trees per plot (1250 whole fruits) were examined for presence of codling moth injury. At harvest 100 fruits from 25 bins per plot were examined and the number injured by insects, including codling moth and leafrollers, was recorded.

Results and discussion – 2001:
3M sprayable codling moth pheromone – Royal Slope: During the first flight period there was no real reduction in moth capture in the pheromone treated area relative to the untreated area. After the first generation there was no difference in the level of damage by CM in either treatment area.

In the second flight the Isomate-C+ treatment had the lowest moth capture relative to the 3M-CM treatments. The 3M-CM treatment without sticker had the least reduction in moth activity, followed by the 3M-CM treatment with sticker, and then the Isomate-C+. Moth pressure was very high, and the Isomate treatment only suppressed moth activity by about 80%.

3M sprayable codling moth pheromone – Chelan: During the first flight there was little suppression of CM activity by the 3M-CM treatments. The 1 g AI/acre rate suppressed moth activity about one week. The 20 g AI/acre rate suppressed moth activity for a slightly longer period, but the Isomate treatment provided the greatest degree of suppression.

In the second flight, the 3M-CM treatments with or without sticker suppressed moth activity by only about 60% but did so for almost 30 days. The Isomate treatment suppressed moth activity by 86% relative to the untreated control.

Conclusions: The 3M-CM sprayable pheromone has promise as a new technology for delivering pheromone; however, the new capsule technology does not seem to remain in the foliage or at least does not remain effective in shutting down moth capture. Continued research on the retention of the sprayable pheromone in the tree is needed for this new formulation.

Consep sprayable codling moth pheromone: Pressure by CM was low as indicated by capture of moths in either CSP or Isomate treated blocks at the Naumes Pateros Orchard. The average number of moths per trap per week for the entire growing season is shown in Table 1. Fruit injury after the first generation was low, as it was at harvest in most blocks. Actually, the highest damage was noted in the Isomate treated blocks at harvest. Almost all this injury was on the orchard border. The CSP formulation was easy for the grower to work with and, at least under the low pressure situations experienced at the Naumes Pateros orchard in 2001, performed as well as Isomate-C+ when supplemented with one insecticide. The pressure of CM in the 2001 trial location was not sufficient to ascertain how long the CSP applications suppressed CM flight or if suppression overlapped application intervals.

Table 1. Average moth capture (±SEM), accumulated moth capture and fruit injury by CM in blocks treated with ESP or Isomate-C+.

	Average moths per trap per week		Accumulated moths per trap		% CM fruit injury	
Block-Treatment	SuperLure	DA-lure	SuperLure	DA-lure	First	Harvest
C6-ESP	0.123±0.05	0.035±0.04	7	2	0.10	0.10
C3-Isomate	0.158±0.08	0.000±0.00	6	0	0.00	0.00
F4- ESP	0.224±0.10	0.132±0.04	17	10	0.20	0.00
F5&9-Isomate	0.395±0.09	0.197±0.06	30	15	0.60	0.88
F15- ESP	0.158±0.09	0.026±0.03	6	1	0.20	0.20
F17-Isomate	0.105±0.07	0.184±0.07	4	7	0.00	0.60

3M sprayable leafroller pheromone: Leafroller sprayable pheromone showed consistent reductions in pheromone lure trap catch for the obliquebanded and pandemis leafrollers at both rates when applications were made at the proper timings. Results showed that the higher rate (40 g AI/acre) provided more consistent reduction of moth activity compared to the lower rate (20 g AI/acre). Although there was consistent reduction in trap catch in many of the trials, there is no evidence of a reduction in larval populations using sprayable pheromone at either rate. Bait traps attracted significant numbers of adult moths this year in two of three orchards. There was no difference between the mating success of the untreated and sprayable pheromone blocks.

Leafroller hand-applied pheromone dispensers: All of the leafroller pheromone dispensers showed consistent reductions in pheromone trap catch in both flights for the obliquebanded and pandemis leafrollers at both rates. Although there was consistent reduction in trap catch in many of the trials, there is no evidence of a reduction in larval populations using any of the pheromone dispensers. Bait traps attracted more leafrollers than last season, (2000 - 279 moths; 2001 - 5,660 moths). In all hand-applied dispenser blocks there was a reduction in the percentage of mating success in the high rate pheromone block as opposed to the untreated.

Attract & Kill – leafroller formulation: 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.

In the field wind tunnel almost no moths were attracted to the attract & kill formulations containing 0.16% of pheromone or less. As the pheromone concentrations increased from 0.8 to 4%, moth captures increased but were always significantly less than captures in a trap baited with a 1X pheromone septum. Similar results were recorded when moths were trapped in orchards where traps were loaded with different concentrations of attract & kill formulations. Tests will continue in 2002, and this winter the pheromone release rate from the Last Call attract & kill formulation will be determined relative to the pheromone septum.

Scentry CM fiber pheromone:
The Scentry NoMate fiber pheromone formulations for codling moth showed some promise in suppressing moth activity in traps placed in treated orchards. Where there was high pressure from CM, suppression was not sufficient to prevent fruit injury (Knapp Orchard – Booster Pump block). In the second generation there was good suppression of moth activity in the Old Cherry block of the Knapp Orchard.

In the leafroller test the Scentry fiber pheromone suppressed moth capture in traps throughout the complete first flight compared to the untreated control. There was a lower density of leafroller larvae in one fiber pheromone treatment but not in the other compared to the control.

The application technology used in 2001 was not to the standards of the company, and better equipment to be used in 2002 should trials move forward. The uneven distribution of fibers in the plots was due in part to application equipment and in part to wind drifting the fibers. These are problems that can be overcome by using appropriate equipment and making treatments during early morning hours.

Budget:
Evaluation of sprayable pheromone and attract & kill for codling moth and leafroller control
Jay F. Brunner,
Project duration: This project has lasted three years and terminates with this report. A new proposal in this area is being presented.
Current year: 2002
Original budget request: $35,000

Year	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**TOTAL**
Total	35,000	35,330	16,280	86,610

Current year breakdown

Item	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**1999-2001**
Salaries	16031	15000	0	31031
Benefits (%)	4649	4950	0	9599
Wages	8400	10500	10500	29400
Benefits (%)	1344	1680	1680	4704
Equipment				0
Supplies	3576	2000	2000	7576
Travel	1000	1200	2100	4300
Miscellaneous				0
Total	35000	35330	16280	86610

CONTINUING PROJECT **YEAR 2/3**

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

PI(s): Lawrence A. Lacey and Thomas R. Unruh
USDA-ARS, Yakima Agricultural Research Laboratory

OBJECTIVES: Year 1 (revised)

1. Assess the effects of chlorine concentrations that are used in packing lines on nematode efficacy.
2. Evaluate *Steinernema feltiae* over a range of concentrations for control of codling moth in fruit bins.
3. Study the compatibility of entomopathogenic nematodes and hymenopterous parasitoids of codling moth.

Years 2 & 3

4. Continue research on the improvement of nematode and viruses through formulation
5. Investigate several sprinkler types for automated post application wetting to enhance nematode efficacy.
3. evaluate the effects of the frequency and timing of application of the Carpovirusine *Cp*GV formulation on insecticidal efficacy, fruit damage and density of subsequent generations
4. Evaluate the combined effect of application of *S. feltiae* on overwintering populations of CM nematode and *Cp*GV application after biofix for control of neonate larvae on insecticidal efficacy, fruit damage and density of overwintering population

Year 1 deviates from original objectives in that other research was addressed while waiting for virus registration and the securing of large supply of nematodes; Years 2 and 3 now include formulation which was originally addressed in another proposal.

SIGNIFICANT FINDINGS:

6. Nematode efficacy for control of codling moth in orchards and fruit bins can be improved after formulation with wetting agents, humectants or other compounds that retard evaporation.
7. Chlorine concentrations that are used in packing lines can negatively impact nematode efficacy.
8. Nematode concentrations of 25 infective juveniles per ml of water and higher are needed for effective control of codling moth prepupae in fruit bins.
9. Entomopathogenic nematodes that provide effective control of cocooned codling moth larvae are compatible with hymenopterous parasitoids of codling moth provided that the parasitoids are released after application of nematodes.

PROCEDURES:

Humectants, wetting agents and adjuvants that slow desiccation were tested for their effects on survival and infection of codling moth larvae in laboratory bioassays, orchard trials and tests in fruit bins. The adjuvant included Glycerol (4 and 10%), Silwet 77 and 408 (silicone based wetting agents) , Stockosorb (absorbent polymer; used at 0.2%); Leafshield (wax emulsion concentrate); LI 700 (surfactant-penetrant), Freeway (silicone surfactant); and AquaGro 2000 non-ionic surfactant. Except where otherwise noted, label rates were used for each of the adjuvants.

Nematodes for most tests were produced in waxmoth larvae (*Galleria mellonella*) using methods described by Kaya and Stock (1997). The laboratory assay designed by Lacey and Unruh (1998) was employed for the evaluation of *Steinernema feltiae* with and without adjuvants against cocooned codling moth larvae in cardboard strips. The treated cardboard strips were left uncovered in Petri dishes and incubated in air current at 25°C and compared with strips that were treated with nematodes in water only (10 IJs/cm^2) and incubated in sealed Petri dishes. Laboratory bioassays were also conducted with aqueous suspensions of *S. carpocapsae* IJs with and without Silwet (0.05%), Stockosorb (0.2%) or a combination of the two adjuvants. The treated cardboard strips were incubated in sealed Petri dishes, or open dishes protected from air currents, or in strong air currents.

Field trials of *S. feltiae* IJs in water only, *S. feltiae* in water + Silwet , and *S. feltiae* in water + Stockosorb (5 trees per treatment), were conducted in October, 2001 against sentinel larvae in a 20 year old Golden Delicious orchard using methods described by Lacey et al. (2000). Sentinel larvae in grooved apple logs and cardboard strips were attached to each tree. After application of the various treatments, the logs and strips were left in the orchard for 24 h before transferring them to a walk-in incubator at 25□C for at least 3 days. After incubation, mortality and nematode infection was assessed.

Trials of *S. feltiae* in fruit bins were conducted in greenhouses in low and high humidity using IJs in water only, *S. feltiae* in water + Silwet, *S. feltiae* in water + Stockosorb, and *S. feltiae* in water + Silwet + Stockosorb using modified methods described by Lacey and Chauvin. (1999). Grooves were cut in one of the corner supports of each fruit bin and subsequently infested with mature diapausing codling moth larvae. After the larvae produced cocoons the corner supports were returned to the bins just prior to treatment. The bins were immersed in suspensions of the appropriate treatment for one minute and then incubated in a greenhouse at 25□C at either low humidity (39% RH) or high humidity (89% RH), for 24 hours. After incubation in the greenhouse, the infested corner supports were removed and incubated at 25□C for at least 3 days after which mortality and infection were assessed.

Trials of *S. feltiae* in fruit bins were also conducted in greenhouses using IJs in water only and, *S. feltiae* in water + Silwet, to study the effects of chlorine concentration (100 ppm) on efficacy. The bins were immersed in suspensions S. feltiae without chlorine as untreated controls. Then chlorine was added and groups of 5 bins were treated after 0, 1, 2, 4 and 6 hours. The bins were then incubated in a greenhouse at 25□C at 70% RH, for 24 hours. After incubation in the greenhouse, the infested corner supports were removed and incubated at 25°C for 5 days after which mortality and infection were assessed.

The potential for compatibility of hymenopterous parasitoids and *S. carpocapsae* as studied in the laboratory using *Mastrus rudibundis* and *Liotryphon caudatus* that were originally collected in native apple forest in Kazakstan and maintained at the Yakima Lab in colony reared cocooned codling moth larvae. Mated females, 3-4 days old, were used for all experiments. After emerging, the parasitoids allowed to mate and have access to honey and cocooned codling moth larvae in cardboard strips. Twenty-four hours prior to each experiment, females of each species were placed in large Petri plates (15 cm diam), with a source of honey, but without host larvae.

Perforated 15.2 cm^2 cardboard strips (8 x 1.9 cm) containing approximately 20 cocooned CM larvae were prepared according to procedures prescribed by Lacey and Unruh (1998). For each parasitoid-nematode combination, diapausing cocooned CM larvae in 25 cardboard substrates were treated with an approximate LC_{95} of nematodes (380 IJs/cm^2), and incubated for 12, 24, or 48 hours before adding four mated female parasites to each of five large Petri dishes (15 cm diam.) containing one treated and one untreated cardboard strip. Parasitoids were left in contact with treated and untreated cardboard strips for 24 hours at 25°C (16L:8D) after which time they were be removed and the plates incubated for an additional 4-5 days. After incubation, the cardboard substrates were opened and the total number and number of moribund, dead, and parasitized larvae were recorded in treated and untreated strips. Controls consisted of five nematode treated strips, five water treated strips and 10 water treated strips exposed to 20 parasitoids (4 parasitoids and two strips in each of five Petri dishes) The experiments were repeated on three separate dates for each nematode-parasite combination.

RESULTS AND DISCUSSION:
A range of effects from detrimental to protective were observed for the formulation components on infective juveniles of *S. feltiae* (Table 1). The most promising agents in terms of increasing mortality of codling moth in laboratory bioassays were the silicone based wetting agents, (Silwet L-77 and 408, Freeway). The wax emulsion, Leafshield provided limited improvement over IJs in water alone. Control obtained with *S. feltiae* and Aqua Gro 2000-L or LI-700 was not significantly different from that observed with the nematode and water. Formulation with glycerol resulted in a marked decrease in nematode efficacy, especially at the higher concentration. Tests conducted with *S. carpocapsae* IJs and Stockosorb and/or Silwet L-77 in unsealed Petri dishes with reduced air circulation showed enhanced activity with the two agents compared to water suspensions of IJs (Figure 1). Surprisingly, IJs combined with both adjuvants produced slightly less mortality than IJs in water alone under the same conditions. Activity of the IJs was sharply reduced for all treatments when the cardboard substrates were placed in strong air currents.

The larvicidal activity of formulated and unformulated *S. feltiae* against cocooned sentinel larvae in log and cardboard substrates in late fall orchard trials is shown in Figures 2 and 3, respectively. Larvicidal activity was significantly higher in cardboard strips than in logs and in treatments that included post application wetting of trees compared to those not receiving additional wetting. Significant improvement in the larvicidal activity of *S. feltiae* was observed in both types of substrates that received Silwet or Stockosorb and post application wetting.

Larvicidal activity of *S. feltiae* formulated with Stockosorb, Silwet L-77 or a combination of the two in fruit bins against cocooned codling moth larvae is presented in Figure 4. The best control was achieved in high humidity with Silwet formulated IJs. In low humidity, treatments that included Stockosorb provided greater control. Wet aggregations of Stockosorb were obvious in the grooves of the corner supports. IJs that were formulated with Silwet provided the least amount of control in the low humidity test.

Rapid evaporation due to strong air currents appears to especially effect *S. carpocapsae*. This species is regarded as an “ambusher” and waits for insects hosts to pass by. This propensity to linger on the surface ostensibly renders them more vulnerable to desiccation. *Steinernema feltiae* on the other hand is regarded as a “cruiser” and will actively seek out hosts. *S. feltiae* also tends to be more temperature tolerant than *S. carpocapsae,* being able to infect insect hosts at 10°C.

In earlier tests and the orchard tests reported here, assessment of activity in cardboard strips on tree trunks and in fruit bins may have provided more moisture and permitted penetration of cocoons than would have occurred in natural substrates (*i.e.* under bark). The grooved logs on the other hand represent a worst case scenario for the drying of IJs. In this situation, aqueous suspensions of *S. feltiae* IJs plus Silwet with supplemental wetting apparently enabled rapid penetration of insect hosts and IJs plus Stockosorb slowed the evaporation. In the absence of supplemental wetting, both agents improved control over that obtained with IJs in water alone, especially in the cardboard substrates. In fruit bins, Silwet significantly improved efficacy, but only in high humidity. Apparently in low humidity and the strong air currents in the greenhouse, rapid evaporation curtailed the time available to IJs to find and penetrate a host. Conversely, under low humidity, Stockosorb provided more moisture enabled a broader window of opportunity for host location and penetration. The combination of the two adjuvants in high humidity was apparently antagonistic, providing lower levels of control than with unformulated IJs.

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

Chlorine concentration of 100 ppm clearly affects nematode efficacy, especially when no wetting agent has been added to the treatment tank (Figure 5). The addition of the wetting agent, Silwet-L77 increases efficacy of the nematode and appears to reduce the negative effect of chlorine (Figure 6).

The effect of nematode concentration on the efficacy of *S. feltiae* for control of codling moth larvae in fruit bins is presented in Table 2. Concentrations of at least 25 infective juveniles or higher per ml of tank water are required for reasonable levels of control in fruit bins.

When codling moth larvae that had been parasitized by *Mastrus rudibundis* and *Liotryphon caudatus* were exposed to *S. carpocapsae* the wasp larvae were as susceptible to the nematode as were the codling moth larvae. However, when the parasitoids were given the choice between parasitized and healthy codling moth larvae, they preferred healthy larvae especially 24-48 hours after nematode treatment. Figures 7 (*M. rudibundis*) and 8 (*L. caudatus*) show the progressive preference for healthy larvae 12-48 hours after nematode parasitization. Nematodes and parasitoids of codling moth can be compatible if release of wasps are made after application of nematodes.

REFERENCES CITED:

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Lacey, L. A., A. Knight, and J. Huber. 2000. Microbial control of lepidopteran pests of apple orchards. *In* "Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests" (L.A. Lacey and H. K. Kaya, eds.) pp. 557-576. Kluwer Academic Publishers, Dordrecht.
Unruh, T. R. and L. A. Lacey. 2001. *Biol. Contr.* **20**: 48-56.

BUDGET:

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

Project duration: three years (2001-2003)

Current year request 2002: **$28,097**

Item	Year 1 (2001)	Year 2 (2002)	Year 3(2003)
Salaries & Benefits GS-5 0.8 FTE	$20,700	**$20,916**	$21,150
GS-3 0.27	5,185	**5,681**	5,700
Supplies	1,000	**1,000**	1,000
Miscellaneous	500	**500**	500
Total	$27,385	**$28,097**	$2,350

WTFRC funded $25,000 in 2001 - USDA - ARS funded remaining portion.

Table 1. The effect of formulation components on larvicidal activity of *Steinernema feltiae* against prepuae of *Cydia pomonella* in circulating air currents.

	Mean % mortality ± s.e.m.
controls	0
water only	55.3 ± 8.8
water only (sealed)	93.0 ± 1.2
Silwet L-77	85.2 ± 5.7
Freeway	84.0 ± 6.7
Silwet 408	78.3 ± 4.9
Leafshield	64.9 ± 2.7
Aqua Gro 2000-L	58.6 ± 16.1
LI - 700	57.7 ± 1.5
glycerol 4%	27.7 ± 7.0
glycerol 10%	4.9 ± 1.8

Table 2. *S. feltiae* at concentrations plus Silwet L-77, for control of codling moth larvae in fruit bins.

	% Total Mortality ± s.e.	
Test dates	10/31/01	11/14/01
Silwet L-77	1.5 ± 0.9	0
5 IJ's/ml	33.3 ± 5.4	28.5 ± 3.8
10 IJ's/ml	53.8 ± 6.3	49.8 ± 11.5
25 IJ's/ml	71.9 ± 6.2	74.1 ± 7.0
50 IJ's/ml	74.7 ± 7.7	87.9 ± 3.9
100 IJ's/ml	91.0 ± 2.9	90.6 ± 3.2

Figure1. The comparative activity of *Steinernema carpocapsae* formulated with Silwet L-77 and/or Stockosorb

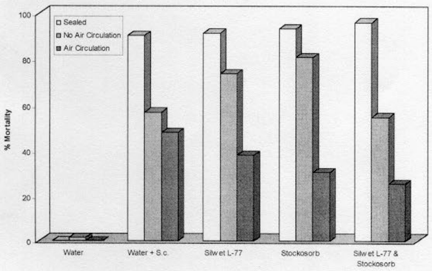


Figure 2. The effect of formulation components on the larvicidal activity of infective juveniles (IJs) of *Steinernema feltiae* against prepupae of *Cydia pomonella* in log substrates under late fall orchard conditions

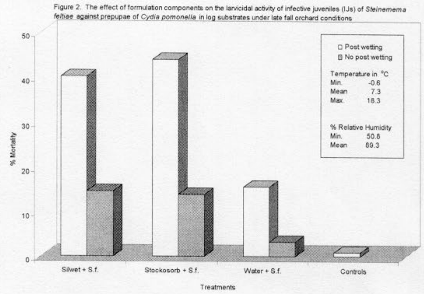


Figure 3. The effect of formulation components on the larvicidal activity of infective juveniles (IJs) of *Steinernema feltiae* against prepupae of *Cydia pomonella* in cardboard substrates under late fall orchard conditions

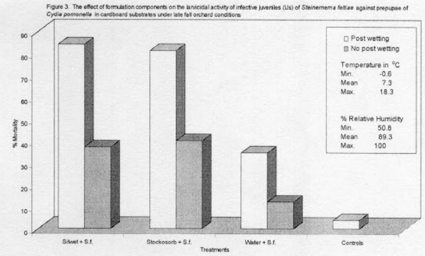


Figure 4. The effect of formulation components on the larvicidal activity of *Steinernema feltiae* against prepupae of *Cydia pomonella* in fruit bins in low and high humidity

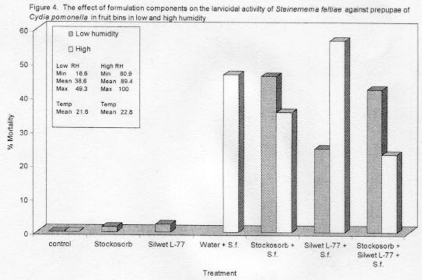


Figure 5. The effects of chlorine on *S. feltiae* at timed intervals, mean of four tests

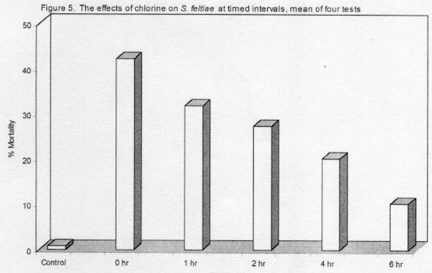


Figure 6. The effects of chlorine on *S. feltiae* with the addition of Silwet L-77 at timed intervals, mean of three tests

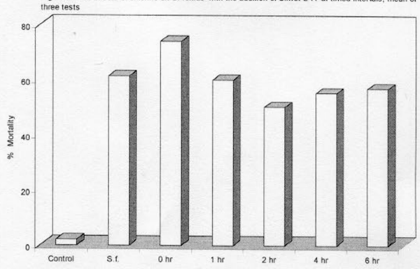


Figure 7. Number of parasitized codling moths by the *Mastrus ridibundus*

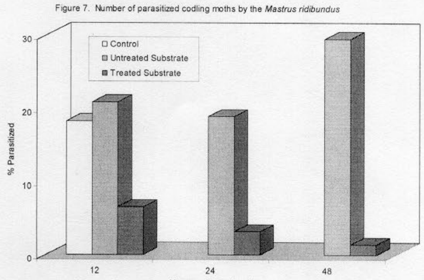
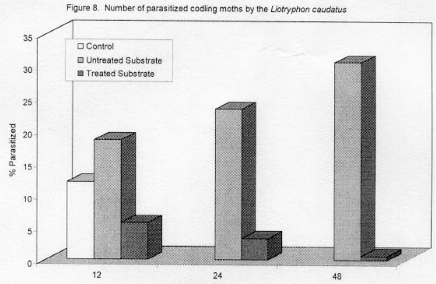


Figure 8. Number of parasitized codling moths by the *Liotryphon caudatus*



CONTINUING PROJECT **YEAR 3/3**
WTFRC Project #: 6408

PROJECT TITLE: Feeding enhancements for insecticides targeting neonate lepidopteran larvae.

PI: Maciej Pszczolkowski,
ORGANIZATION: Dept. of Entomology, Washington State University, Pullman

CO-PIS: John Brown, Yooichi Kainoh, Sandye Bushman, Luis Matos, Kathrine Johnson. All Co-PIs affiliated at Dept. of Entomology, Washington State University, Pullman.

OBJECTIVES:
Lepidopteran apple pests have grown in importance because of potential loss of older and uncertainty about newer chemistries. Increased pest pressure from abandoned or not sprayed orchards make the pests such as codling moth likely to cause serious problems in years to come. Research is needed to address chemical control in such a situation. Historically the problem of excessive fruit damage was resolved with multiple applications of broad-spectrum insecticides until the implementation of the Food Quality Protection Act restricted the amount of Guthion® that could be applied each season. Newer chemicals are more active if ingested by the larval pest and some pest populations are so well understood and monitored that we can estimate when 97% of the generation can be found in the egg stage at one point in time. Our general objective is to reduce the amount of pesticide toxic ingredients, needed for effective control of pest populations, by combination of the pesticide with feeding stimulants.

GOALS AND ACTIVITIES FOR THE NEXT YEAR:
1. **2002 January - March:** Finalize our characterization of Sweet'n Low®'s active ingredients.
2. **2002 January – June:** Acquire pre-formulated particle layer matrixes from commercial sources and test them under our sensitive bioassay system.
3. **2002 January – June:** Continue studying exploratory behavior of neonates on apple leaves
4. **2002 January – December:** Investigate hydrophobic glutamate receptor agonists that could be tank mixed by growers without commercial pre-formulation of additives.

SIGNIFICANT FINDINGS:
1. Showed that codling moth neonates are capable of feeding and development on apple leaves.
2. Established and optimized a rapid bioassay for evaluation of stimulation and spatial characteristics of codling moth feeding on apple leaves.
3. Found monosodium glutamate and Sweet'n Low to be feeding stimulatory in codling moth. Identified saccharine as feeding stimulatory ingredient of Sweet'n Low. Tested persistence of monosodium glutamate and Sweet'n Low in the field. Tested effects of two feeding stimulators (monosodium glutamate and saccharine) on spatial characteristics of feeding in codling moth neonates.
4. Revised our strategy for recording of codling moth exploratory behavior on leaf surface.
5. Tested and analyzed codling moth exploratory behavior on leaf surface.
6. Suitability of four particle layers and five micro- encapsulation substrates for providing rain-fastness of feeding enhancements have been tested. Moreover, subtypes of glutamate receptors involved in feeding stimulation by MSG (Pszczolkowski 2002b, this report) were characterized as baseline data for alternative strategy of obtaining rain- fast feeding stimulator for lepidopterans.

METHODS:
Sweet'n Low®: A further characterization of Sweet'n Low®'s active ingredients is needed because our optimum concentration of saccharine alone 0.1% still exceeds the concentration that the manufacturer of Sweet'n Low® reports to be in the commercial sweetener. Most of Sweet'n Low®'s ingredients were tested alone and only saccharine enhance feeding behavior, but time restricted our testing of saccharine mixed with cream of tartar, or saccharine with dextrose, etc. This could represent an opportunity to reduce the cost of the additive, since saccharine at $68/lb is the most expensive ingredient in Sweet'n Low®, whereas dextrose or corn sugar cost very little. Even if the best formulation resulted from less saccharine and the addition of a small amount of cream of tartar at $40/lb it should prove cost effective.

Particle layer matrixes: Last year 3-M Canada provided us with four additives, Dr. Larry Schrader WSU-Wenatchee provided two, and Pacific Biocontrol sent polystyrene beads. These materials were tested alone for their influence on neonate ingestion and all were found to be detrimental to feeding behavior. Our attempts to re-formulate these additives with either saccharine or MSG were disappointing. Re-formulation efforts using freeze-drying caused the matrixes to form polymeric structures that then needed to be pulverized and re-tested, which did not improve their performance and would be an unacceptable procedure for growers to use. Based on our 2001 research into acceptability of re-formulation and adherence to leaf-surface. We will request commercial suppliers of matrixes that seem to have promise (Xantan 3-M and others as discovered).

Behavioral Research: We will continue our behavioral research using the monitoring system that we have established. Neonates' exploratory behavior on a leaf treated with MSG or Saccharine (with an increased number of replications), with Success®alone, and with Success®/feeding stimulant combination will be analyzed. Effects of hydrophobic MSG analogs (see **Pharmacological Research**) on neonates' behavior, with or without Success® will be examined too.

Pharmacological Research: Pszczolkowski et al. 2001 reported 25 ppm MSG increased codling moth larval feeding on apple leaves by 20 – 30 % and tripled the mortality of 12.5ppm Success® over larval mortality exposed to that concentration of Success® alone. In a more recent manuscript, Pszczolkowski et al. 2002b identified glutamate receptor antagonists (GluR antagonists) that were then used to characterize two distinct phases of feeding, regulated by different sets of glutamate receptors; ionotropic GluR and metabotropic GluR. This research suggests a different approach to resolving the rain fastness problem, rather than depending on MSG itself to stimulate feeding, we propose using non-polar analogs of MSG that target specific subtypes of GluRs. Such analogs, being hydrophobic, would be much more rain-fast than MSG. This approach would be more compatible with the codling moth larval attractant ethyl (2E, 4Z)-2,4-decadienoate recently reported by Knight and Light (2001). It would be more helpful for the grower, and possibly more economic, since prospective rain-fast analog of MSG could be tank- mixed and delivered to the tree with conventional spraying systems.

RESULTS AND DISCUSSION:
Accomplishment #1: For baseline data, untreated apple leaves were individually placed in water, in plastic 1oz cups. Per each leaf, one neonate was released in the middle of the leaf, on the upper surface. After 24 hours the leaves were examined under microscope and spatial characteristics of feeding was evaluated. We monitored the percentage of larvae that started to feed and the side of the leaf where feeding occurred. Additionally, to evaluate topography of feeding, each feeding place was categorized according to its location. To that end, each feeding place was categorized more specifically, depending on its exact location. We found six distinct locations of feeding places; main vein, sub- veins, confluence of main and sub- vein (delta), inter-veinal "open" space of the leaf, and

stem. At present stage of our study, we pooled these results into two broader categories: “veins or stem” and “open space”. Such an assay was used for testing TritonX, Saccharine or MSG solutions.

Accomplishment #2: Saccharine hemicalcium salt has been identified as feeding stimulatory ingredient of Sweet’n Low. We obtained a concentration-dependent response. Saccharine increased feeding even at 0.01% concentration (which is 100x less than SNL), however 0.1% concentration seems to be optimal and higher concentrations do not significantly increase ingestion of leaf tissue. Other components of Sweet’n Low (cream of tartar, dextrose, and calcium silicate) did not stimulate feeding in wide range of concentrations (0.001% - 1%).

In tests on untreated leaves, 59% of codling moth neonates (N=149) started feeding. All feeding neonates began and continued feeding on the lower side of the leaf. Larvae had a distinct tropism towards leaf’s veins in their choice of feeding place; 83% started feeding in close neighborhood of the veins or in stem, whereas 17% preferred the areas between the veins. Triton X (0.02%), MSG (1-1000 ppm) and Saccharine (0.0001% -0.1%), applied on the upper surface of the leaf, changed the dynamics and spatial characteristics of feeding. Exposure to feeding stimulants resulted in a concentration-dependent increase of percent of larvae that started to feed (N=17-37, increase from 57-59% at 0.02%Triton X, up to 92-100% at 1000ppm MSG and 0.1% Saccharine). Also larval (N=82-149) preference of inter- veinal “open” space for feeding increased in the presence of Triton X (by factor of 2.2x), MSG (by factor of 2.7x) and Saccharine (by factor of 3.3x). However, all the larvae still preferred to feed on the lower side of the leaf.

Accomplishment #3: Preliminary, we wanted to build a manually operated tracking system that would be inter- faced with a computer, and recording the behavior by using custom made software. However, during the course of our work, we established a more economic recording system. Finally, we have been using a very high- resolution camera hooked up to an inexpensive digital time-lapse recorder. Next, registered neonates’ behavior may be analyzed by PC, and commercially available software for video editing. Our system uses SVHS videotapes that are the least expensive registering carrier available. Such a solution also allows for more economical use of man- power, since the operator (who in originally designed system would manually track the wandering neonate in real time) may analyze the behavior in many aspects reviewing the tape several times. We are improving our system so it will register neonates’ behavior on both sides of the leaf simultaneously.

Accomplishment #4: Apple leaves of uniform size (5.5-6 cm high, 4 cm wide) were individually placed in water, in plastic 1oz cups. Per each leaf, one neonate was released in the middle of the leaf, on the upper surface. Using our tracking system, we registered and analyzed exploratory behavior of the neonates. Two behavioral parameters were evaluated at the present stage of our study: average time spent on the upper side of the leaf, and average time spent on the peripheral part of the leaf (5mm wide rim around the leaf). Peripheral rim equaled about 32% of total leaf area. Our results indicate that feeding stimulants remarkably extend time spent on the upper side of the leaf (0.01% Saccharine by factor of 2.1x, and 100ppm MSG by factor of 4x). Feeding stimulants also decreased neonates’ tropism towards peripheral parts of the leaf (0.01% Saccharine by factor of 0.43, and 100ppm MSG by factor of 0.8).

Accomplishment #5: We tested suitability of adhesives identified as Matrix A and RayNox (developed by Dr. Larry Schrader from WSUTFR Wenatchee, WA), an acrylic microsphere adhesive (Acrylic) supplied by 3M (Canada), and Placement®, commercially available deposition and retention agent manufactured by Wilbur Ellis, Fresno, CA. All tested chemicals had inhibitory effects on feeding when applied at concentrations of 10% stock solution. At 1% concentrations, Acrylic and Matrix A did not inhibit, and Placement® slightly inhibited feeding (by about 10%). RayNox, at all concentrations ranging from 0.1 to 10 %, significantly inhibited feeding (by 17 to 88%) and therefore has been discarded from further experiments.

MSG and Saccharine at feeding stimulatory concentrations (100 ppm and 0.1% respectively) were mixed into 1% solutions of Acrylic, Matrix A or Placement. Surprisingly, those feeding stimulators, at concentrations that are stimulatory alone, did not stimulate feeding if mixed into these adhesives. In the case of Matrix A, even a slight inhibitory effect was observed.

Five micro-encapsulation substrates were tested; polystyrene beads approx. 0.5mm in diameter (from Tom Larsen, Consep Inc., Bend, OR), starch [formulated ourselves according to McGuire et al. (1990), J. Econ. Entomol 83:2207-10], and three substrates from 3M, Canada. These were; gelatin from calf skin (B275), Sodium alginate from sea plants (ALG) and Xantan gum (Xantan). We loaded all these substrates with a control solution (0.02%Triton X), 0.1% Saccharine, or 100 ppm MSG. First physical properties were evaluated in non-loaded (non-formulated) substrates, and in substrates loaded with control solution (re-formulated). Two parameters were checked; how easy re-formulated (loaded with control or experimental solutions) matrix subjects to pulverization, and how well non- or re-formulated matrix sticks to each side of the leaf. The results indicated that only the starch re-formulated substrate could be pulverized, providing granules suitable for employing as bait stations. The bait stations can also be provided by polystyrene beads (here pulverization is not needed).

However, the substrates not needing pulverization, or those that tolerate re-formulation (polystyrene beads and starch, respectively) did not stick to either the upper or the lower side of the leaf. The ability of sticking to the lower side of the leaf is particularly important here, because some our other experiments indicate that in "open" leaf tests, when larvae are not constrained in test feeding stations, they start feeding only on the lower side. Only Xantan gum exhibited good or very good stickiness to both the upper and the lower leaf surface. However, Xantan does not pulverize after re-formulation.

Thus only non-formulated Xantan was tested in feeding assays. Two types of 24h assays were employed here; the assay with larvae constrained in feeding stations (Pszczolkowski et al. 2001), and choice assay on the leaf. In the latter case, the upper, the lower, or both sides of the leaf were treated with non-formulated Xantan. Larvae were individually placed in mid- area of the upper side, and could freely crawl on both sides of the leaf. In feeding station assays all (N=24) the larvae avoided Xantan- treated surfaces by boring into the leaf and feeding on the opposite side of the leaf section. In assays on the leaf, 94-96% (N=27-30) of the larvae avoided Xantan- treated surfaces by escaping from the leaf or from treated side of the leaf.

To determine an alternative way of providing rain-fastness for our feeding stimulatory formulation, we pharmacologically characterized the glutamate receptors (GluR) involved in codling moth feeding stimulation by MSG. Our study (Pszczolkowski et al. 2002b) showed that the first 3h after hatch are crucial for feeding stimulation by MSG. If larvae are not exposed to MSG within the first 3h after hatch, subsequent exposure to MSG does not result in elevated feeding ratios. Two distinct sets of glutamate receptors play roles in these two phases of feeding stimulation. Ionotropic GluR predominantly regulate food intake in 3-4h period after hatch, and both ionotropic and metabotropic GluR are involved in feeding stimulation after this initial period (Pszczolkowski et al. 2002b). Our recent research indicates that MSG stimulated food intake by freshly hatched neonates is controlled via NMDA subtype of glutamate receptors. Feeding is suppressed by NMDA receptor blocker, and stimulated by NMDA receptor excitant, whereas drugs having affinity to other subtypes of glutamate receptors have little or no effect at this stage of neonates feeding behavior. However, overall leaf consumption within first 24h after hatch is controlled by three subtypes of glutamate receptors (NMDA, kainate, and metabotropic), since all the drugs having affinity to particular subtypes of glutamate receptors exert some stimulatory effect (excitants) or inhibitory effect (blockers) on feeding. Kainate subtype appears to be predominant here, but some role of NMDA subtype and (to less extend) metabotropic subtype is also observed.

Conclusions: Saccharine is the active ingredient of Sweet'n Low, and, as well as MSG may be successfully used to stimulate feeding. Saccharine and MSG increase percentage of larvae that start feeding on leaves, and stimulate larvae feeding in intra- veinal spaces, that would not be preferred normally. Moreover, these chemicals extend the exploration time of the upper side of the leaf and suppress neonates' tendency of peripheral leaf exploration.

The adhesives and micro-encapsulation substrates that we have tested are not suitable for improving of our formulations. Adhesives may mask feeding stimulants making them unable to detect by the neonates. Most of micro-encapsulation substrates tested so far had not suitable physical properties that would make them promising in field research. Xantan, the only substrate that has good stickiness to the leaf, is a repellent to codling moth larvae.

However, the pharmacology of feeding stimulation by MSG suggests alternative strategy for improving rain-fastness of our formulations. Instead of loading micro- encapsulation substrates with hydrophylic MSG, we could use a direct spray with a formulation containing hydrophobic analog of MSG, that would act on specific subtype of glutamate receptors that we have characterized in our feeding assays. We have found nine such candidate substances. One of them, (RS)-willardiin seems to be particularly promising since it is not soluble in water. Such an approach also may make it easier to combine a feeding stimulant with a potent, newly discovered codling moth larvae attractant, ethyl (2E,4Z)-2,4-decadienoate. This compound attracts codling moth neonates (Knight and Light, 2001) if dissolved in hexane. Our own research showed no attractiveness if the same compound was dissolved in more polar water. Thus, loading any micro-encapsulating substrate with combination of water–soluble feeding stimulator and ethyl (2E,4Z)-2,4-decadienoate may be difficult and time- and cost-consuming.

Anticipated benefits are summarized as follows:

1. Successful control of pest populations by enhanced formulation, precision application equipment, and mating disruption, at concentrations of toxic ingredients reduced by 50-90%.
2. Reduced costs and greatly improved the tree fruit's industry's ability to remain under limitations that EPA and FQPA may place on the amount of active ingredient that can be sprayed during the growing season.
3. Mating disruption reduces number of mated codling moth females. Enhanced formulation of insecticide sprays used to target progeny of those moths that do succeed in laying eggs despite mating disruption will not only protect that year crop, but also greatly delay the development of population resistance to the mating disruption.

PUBLICATIONS AND REFERENCES:

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Pszczolkowski, M.A., Matos, L.F., Bushman, S.M., and Brown, J.J. (2001). Feeding enhancements for insecticide targeting neonate lepidopteran larvae. In: Proceedings of 6th International Symposium on Adjuvants for Agrochemicals ISAA 2001. H.de Ruiter (ed.). ISAA 2001 Foundation. Amsterdam, The Netherlands. pp. 420-424.

Pszczolkowski, M.A., Matos, L.F., Brown, R., and Brown, J.J., (2002a). Feeding and development of codling moth larvae on apple leaves. Ann. Entomol. Soc. Am. (submitted)

Pszczolkowski, M.A., Matos, L.F., Bushman, S.M., and Brown, J.J. (2002b). Effects of monosodium glutamate and glutamate receptor antagonists on codling moth larvae fed apple leaves. Entomol. Exp. Appl. (In review)

Pszczolkowski, M.A. and Brown J.J.. (2002c). Prospects of monosodium glutamate use for enhancement of Spinosad toxicity against codling moth neonates. Phytoparasitica. (In revision)

BUDGET:
Feeding enhancements for insecticides targeting neonate lepidopteran larvae.
Maciej Pszczolkowski (Brown)
Current Year: Fiscal Year 2002

Original Budget Request:

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

Item	Year 1 FY 00/01	Year 2 FY01/02	**Year 3 FY 02/03**
Salaries	$6,000	$8,000	**$6,675**
Benefits	$1,000		**$2336**
Wages	$6,000	$4,000	**$1500**
Benefits		$360	**$135**
Goods & Services	$3,000	$300	**$3000**
Travel	$500		
Equipment	$3500	$8200	**$500**
Total	$20,000	$20,860	**$14,146**

*Dr. Pszczolkowski's salary is partially (78%) paid from federal grants IFAFS and RAMP.

This research is partially supported by federal funding IFAFS and RAMP $23,949.

Washington State Commission on Pesticide Registration has committed $14,146 contingent on WSTFC's contribution.

CONTINUING PROJECT **YEAR 3/3**

Title: Development of Feeding Attractants to Control *Lacanobia*

PI: Peter J. Landolt, USDA-ARS, Wapato, WA

Cooperators: Jay Brunner and Mike Doerr, WSU, TFREC, Wenatchee
Everett Mitchell, USDA, ARS, Gainesville, Florida

Objectives:

Objectives for Year 2:

1. Determine bait station efficacy for killing moths attracted to feeding stimulants
2. Develop improvements in bait station design
3. Evaluate the local impact of bait stations on moth reproduction

Objectives for year 3.

1. Demonstrate knockdown of *Lacanobia* moths in orchards using bait stations.
2. Determine a minimum effective density of bait stations for reducing *Lacanobia* moth populations in the field.
3. Demonstrate effects of reductions in female *Lacanobia* populations on subsequent larval populations in apple orchards.

It was expected that this work would require an additional year to conduct field trials, with the above indicated objectives for year 2 permitting the conclusion of development and selection of a bait station design. The overall goal of year 2 was to finalize selection of a bait station design among several being considered. This was done by evaluating mortality of moths attracted to feeding attractants used to bait the stations and by observing close range behavior of attracted moths in order to make design improvements that will improve the percent killed. Bait stations were evaluated for *Lacanobia* fruitworm, using moth feeding attractants and pheromone, in flight tunnel and field cage assays. Feeding attractants were tested for codling moth in a flight tunnel but were thought to be too weak to pursue. Limited field testing was conducted for bait stations for *Lacanobia* fruitworm and for *Pandemis* leafroller moths.

Although preliminary field testing was done with a final version of a bait station, we did not evaluate any effects of baiting on moth reproduction. The purpose of the field tests conducted was to validate laboratory findings in terms of moth contact with bait stations under field conditions. This work was problematic because, as indicated in laboratory tests, moths did not die immediately, making field confirmation of contact and mortality difficult. Thus, after contacting bait stations, moths flew and were not recovered. Contact by moths with bait stations was, however, confirmed.

Significant Findings:

1. A lure and kill station design was proven effective in killing 100% of attracted moths.
    a. Moths are killed following contact and a feeding stimulant is not required.
    b. The bait station is long lasting and cheap to construct.
    c. The bait station incorporates current lure technology into its design.
2. Feeding attractants presently available are relatively strong for both sexes of *Lacanobia*, weak for codling moth and intermediate for *Pandemis*.
3. The bait station does not kill moths immediately, making confirmation of death problematic under field conditions.
4. Moths contacting stations are soon incapacitated, which will prevent any oviposition or mating before death.

Methods to be employed in 2002.

Three field tests will be conducted in 2002, evaluating bait station densities, evaluating bait station lures, and evaluating effects on oviposition and larval numbers in the subsequent generation. The focus in these experiments will be on *Lacanobia* because the feeding attractants available for *Pandemis* and for codling moth are weaker.

In the first experiment, the objectives will be to demonstrate knockdown of moths in orchards using bait stations, and to compare different densities of bait stations in order to determine an optimum density for use in reducing female moth activity. Five acre blocks of apple orchards will each be monitored daily for one week with 2 small blacklight traps and with 2 Universal Moth Traps baited with low-load sex pheromone lures, during late May/early June for determination of *Lacanobia* moth flight activity. After one week of daily monitoring, bait stations will be deployed and the blocks of apple will continue to be monitored with the light traps and pheromone traps for an additional 10 days. This protocol will be followed to evaluate four densities of bait stations (0, 10, 30, and 100 stations per acre). These treatments will be replicated over 5 orchards, for a total of 20 orchard blocks in the experiment. Trap data will be analyzed to determine changes in moth activity in orchard blocks before and after bait station deployment and between treatments (station densities).

In the second experiment, the objective will be to determine if there are any additive effects of baiting with both sex pheromone and feeding attractant lures. Five-acre orchard blocks will be monitored as indicated above, but only with blacklight traps, bait stations will be deployed, and then blocks will be monitored for 10 days following bait station deployment. Bait stations will be baited either with *Lacanobia* sex pheromone in rubber septa or with feeding attractant (acetic acid and 3-methyl-1-butanol) in vials. Treatments will be 1) 30 low load pheromone bait stations per acre, 2) 30 feeding attractant bait stations per acre, 3) 30 low load pheromone and 30 feeding attractant bait stations per acre, and 4) no bait stations. This experiment will follow the results of the first experiment and bait station densities may be changed pending those results. Trap catch data will be analyzed to determine changes in moth activity in orchard blocks before and after bait station deployment and between treatments. It is hypothesized that female *Lacanobia* may be more strongly attracted to feeding attractants with a reduced presence of males.

In the third experiment, results of the preceding two experiments will be used to select an optimum treatment regime that will be more rigorously tested for effects on reproduction. Again, 5-acre blocks of apple will be monitored with blacklight traps for one week prior to bait station deployment. Blocks will be monitored with traps for 10 days following bait station deployment, and will be sampled for *Lacanobia* larvae on apple foliage twice weekly for four weeks following treatment. This experiment will be one treatment in comparison to untreated orchards, replicated over 5 orchard blocks.

With all 3 experiments, monitoring and bait station deployment will have to be scheduled around the full moon of each month, in order to obtain adequate sampling with the blacklight traps. Additionally, an adequate amount of moth activity must occur before bait station deployment, because the objectives are to see how bait stations knock down moth numbers. If moth numbers are too low to provide useful data, orchard blocks will be selected in other areas. It is anticipated that the first and second experiments can be conducted during the first moth flight, which lasts from mid-May through much of June, while the third experiment can be conducted during the second moth flight in August into September.

Results and Discussion

A lure and kill station design was selected as generally superior for all insects tested and all lures tested. It consists of a tubular flower shaped design with perforations that permits airflow through it. The lure (septum or vial) is glued into place inside of this design and odor passes from within the station to the outside and through the staton. The flower design is coated with a pesticide formulation that is contacted as moths attempt to locate the source of the odor. In observational assays, moth kill was virtually 100% among moths that were attracted. That is, all moths observed to track the odor plume and approached the station contacted the station surface and were killed. In flight tunnel tests, moths were recovered and placed in vials for observation at regular time intervals. In all cases, death was not immediate, but took up to several hours, although moths were generally incapacitated in a much shorter time. In a laboratory setting, these stations were effective for several months. However, in full sun in field trials, stations were replaced every two weeks because of wear and drying of the pesticide formulation. There are recommended additives to further extend the field life of the formulation.

It is anticipated that these lure and kill stations for Lacanobia will be relatively inexpensive, if experiments to demonstrate control at these bait station densities are successful. The materials for the bait stations cost less than one dollar retail at this time, which certainly would be reduced under commercial development and large scale production. Expectations of effective station densities come in part from calculations made by the late Dr. Edward Knipling of USDA-ARS for mathematical modeling of mass trapping of female versus male insects. These densities are dramatically less than that recommended, for example, for control of codling moth with lure and kill applications (Last Call) using sex pheromone. Preliminary discussions with EPA personnel have been very encouraging, primarily because these bait stations are discrete, use pesticides already registered for use on apple, and can be deployed with no contact with the fruit crop.

Data obtained to date on the reproductive status of female moths captured in traps baited the feeding attractant is also encouraging. These results indicate a strong feeding response of female moths before they have developed eggs and yet when they are carrying many eggs, setting up an expectation that moths can be killed before they oviposit. Then experiments outlined above will provide the first determination of the effects of killing Lacanobia moths attracted to the feeding attractant and will tell us if we are on a productive course of research.

While it is thought that the Lacanobia lures are more likely to be effective in a bait station strategy to manage moth populations, compared to codling moth and Pandemis leafroller, it is also thought that the technology and strategies we are developing might still be used against these moths with additional chemical attractants and combinations of attractants.

Budget:
Development of Feeding Attractants to Control *Lacanobia*
Peter J. Landolt,
Project Duration: 2000-2002
Current Year: 2002

Year	Year 1 (2000)	Year 2 (2001)	**Year 3 (2002)**
Salary	20,800	22,000	**19,500**[1]
Benefits	6.93		**2,500**
Supplies	4,000	3,000	**4,000**[2]
Travel	500		**1,000**[3]
Total	32,313	25,000	**27,000**

This work complements funding from and efforts made under IFAFS and RAMP grants.

[1] Salaries for 1/3 of a GS-7 biological technician position and 2 summer student assistants.
[2] Lure and kill station materials, light traps
[3] For travel to field sites.

CONTINUING PROJECT **YEAR 2/3**

Project Title: Insect Responses to Induced Defensive Chemistry of Apple and Pear

PI: Peter J. Landolt, USDA-ARS, Wapato, WA

Objectives:

2001 Objectives

1.Determine effects of induced chemistry and leaf age on suitability of apple foliage as food for *Lacanobia* larvae.
2. Determine effects of apple foliage quality on *Lacanobia* larval movement
3. Determine effects of old codling moth infestation of fruit on new codling moth infestation.

2002 Objectives

1. Characterize defensive chemistry of apple leaves.
   - Quantify terpenoids from leaves May and August
   - Quantify protease inhibitors in leaves in May and August
2. Determine effects of apple foliage quality on *Lacanobia* and *Pandemis* larval movement.
3. Determine effects of infested fruit on codling moth oviposition in laboratory assay

Significant Findings:

1. *Lacanobia* larvae fed apple foliage in August developed more slowly than larvae fed apple foliage in June.
2. *Pandemis* larvae fed apple foliage in August developed more slowly than in May. \
3. *Lacanobia* larval weight gain was not related to leaf position (which indicates relative leaf age).

Methods for 2002 research:

Five batches of Fuji apple leaves will be picked from five different apple trees in each of 3 different orchards and will be analyzed for quantities of 2 types of defensive chemicals: terpenoids and protease inhibitors. These analyses will be conducted monthly from May to September. Because of the time involved to process these samples, they will be stored in an ultra freezer until analysed. It is expected that amounts of some or all of these types of chemicals will be negatively correlated with *Lacanobia* larval growth rates and weight gain on apple foliage.

The activity of protease inhibitors in leaf foliage will be assessed you methods described by Thaler et al. (1996, J. Chem. Ecol. 22: 1767-1781). This involves measuring the rate of digestion of benzoyl tyrosine ethyl ester by commercial proteases, using a spectrophotometric method, in the presence of a suitable leaf extract containing the protease inhibitors.

Leaf terpenoids will be quantified using a volatile collection system. Volatile collections will be made off of individual leaves to measure amounts of selected terpenoid compounds that are released as leaf odor. Again, final extracts of volatile collection system traps will be made by GC-MS for characterization and quantification by comparison to hydrocarbon standards. Apple leaf and fruit volatiles have been characterized and we have a lot of experience to date on characterizing the compounds (including terpenes) that are emitted by apple and pear fruit, as well as pear foliage. The equipment is in place both for the sampling of leaf volatiles and for the analyses of volatile samples.

Additional rearings of *Lacanobia* and *Pandemis* larvae will be made using foliage batches from Bartlet pear to determine if the same seasonal pattern to host suitability holds with pear as with apple.

An arena type assay will be used to assess codling moth oviposition in the presence of fruit. Pairs of uninfested and infested apple fruit will be tested in a 4 hour assay to determine how many eggs are deposited in the presence of each fruit. Assays will be replicated 20 times. The experiment will be repeated using infested and un-infested Bartlett pear fruit. Additionally, the previous approach of comparing rates of infested fruit near treated (previously infested) fruit and control fruit will be attempted again under more controlled circumstances, using caged apple trees and released mated female codling moths.

Bioassays will be conducted by Jamie Dedlow (WTFRC funding) during the field season, with assistance from a student (WTFRC funding). Quantitative assays for terpenoids and protease inhibitors will be set up by Connie Smithhisler (YARL chemist on base funding) and will be conducted by Jamie under the supervision of Connie.

Results and Discussion:

Results of experiments in 2001 confirmed that larvae of *Lacanobia subjuncta* develop more slowly on foliage of apple picked in August compared to foliage picked in May. This was suggested in previous preliminary data but needed to be confirmed in controlled and replicated testing. These experiments also provide the first evidence that larvae of *Pandemis* leafrollers develop more slowly on apple foliage in late summer compared to early summer. These findings indicate that larval development rates are not strictly correnlated to degree day accumulations, but are affected strongly by food quality that varies seasonally. Hypotheses to explain the decreased rate of development on apple foliage late in summer are 1) defensive chemicals accumulate with leaf age, 2) trees may put more energy into growth early in the season, and more into defensive chemistry late in the season, 3) defensive chemistry late in the season may be induced, as an accumulation of responses to insect and pathogen challenges over weeks and months, and 4) overall tree vigor may be greater in August than in June in relation to seasonal growing conditions (temperature, insolation).

Results of tests of apple leaves in June of different relative ages (at different positions on branches relative to the growing tip) did not indicate a relationship between leaf position and larval weight gain. Although the leaf ages (in days) were not known in this test, leaves at the terminals are older than leaves more proximal on a branch. The results do not support the hypothesis that new leaves are better as food for *Lacanobia* larvae, compared to older leaves.

Results of tests of codling moth infestation patterns in the field in response to infested apples failed to produce meaningful results because of very low rates of infestation patterns in most field plots. This test involved artificially infesting apples on trees and then comparing percentages of apples infested naturally near these artificially-infested apples in comparison to controls. In all replicates conducted in commercial orchards, rates of infestation were too low to obtain data suitable for statistical analyses. In a replicate of this test conducted at the Moxee farm, nearly all fruit were infested, both near treated and untreated fruits. This experiment was conducted during both codling moth flights, with changes in experimental sites from May to August. For the next season, we will change the methods and try this experiment under more controlled conditions with field caged trees and in laboratory arena assays to look for evidence of oviposition in response to the presence of infested fruit.

Experiments to determine *Lacanobia* larval movement in relation to food quality (do larvae move more or wander and then damage apple fruit when leaf quality is poor) were not conducted this past season because we did not rear enough larvae to support all of the experiments outlined. A significant part of the effort for this work is put into obtaining and maintaining a colony of *Lacanobia* to provide larvae and moths for the experiments.

At this time, the experiments are focusing on determining why larvae develop more slowly on apple foliage late in the season. Answers to that question will lead to other avenues of developing pest management tools and strategies. For example, this work should lead to improved forecasting of moth phenology in the second generation, and the identification of sources of apple resistance to fruitworms and leafrollers could lead to efforts to improve resistance or manipulate resistance to impact pest populations (for example, with chemical inducers).

Budget:
Insect Responses to Induced Defensive Chemistry of Apple and Pear
Peter J. Landolt,

Project Duration: 2001-2003
Current Year: 2002

Year	Year 1 (2000)	**Year 2 (2002)**	Year 3 (2003)
Salaries	18,000	**18,600**	
Benefits		**3,800**	
Supplies	4,000	**2,000**	
Travel	1,000		
Total	23,000	**24,400**	

[1] Salary for ½ time GS-5- term appointment (Dedlow) and a summer student assistant.
[2] Reagent chemicals, solvents, glassware

CONTINUING PROJECT **YEAR 2/3**
WTFRC Project # AE-01-54 **WSU Project # 3366**

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

PI: Vincent P. Jones, Assistant Entomologist
WSU-TFREC, 1100 N. Western Avenue, Wenatchee, WA 98801

Co-PI and Jay F. Brunner, Entomologist
WSU-TFREC, Wenatchee

Collaborator: Tom Unruh, USDA-ARS, Wapato

Objectives:
1. Develop sampling plans for PLR and OBLR that minimize the number of samples per acre, maximize repeatability of the estimate and reduce the time and cost.
2. Improve the phenology models for PLR and OBLR so that the time for sampling parasitism of these two species can be standardized. This will improve accuracy in estimates of parasitism and reduce the number of times samples need to be taken throughout the season.
3. Develop sampling plans for parasitoids of PLR and OBLR to accurately estimate the effect of the natural enemies with minimized sampling.

Significant findings:
- The spatial distribution of leafrollers suggests that at low population levels leafroller hot spots are about 60 feet in diameter.
- Large-scale spatial patterns are obscured when populations are high or extremely low, suggesting that sampling programs should be based on randomly choosing areas within the orchard separated by 300+ feet and sampling areas about 30 feet in diameter.
- The usefulness of sampling for damage in the first generation as an indicator of leafroller population level decreases linearly with increasing degree-days.
- Phenology samples provided data on stage specific timing of the different stages of leafrollers and their parasitism rates.
    - The best time to sample for *Colpoclypeus florus* is during the 5^{th} – 6^{th} instar of *Pandemis* larvae, from 1,100-1,600 DD°C or 1,980-2,880 DD°F.
    - *Apanteles* can be best detected during the 6^{th} instar, from 700-900 DD°C or 1,260-1,620 DD°F.
- Lab studies have begun on the developmental time and the effects of *Bt* on developmental time.

Methods in the coming year:
The methods described in the original proposal for all objectives are still going to be used with the following modifications.

Objective 1. This winter, we will be performing a comprehensive analysis of the data collected this year. We expect to be able to develop sampling protocols, based on our current data, on the spatial distribution that we can test in the second year. During the next season, we will focus on testing these sampling programs and increasing the size of the data set for further optimization of the sampling protocol. During the final season, sampling protocols will be evaluated for time efficiency, repeatability and ability to detect leafrollers at low population densities.

__Objective 2__. Sampling will continue at the PLR sites we found this year, and we will increase the number of sampling locations for OBLR. Laboratory studies on developmental rate should be finished by next spring, and model building will begin next fall, with validation in the third year.

Objective 3. This winter, we will identify our unknown parasitoids and focus our efforts on parasitoids that hit at different times in the life cycle of the leafrollers. Our data from objective 2 should provide time-specific parasitism rates and suggest the best times to increase our sampling effort. We will also work on our rearing techniques to increase the percentage of parasitoids that we are able to recover.

Results and discussion:
Objective 1. We sampled 11 large orchard blocks and collected 1,800 samples consisting of 15-25 sampling units each. Our data sets are about the same size for PLR and OBLR. Sampling units early in the season were infested buds and later were infested terminals. Each sample collected was identified as to the exact location within the orchard so that we could examine the spatial distribution of leafroller damage. When a damaged sampling unit was found it was removed from the tree and brought to the lab, and all leafrollers were collected and placed on diet to determine percentage parasitism. All cups were marked so that we knew exactly from where the parasitoid was collected within a field.

We found that by sampling every tree within a small area the small-scale patterns of leafroller distribution were quite evident and clearly showed that leafroller damage was confined to small patches of about 30 feet in diameter (Fig. 1). When we sampled with a greater distance between samples, the apparent size of the areas increased to approximately 120 m in diameter (Fig. 2).

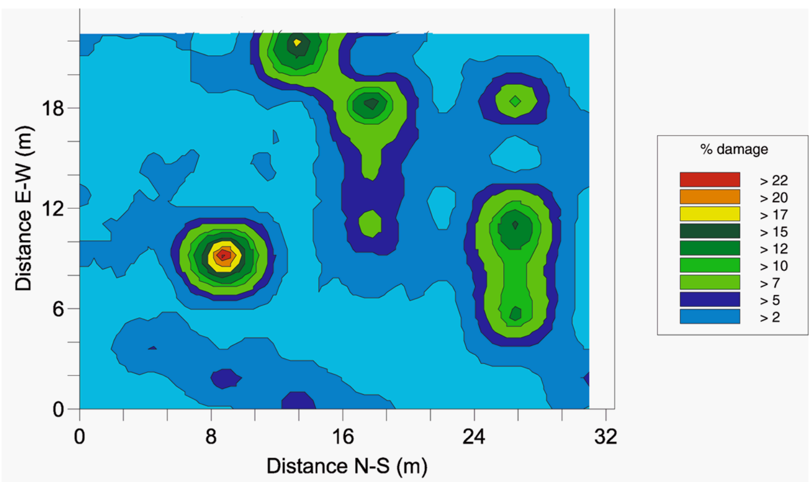
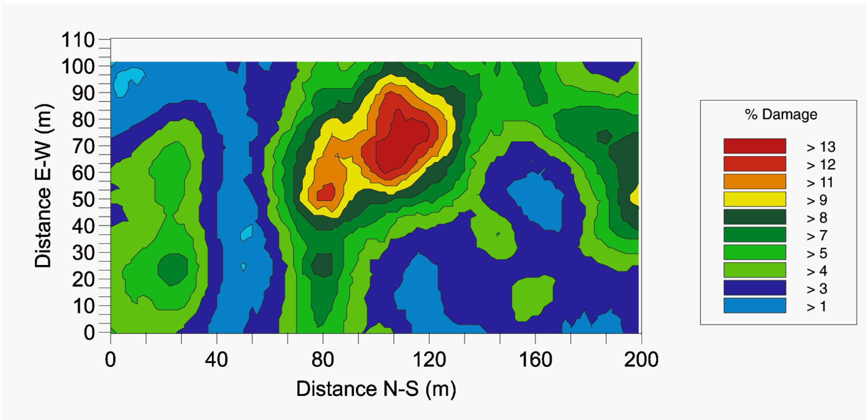


Fig. 1. Small-scale distribution of PLR early in the season.

Fig. 2. Large-scale distribution of PLR with a moderate population level.



This means that fewer samples per acre are necessary for detection of moderate population levels. However, if leafroller populations are large or very low, spatial pattern cannot be discerned. In the case of low population levels, only one small area may have LR present, meaning that it is easy to miss a cluster of leafrollers (Fig. 3). In a situation with large population levels, virtually all the trees are infested, and sampling nearly anywhere gives a good estimate of the population level.

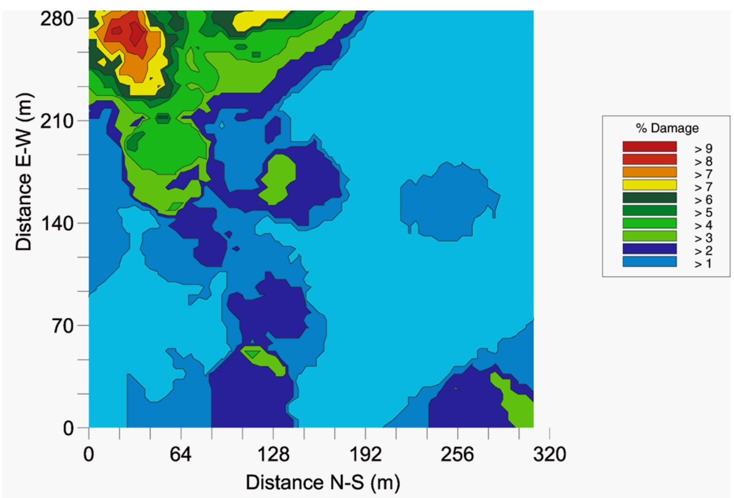


Fig. 3. Large-scale distribution of PLR with a low population level.

More large-scale sampling data need to be collected and analyzed to allow us to design a better sampling pattern for PLR and OBLR. On a preliminary basis, perhaps the best way to sample is to choose locations spaced widely apart (about 300+ feet) but for each location sample several trees within a 30-foot area.

We have not yet begun to analyze the data sets for parasitoid spatial distribution, but collection levels were fairly low at all locations. That analysis will be finished this winter.

Sampling using only damage as an indicator of presence of PLR larvae is efficient only because you can easily spot the damage. In our surveys, we determined the damage in the field, brought all the damaged terminals to the lab and searched for the presence of larvae. By dividing the number of larvae found by the number of terminals or buds damaged by leafrollers, we were able to determine the percentage of terminals infested with larvae when damage was evident. This figure was then regressed versus the degree-days accumulated at that particular location (Fig. 4). When this analysis was performed, it was obvious that, even early in the season, nearly 40% of the buds damaged do not contain leafrollers. By 600 degree-days (near the end of the first generation), only about 15% of damaged terminals contain larvae.

By the second generation, damage was simply not an acceptable indication of LR population levels. The usefulness of damage at predicting LR populations would be reduced further if a pesticide spray were applied that killed large numbers of LRs because the damage would still be present.

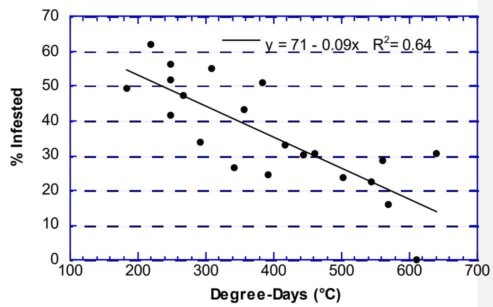
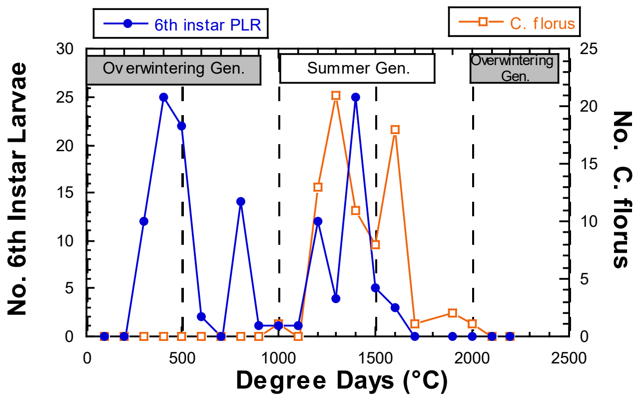


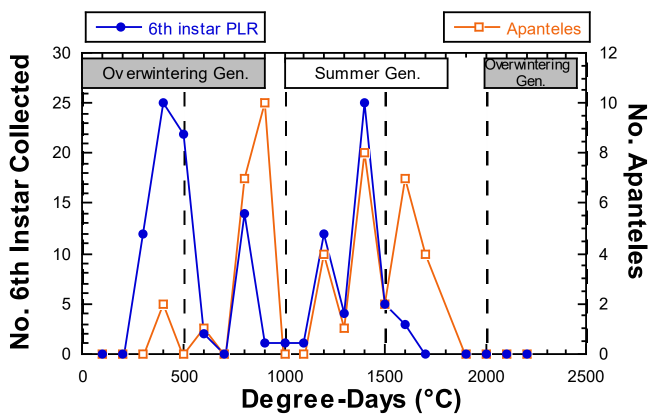
Fig. 4. Percentage of sampling units showing damage that also contain larvae versus degree-days

Objective 2. We set up six sampling locations with PLR and two sites with OBLR. *So far, only the PLR data set has been analyzed.* We collected nearly 3,600 PLR larvae at the different sites. For each larva, we measured its head capsule (to determine the stage of the larva), assessed parasitism and recorded the date and degree-days at collection. Our data show that any particular stage occurred in reasonably narrow windows in time but overlapped considerably.

Over all sites we collected 13 species of parasitoids. Parasitism was 6.3% over all the different sites, with the majority of the parasitoids being *Colpoclypeus florus* and *Apanteles* spp. (44 and 29% of the parasitoids collected, respectively). Overlaying the stage distribution of PLR larvae on the parasite collection data shows that the 5th-6th instars of PLR provide the optimal timing for detection of parasitism. *Apanteles* was recovered from the overwintering and the summer generations of PLR. However, *C. florus* was only recovered in the summer generation. On a degree-day scale this corresponds to 1,100-1,600 DD°C or 1,980-2,880 DD°F for the summer generation (Figs. 5, 6).

Figures 5-6. PLR 6th instar larval collections vs collections of *C. florus* (left) and *Apanteles* sp. (right)





The remaining OBLR data and the final identification of the currently unknown parasitoids will be finished this winter.

Objective 3. The data from objective 2 give us the broad times during which parasitism can be detected. However, before a reasonable model can be developed, more information on parasitoid abundance must be gathered. We also still have sticky traps that need to be counted to determine their usefulness in the field for detection of parasitoid abundance. This will be finished this winter and will be correlated with our larval collection data. If the trap data are well correlated with the parasitoids reared from the field, then we can develop a preliminary model and test it this year. Otherwise, the second year of the project will be used to collect more data, and the third-year effort will be focused on the validation phase.

Budget:
Developing sampling plans for leafrollers and their natural enemies
Vincent P. Jones
Project duration: 2001-2003
Current year: 2002
Original budget request: $49,740

Year	Year 1 (2001)	**Year 2 (2002)**	Year 3 (2003)
Total $126,859	$49,740	$41,734	$35,385

Current Year Breakdown

Item	Year 1 (2001)	**Year 2 (2002)**	Year 3 (2003)
Salaries	$8,750	**$14,560**	$15,142
Benefits[1] (30%)	2,800	**4,368**	4,543
Wages	14,000	**14,251**	10,000
Benefits (16%)	2,240	**2,280**	1,600
Equipment	15,900	**0**	0
Supplies[2]	3,200	**3,475**	2,100
Travel	2,850	**2,800**	2,000
Miscellaneous			
Total	$49,740	**$41,734**	$35,385

[1]Benefits changed from 32% in 2001 to 30% in 2002.
[2]Supplies increased to reflect costs of rearing supplies. Total cost of the proposal per year is the same, the decrease in benefit costs are applied to the supplies category.

CONTINUING PROJECT **YEAR 4/4**

Project Title: Leafroller Biological Control

PI: Tom Unruh, USDA-ARS Yakima

CO-PIs Jay Brunner, WSU-TFREC, Wenatchee
Vince Jones, WSU-TFREC, Wenatchee

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

Significant findings in 2001
A detailed report will be presented as a poster and handout at the Entomology Review in January. The major findings of 2001 efforts are 3:

- Assessment of gardens planted in 2000 demonstrated that important alternative hosts for leaf roller parasitoids can be fostered near orchard boundaries where they did not previously exist.
- The discovery and evaluation of leafroller parasitism near the Ahtanum rose showed that parasitoids can produce virtually complete suppression of leafrollers.
- Survival and exclusion cage studies show that predators and unknown mortality suppress leafrollers by about 80 to 90 percent but parasitoids were the major mortality factor eliminating the survivors after predation.

Methods (for 2002):
1) Evaluate and Expand Gardens. The four experimental gardens planted in 2000 and evaluated in 2001 will be evaluated again with transects in 2002. Simultaneously, 2 or these garden areas will be expanded to include a large planting of roses and another of strawberries. These sites will be infested with *Ancyllis* by mid-summer 2002 and monitored in spring of 2003 for their effect.
This will end our demonstration of the extent which these habitats can be modified. As in 2001, short transects at gardens and control sites will consist of 3 groups of 5 trees at 5, 50 and 250 m distant from target habitats. Transects extending from gardens will also be continued the orchard area to near riparian areas along the Yakima River. Deployed trees will be infested with 3rd instar larvae during spring and summer generations and harvested 2-3 weeks following deployment.
2) Evaluate source of mortality as a function of management system.
Studies will employ potted tree deployments and, if growers are amenable, will use bearing trees, both infested with mid-stage leafrollers. A subset of each exposure will be covered with screening which studies in 2001 showed it to reduce predation but allow parasitism. Ideally 15 paired sites will be infested covering a transect begining in Yakima, into the Basin, Wenatchee, and Brewster growing districts. Leafroller pheromone and food-bait traps will be deployed at sites following generation-specific exposures to document the dominant species at each site and provide an estimate of abundance.
3) Test ways to grow strawberries/roses congruent with orchard practices. To optimize our ability to create and maintain alternate host plants for the strawberry leafroller near orchards, we will complete testing of several wild and multi-floral roses and strawberry varieties for their suitability in three ways:
a) their 'horticultural suitability for these near orchard habitats (both natural arid settings and irrigated), b) as hosts for both leafroller and parasitoids (in the laboratory and in the field), c) measure

the extent to which these plantings are utilized by pest species including stink bugs, lygus, and pest leafrollers. Currently 22 accessions of wild strawberry and two rose clones are being propagated in the greenhouse. These will be studied for objective 3b through the winter. Greenhouse-grown plants will be tested in the laboratory for Ancyllis oviposition preference, larval development, and larval suitability for parasitoid development (*Colpoclypeus* only). Candidate accessions/clones from these tests will be planted in a common garden experiment in spring. Plots will be infested with *Ancyllis* and observations made on abundance in the field. Possible weedy traits of strawberries for in-orchard use will be described qualitatively. This work will be supplemented with pilot studies of ways to cultivate strawberries that will be compatible with conventional weed control, irrigation, and pesticide use practices.

Results and Discussion

1) Measure the effect of rose/strawberry gardens that act as alternative host habitats for *Colpoclypeus florus* on enhancing parasitism in orchards distant from riparian habitats.

This effort was very successful. At 3 planted gardens spring parasitism by *Colpoclypeus* increased dramatically from the complete absence of parasitism observed in 1999 and 2000 to parasitism in excess of 50% in or within 50 m of gardens. Pesticide pressure and unknown factors caused parasitism to drop off very quickly with distance from gardens. Figure 1. Displays an example of pattern of parasitism near gardens in Spring 2001 in Wapato. No parasitism was observed in these areas in the Spring of the previous 2 years.

2) Measure immature mortality schedule of *Pandemis* leafroller, the extent to which mortality is from predation versus parasitism, and how these vary with a) organophosphate use, and b) season (spring and summer leafroller generations).

Two exposures, one for each generation were conducted at a young apple planting in Wapato and one exposure was conducted in the spring generation only at Moxee. The late summer studies at Moxee were destroyed by a tremendous storm. Comparisons among chemical exposure plots were not conducted. Predation and natural attrition of larvae accounted for roughly 90% of mortality observed. In the high parasitism site (Wapato) virtually all remaining larvae were parasitized. At the low parasitism site (Moxee) nearly all remaining larvae survived to pupate. These results suggest that predation and parasitism work in concert and that parasitism is crucially important to prevent pest population growth. Figure 2 shows example of exclusion cage studies at Wapato compared to Moxee in spring. Bars indicating total alive compared to living leafrollers represent a measure of importance of parasitism in the final stages of the immature stage of leafrollers. Almost no leafrollers survive parasitism at Wapato.

3) Describe horticultural qualities of rose and strawberry varieties as plantings adjacent to orchards and their entomological qualities as hosts for *Ancyllis* and key parasitoids, and as possible sources of pest problems.

Little effort was directed at this objective in 2001 because strawberry and rose plantings were not extensive enough for collecting good data. This objective will be readdressed in 2002.

3alt.) Measure the effect of a well established rose patch housing alternate hosts on parasitism in a commercial orchard.

An alternative effort was made to study the effect of a newly discovered rose patch near commercial orchards in the upper Yakima Valley. Using 4 sites within two orchard (2 in apple and 2 in cherry) and a site in the rose patch, season-long parasitism was monitored with sentinel host larvae. This work demonstrated more effectively than all previous ones that a significant source of alternate hosts can dramatically enhance parasitism of leafrollers. Specifically in both apple and cherry orchard, even at sites over 200 m from roses, parasitism of the spring generation was ca. 90%. Summer parasitism was similarly high. These results are summarized in Figure 3.

Budget
Leafroller Biological Control
Tom Unruh
Project Duration: **1999-2002**

Although three years of support were requested in the 2000 Apple Entomology review. I think that this will be the final year of this effort except possibly some minor funding in 2003 to assess large garden manipulations in the spring of 2003. Leafroller biological control studies will continue for several more years but are more likely to address new exotic parasites and biochemical studies of predation.

Current Year: 2002

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

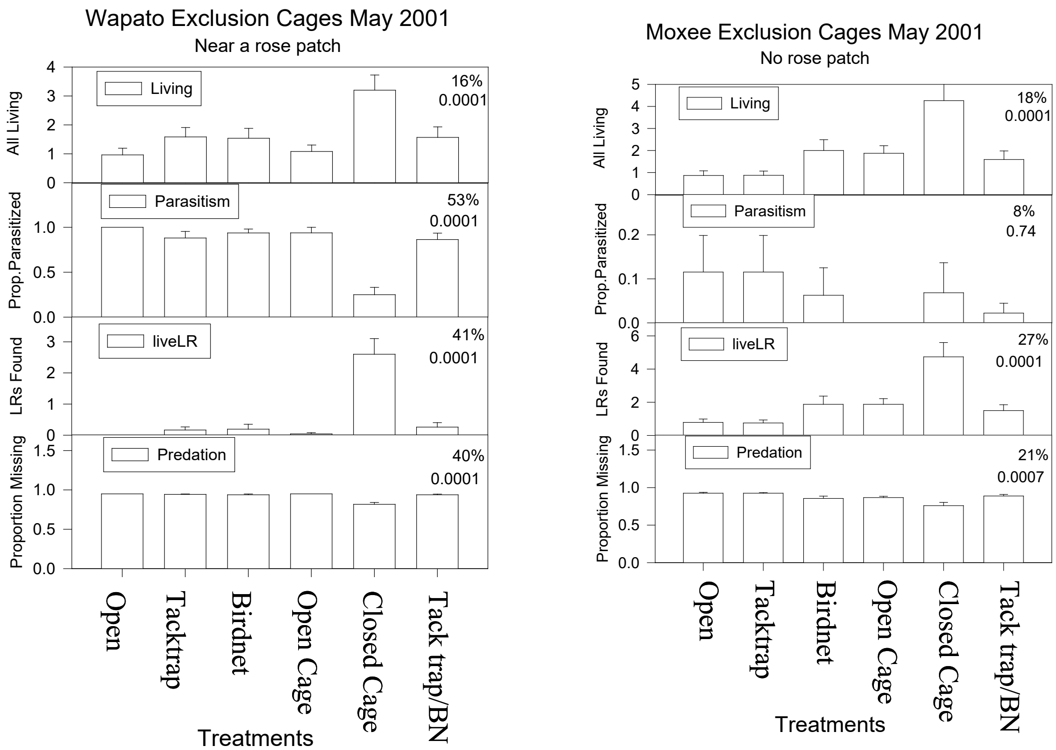
Current Year Breakdown:

Item	Year 4 (2002)
Salaries	55,000
Benefits	5,000
Travel	2,400
Supplies	1,000
Total	63,400
TOTAL (WTFRC)	**35,000**

Figure 1. Pattern of leafroller parasitism near gardens in spring 2001. The red pie slices are measure of parasitism by C. florus near 2 gardens**.** No parasitism by Colpoclypeus was seen in these areas prior to the garden (ie. 1999 and 2000).

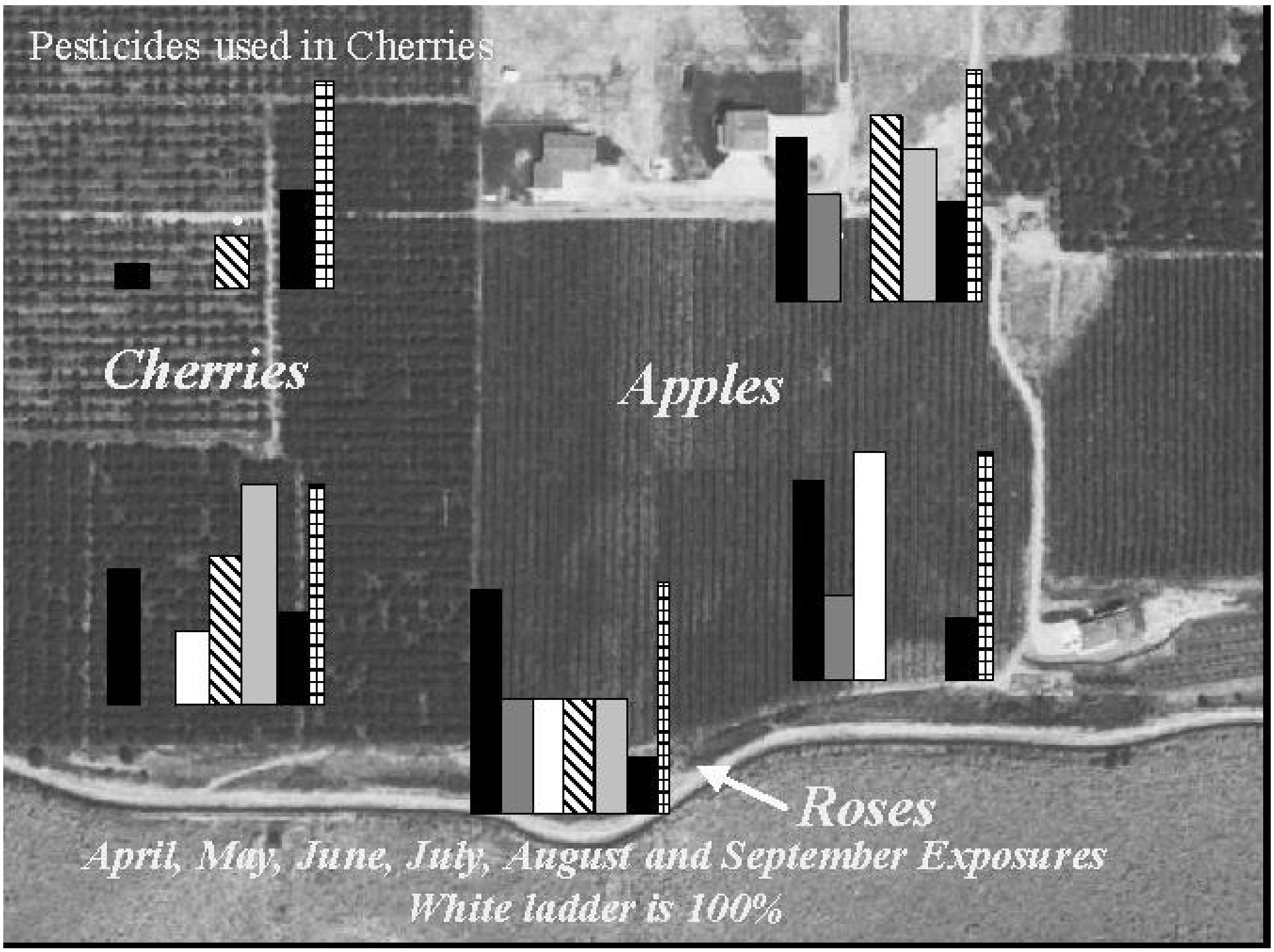
Figure 2. Pattern of spring survival in exclusion cage studies at Wapato and Moxee.

Compare parasitism at Wapato (approaching 100% in all cages except closed-fine mesh cage) which is adjacent to a rose patch with Moxee (10% or less). AT Moxee virtually all parasitism was by flies whereas at Wapato it was by Colpoclypeus



3. Pattern of parasitism at Ahtanum Orchard/Rose Patch

Parasitism approaching 100% was observed in rose patch and around 90% at both apple sites in spring. Parasitism in cherries was reduced by pesticide use for fruit fly control



CONTINUING REPORT **YEAR 3/3**

TITLE: Leafroller resistance to Confirm, Intrepid and Avaunt

PERSONNEL: Michael Smirle, Agriculture & Agri-Food Canada, Summerland, B.C.

OBJECTIVES:

1) To continue to expand the database on baseline susceptibilities in leafrollers to insect growth regulators and other materials, including Success® (Spinosad) and Foray® (Bt).
2) To evaluate insecticide synergists for their effects on the efficacy of Confirm, Intrepid and other materials against resistant leafroller populations.
3) To compare activities of enzymes responsible for insecticide detoxification in leafrollers from resistant and susceptible populations.
4) To compare the metabolism of Confirm and Intrepid in resistant and susceptible leafroller populations using radiolabelled insecticides.

Several of these objectives will continue in 2002. Baseline studies on Success and Foray will be expanded to other orchard sites. As in past years, suggestions from the Commission to include other insecticides of interest will be followed if resources allow. The recent finding that Avaunt resistance is likely due to elevated esterase activity will shift the emphasis of the biochemical studies slightly; work in 2002 will focus on verifying this result.

SIGNIFICANT FINDINGS:

- Considerable variation in response to insecticides, including materials not yet registered and used in Canada, is present in populations of three leafroller species in interior British Columbia.
- Resistance to azinphosmethyl is correlated with resistance to Confirm and Intrepid, indicating cross-resistance and likely similar resistance mechanisms. Although correlated with azinphosmethyl tolerance, levels of tolerance to Intrepid are low and would not be predicted to cause failure in the field.
- Most leafroller populations are susceptible to Avaunt. However, two orchards had leafrollers that were resistant to Avaunt, to the extent that field failure might be possible. Pre-screening for resistance levels is desireable before Avaunt is included in a leafroller pest management program in British Columbia.
- Synergist experiments indicate that elevated activities of esterase enzymes is the mechanism responsible for Avaunt resistance in obliquebanded leafroller (OBLR).
- All populations tested were highly susceptible to Success (spinosad). There was variation in tolerance to Foray (Bt), but not at levels high enough to predict failure in the field.
- The low levels of resistance seen in this study would not likely cause field failure by themselves, but would contribute to a reduced margin for error in other factors such as timing and calibration.

METHODS:

Leafroller sampling. Larval OBLR were collected from orchards each spring; these were from the first generation, not overwintering individuals. A single collection from each orchard was used to establish a colony from which the larvae used in the bioassays were obtained. These individuals were the first-generation offspring of the leafrollers collected from the field. For the single-generation species (European and fruit tree), overwintering eggmasses were collected in winter or early spring (Dec. - Feb.) and kept in cold storage (2°C) until used in bioassays.

Bioassays. Apple leaf disks were dipped in insecticide solutions, then placed in 1 oz. plastic solo cups containing 5 ml agar to provide adequate moisture. Four to six doses of insecticide plus a control (water + 0.25% Agral 90 surfactant) were used for each bioassay. Five newly-emerged larvae were placed in each cup and allowed to feed on the treated disks for 48 hours, 72 hours or seven days, depending on the insecticide being tested. Cups were held in an incubator at 20□C ; the number of larvae treated at each dose ranged from 50-300.

Synergist studies. Leaf disks were dipped in solutions of Avaunt (six concentrations), either alone or in combination with insecticide synergists PBO (mixed-function oxidase inhibitor) or DEF (esterase inhibitor), and were then placed in plastic solo cups as described above. The control treatment was water + 0.25% Agral 90 surfactant + synergist; the concentration of synergist compounds was 60 ppm. Five newly-emerged OBLR leafroller larvae from a previously identified resistant population were placed in each cup and allowed to feed on the treated disks for seven days 20□C ; approximately 100 larvae were treated at each dose.

RESULTS AND DISCUSSION:

Resistance monitoring: Azinphosmethyl, Confim®, Intrepid® and Avaunt®. Resistance was detected to azinphosmethyl and Confirm in 1999 and was present in three leafroller species: single-generation fruit tree and European leafrollers (*Archips argyrospilus* and *Archips rosanus*, respectively) and obliquebanded leafroller (*Choristoneura rosaceana*, OBLR). Intrepid (methoxyfenozide) and Avaunt (indoxacarb), were included in resistance testing in 2000; Success® (spinosad) and Foray® (Bt) were added in 2001.

Twenty-four orchard populations of the three leafroller species were tested in 1999, 2000 and 2001. 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 European leafrollers that were 41-fold more resistant than a population from an organic site. For OBLR, the highest resistance ratio (most resistant/most susceptible) was 17.6-fold.

Twelve orchards were tested for possible leafroller resistance to Intrepid®. LC 50 values ranged from 0.030 ppm to 1.79 ppm. The resistance ratio (highest/lowest) is almost 60-fold, although even the most “resistant” population was much more susceptible to Intrepid than they were to Confirm, making field failure unlikely. 15 orchards were also tested for resistance to Avaunt. Some orchards were, by conservative estimate, 300-fold higher than the most susceptible sites. The introduction of Avaunt into the Canadian market must therefore proceed cautiously, with pre-screening of resistance levels desirable in some locations.

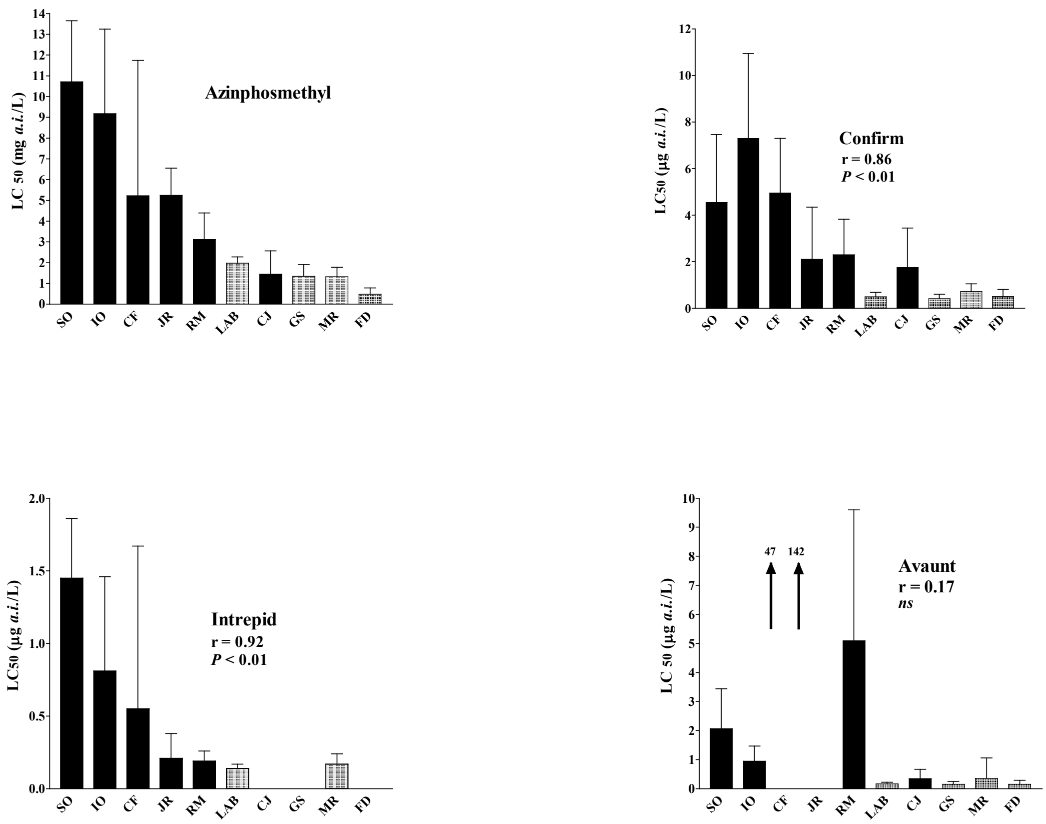


Figure 1. Response of larval obliquebanded leafrollers to four insecticides. Correlation coefficients (r) refer to response compared with azinphosmethyl; significant correlations indicate cross-resistance. Black bars represent responses of leafrollers from orchards under conventional management practices; grey bars represent responses of leafrollers from organic orchards or the laboratory colony. Arrows in the Avaunt graph indicate LC 50 values outside of the graph scale: 47 and 142 for populations CF and JR.

Resistance levels in OBLR populations are summarized in Fig. 1. The order of orchards on the horizontal axis is the same in each graph to enable comparisons to be made more easily. Orchards with leafroller populations relatively resistant to azinphosmethyl also show higher levels of resistance to Confirm and Intrepid; LC 50 values are significantly correlated. This shows that, in orchards with a history of leafroller resistance to azinphosmethyl, Confirm and Intrepid are not the best choices as alternative control chemicals. For Avaunt, two populations have high levels of resistance but there is no correlation between this resistance and resistance to azinphosmethyl. Avaunt works well in orchards with susceptible leafroller populations, but efficacy cannot be predicted in advance on the basis of tolerance to azinphosmethyl and baseline tolerance data are highly desirable.

Similar LC 50 values to those for OBLR were found for European and fruit tree leafrollers. Correlations of azinphosmethyl resistance with Confirm and Intrepid were significant, indicating cross-resistance. Fewer populations of the single-generation leafrollers were tested than had been originally planned because of poor emergence from eggmasses collected in the spring of 2001. Data for these assays are not shown because they were similar to the results for OBLR.

Success® (spinosad) and Foray® (Bt): 13 leafroller populations were tested for their response to Success in 2001; 11 populations were tested for response to Foray. Results for OBLR are presented in Figure 2. Although there is variation in the toxicity of Success across populations, all of the leafrollers tested were highly susceptible to this chemical, even leafrollers taken from the orchard showing the highest tolerance to azinphosmethyl and Avaunt. Success would therefore appear to be a good candidate for use in an insecticide rotation program to manage resistance in leafrollers. For Foray, there was more variation among leafroller populations than was evident for Success, but no population showed enough tolerance to suggest that lack of control in the field may result. There have not been any reports of field failure of Bt compounds when applied to control leafrollers in British Columbia.

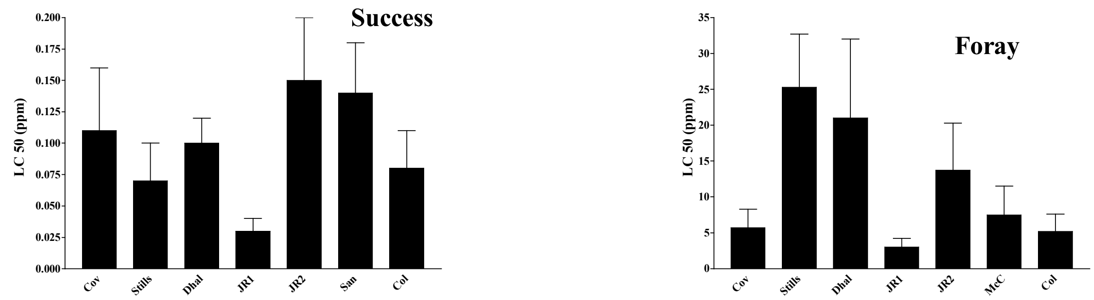


Figure 2. Toxicity of Success® (spinosad) and Foray® (Bt) to obliquebanded leafroller larvae collected from apple orchards.

Synergist studies: Results from synergist experiments with Confirm were reported last year. In summary, PBO (mixed-function oxidase inhibitor) synergized Confirm in one orchard population; DEF (esterase inhibitor) had no effect on the toxicity of Confirm. These results are from one population only and must be repeated.

For Avaunt, DEF strongly synergized toxicity in a resistant population (Fig.3); PBO had no significant effect. This indicates that esterase enzymes are responsible for Avaunt resistance in this particular orchard. These studies will be repeated in 2002, including the evaluation of esterase activity in the resistant population and comparative experiments using a susceptible colony.

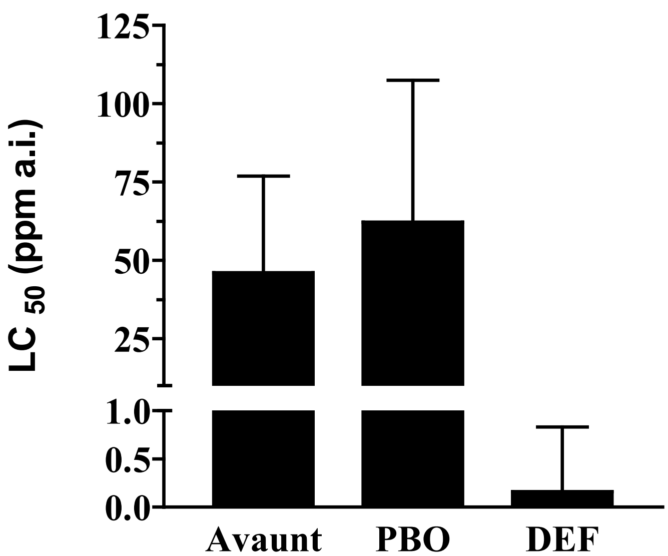


Figure 3. Effect of synergists on the toxicity of Avaunt insecticide to obliquebanded leafrollers.

Enzyme activities and metabolism studies. Studies on the metabolic capacities of leafroller populations are continuing. The obliquebanded leafrollers resistant to Avaunt are of particular interest and will be assayed in detail for esterase activities. The work on radiolabelled (i.e. C-14) Confirm and Intrepid will begin in 2002.

Conclusions. Leafrollers show resistance to several recently-introduced insecticides. Most levels of resistance are low and would not be expected to result in control problems in the field. Resistance to Avaunt may be high enough in some populations to reduce field control, and pre-screening may be necessary before Avaunt is used in some locations. Synergist experiments indicate that resistance to Avaunt is associated with elevated activity of esterase enzymes. No significant resistance has been detected to Success or Foray in the populations tested.

BUDGET:
Leafroller resistance to Confirm, Intrepid and Avaunt
Michael Smirle
Project duration: 2000-2002
Current year: 2002

	2000	2001	**2002**
Salaries	27,200	27,200	**28,500**
- technician, full-time, 12 months (D.T. Lowery, L. Walmbold)			
Benefits [1]	(5440)	(5440)	(5700)
- 20% of salary. All benefit payments are taken from the Agriculture and Agri-Food Canada Matching Investment Initiative (AAFC-MII) portion of the funding.			
Materials/Supplies [2]	4000	4000	**4500**
Travel [3]	1000	1000	**1000**
Total	32,200	32,200	**34,000**
Requested from WTFRC (50%)	16,100	16,100	**17,000**

50% funding from AAFC-MII guaranteed through 2002.

[1] Not included in funding request to WTFRC
[2] Includes all materials used to maintain leafroller colonies in the laboratory: diet ingredients, plastic rearing cups, cages for mating and oviposition. Also includes laboratory chemicals for conducting esterase assays, solvents for HPLC and materials for analyzing radioactive samples.
[3] Travel to orchard sites to collect obliquebanded leafroller populations in spring and summer, and single-generation species in winter. Some sites are distant and require overnight stay.

CONTINUING REPORT **YEAR 2/3**

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

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

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

JUSTIFICATION:

Fear of insecticide resistance, evidence for the disruptive nature of organophophate insecticides, implementation of mating disruption, and the Food Quality Protection Act have acted together to change developing IPM strategies for tree fruit production. Replacement of organophosphates requires effective and selective synthetic pesticides under most management scenarios. Despite claims and preliminary work by chemical manufacturers, the selectivity of many new and upcoming insecticides is poorly known. This is particularly true of sub-lethal effects of many insecticides and behavior modifying technologies on natural enemies of pests.

This proposal is part of a developing coordinated working group to collect complete information on the selectivity of key new pesticides on a couple of model natural enemies and several that are specific to tree fruit production. This coordinated working group represented one of 12 objectives funded in part by the IFAFS/RAMP proposal. Preliminary meetings among participants (listed above) have determined which insect and mite predators will be tested and te objectives in their selection as follows. Species were selected either because they represent good model organisms for general effects of insecticides on natural enemies and can be purchased from commercial insectaries. (Several of these are also important in either pear or apple orchards in the Western Region as indicated by bold.) These include *Orius insidiosus* and ***Forficula auricularia*** (Hilton), ***Chrysoperla carnea*** and ***Mastrus ridibundus*** (Mills)*,* ***Deraeocoris brevis*** (Reidl), ***Galenodromus occidentallis****,and* ***Pnigalio flavipes*** *(*Beers*),* ***Colpoclypeus florus*** (Unruh) and ***Campylomma verbasci*** (Horton). Both ecological and practical considerations went into choosing these species. We have discussed the practical but the ecological is important too. These species represent a range of biologies that are likely to acquire insecticides in different ways because of how they interact with their host, the plant surface, and their seasonality. The following table may make this more clear.

Mode of host attack	**Parasitism** (internal v external) All external	**Predation** Chewing mouthparts **v** sucking Galendromus and Forficula **v** Oius, Campylomma and Deraecoris
Body size	minute **v** large Pnigalio and Colpoclypeus **v** Mastrus	minute **v** large Galendromus **v** Forficula and Chrysoperla
Method of search for suitable hosts/prey	walking on plant Mastrus Pnigalio to mostly by flight Colpoclypeus?	walking Virtually all predators will spend most of life history walking in search of prey
proportion of life history exposed to insecticides	adult stage only higher for parasitoids which walk extensively to locate hosts	all but egg stage
Most likely mode of exposure	Adult acquisition by walking on plant, poisoned honeydew and consuming previously poisoned hosts	Adult and nymph exposure while walking and eating poisoned hosts

OBJECTIVES

Test acute toxicity of 5 insecticides to 9 arthropods using a combined topical, residue and per-os exposure method

Develop bioassay methods to measure sub-lethal effects on beneficial insects

Test sub-lethal effects in those cases where acute effects are trivial or short-lived

Methods:

1) Toxicity is often tested separately for the 3 major modes of poisoning: i) contact (direct spray of formulated material or direct application of technical material),
ii) residue (contact with residue for and extended time), and iii) per-os (by mouth added to food). To reduce the effort required to understand the gross effects of these insecticides we will make all three modes of acquisition simultaneously available. In essence the bioassays will proceed this way. Insects will be anaesthetized by chilling and placed in a petri dish bottom on a piece of damp filter paper. Insects will be sprayed using a Potter's Spray tower (one instrument is at each of 5 locations in the consortium). Insect will be sprayed with 2 ml of formulated insecticide at field rate, or 10% of field rate. They will then be allowed ½ hr in closed petri dish to recover. Survivors will be placed in a vented petri dish containing leaves or other detached substrat which had been sprayed within the previous 4 hr and residue allowed to dry. To satisfy the per-os rout of acquisition detached leaves will receive honey droplets prior to being treated with insecticides. In general, insects will be tested in groups of 5-10 individuals with multiple replicates. Mortality will be assessed at daily for the first 25% of the reproductive life of the predator or parasitoid. Three to five replicates of 10 individuals/dose will be conducted with water controls for each replicate. Correction for control mortality using Abbott's formula will be used and results will be compared by material and dose within material by ANOVA with time of mortality assessment representing a repeated measure.

2) Sublethal bioassays will be developed on the principle that exposures may not have visible acute effects but may influence other life functions that cumulatively represent significant reductions in fitness or reproductive rate. Here bioassays must be developed in a species specific manner. Generally, exposures as described in objective 1 will be used and then specific measures of one or more life functions will be enumerated. These may include mortality schedule, fecundity, propensity to mate, hatching rates, etc. Reproductive rates will be estimated from these indirect measurements. A possible example of a sublethal bioassay may included exposing 5 pair of newly formed adults to chronic exposure at a dose known kill less than 25%. Adults will be placed in petri dishes or other cages with hosts on which they would normally utilize for feeding and reproduction. If possible this will entail exposing the insects to recently poisoned hosts. The reproductive potential of the treated beneficial will be evaluated for the first 25% of its reproductive life for species that will lay multiple eggs over multiple days. For some species, especially the parasitoids, where there are only one to three bouts of oviposition which are associated with new hosts and cyclical development of mature eggs, at least 2 bouts of host attacks will be assessed. Development of these species specific sublethal assays is a major focus of this objective..

3) Methods developed under objective 2 will be used under controlled and replicated designs to test sublethal effects on the 9 natural enemies for 1-2 insecticides in yr 1.

Proposed schedule of accomplishments:

Objectives 1 and 2 should be completed in year 1 for the pesticides listed. Objective 3may begin only in year 2 for some test species and start immediately for others. Subsequent years will address new insecticides under objectives 1 and 3. Although first submitted in 2000, this project has changed. Work proposed in 2001 was not completed although colonies of all insects have been established at all locations. The major reason for delay was the delay in receiving funding from IFAFS. USDA collaborators have not yet received these funds and University collaborators outside of Washington only received the funds at the end of November. Hence the project is being submitted as new. Insecticides to be tested in year 1 of testing are listed by priority below:

Order of				Upper label	High rate	High rate
Priority	Compound/Form	Chem. Name	Class	rate/acre	form/liter	mg AI/liter
1	Provado 1.6F	imidacloprid	Chloronicotinyl	20 fl oz	1.562 ml	300
2	Actara	thiamethoxam	Chloronicotinyl	5.5 oz	412 mg	103
3	Intrepid 2F	methoxyfenozide	IGR: Molt accelerating compound (MAC)	16 fl oz	1.25 ml	300
4	Esteem 0.86EC	pyriproxifen	IGR: JH analog	16 fl oz	1.25 ml	129
5	Success 2SC	spinosad	macrocyclic lactone fermentation product	10 fl oz	0.781 ml	187

Literature review:

One of the primary goals of integrated pest management is the intelligent and rational use of pesticides. In selecting new generation pesticides for occasional treatment of codling moth and secondary pests, it is essential to be aware of the impact of these products on both target and non-target species, particularly those non-target beneficial species that provide effective natural biological control of potential secondary pests in the orchard ecosystem. Traditionally, the impact of pesticides on both insect pests and natural enemies has been assayed by estimation of acute toxicity, through measurement of the effect of topical application on individual mortality (LC50) (Croft 1990; Jepson 1993). Subsequently, testing protocols for the compatibility of pesticides with natural enemies have focused on residual toxicity, the acute effects of field-weathered deposits of pesticides on individual mortality (Jepson 1993). However, one of the most important limitations in ecotoxicology is the extent to which simple laboratory assays of survivorship can be used to predict the effect of pesticides on field populations (Stark et al. 1995). Under natural conditions populations are influenced by a number of life history traits, including survivorship, reproduction, and maturation time, as well as by density dependence acting through changing levels of food supply, enemies and interspecific competitors (Calow et al. 1997; Sibly 1999). In a number of cases, the results from acute toxicity assays have not proved to be predictive of effects detectable either in more realistic laboratory experiments or in the field. While acute toxicity tests may have been effective assays of the impact of the broad spectrum neurotoxic insecticides, the novel modes of action of the new generation of pesticides will require the development of more effective bioassays that are predictive of the impacts of exposure to natural enemies in the field.

The new generation products are more likely to have chronic rather than acute impacts on natural enemies, and may require dietary uptake rather than surface exposure to be toxic (Jepson 1993). Physiological selectivity ratios, based on topical acute toxicity data for both pests and natural enemies, have frequently been used to identify insecticides that are less disruptive to natural enemies (Stark et al. 1995). However, ecological selectivity resulting from differences in biology and behavior of the natural enemies, differential routes of insecticide exposure, and the distribution, timing and mode of action of sprays will be more important in estimating effects of insecticide use on natural enemy populations in the field (Croft 1990).

Due to the difficulty of relating acute toxicity assays to population level effects, life table response experiments have been developed to directly evaluate the population effects of chemical toxicants (Sibly 1996). In these experiments the age specific growth, reproduction and survivorship of low density populations are monitored at regular intervals to estimate the instantaneous per capita population growth rate, a key parameter that links individual performance to population effects (Calow et al. 1997; Sibly 1999). This approach also takes into account the phenomenon of population compensation, a heightened rate of population growth in the presence of abundant resources that become available to individuals that survive acute exposure to a pesticide. However, the disadvantage of life table response experiments, in comparison to acute toxicity tests, is the time, labor and expense associated with continuous monitoring of the performance of a cohort of individuals. This has severely limited their application to studies of insecticide exposure to insects (but see Stark & Wennergren 1995; Walthall & Stark 1997).

The instantaneous per capita rate of increase provides a short-cut method to estimate population growth rate, requiring the population to be monitored only at the start and end of the experiment (Walthall & Stark 1997; Sibly 1999). The correlation between the instantaneous per capita rate of increase and the intrinsic rate of natural increase derived from a life table response experiment is good provided the time interval between observations is sufficiently long (Walthall & Stark 1997; Sibly 1999). In addition, the instantaneous per capita rate of increase appears to show a simple linear negative relationship to insecticide dosage, suggesting that simple regression analysis can be used to analyze dose-response relationships (Walthall & Stark 1997). This approach has been used to predict the effect of new generation insecticides (imidacloprid, neem and dicofol) on pests such as aphids and spider mites (Walthall & Stark 1997; Banken & Stark 1998; Stark et al. 1997). Whether it can be used as effectively in predicting ecotoxicological impacts on natural enemies that have a longer generation time than aphids and spider mites remains to be tested.

Working groups to develop pesticide toxity data against beneficial species has a long history in Europe under the International Organization of Biological Control, Western Palearctic Region Beneficial Insect and pesticides working group (Hassan et.al 1992). Many bioassay methods presented by this group have been directly followed or only slightly modified by work conducted with beneficial insects in tree fruits and this information on acute toxicity has been incorporated into regional or state spray guides. Obviously there are interactions between both direct and sublethal toxicity to natural enemies and the pests for which insecticides are applied. Hence bioassays will tend to test effect of a pesticide on predators and parasites of host to which the insecticide is NOT targeting. Hence, abamectin, used to control psylla and mites, may be tested against leafroller or leafminer parasitoids, but is unlikely to be tested extensively against psylla predators. Also obvious, is that sublethal effects will be sought mostly in new chemistries and especially in materials with hormone-like modes of action. Previous work in Washington has addressed both acute and sublethal effects but with a limited number of species and an emphasis on acute effects (Brunner et al. 2001)

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Budget:
Effects of New Insecticides on Natural enemies: Acute toxicity and sublethal effects
Tom Unruh,
Current year request 2002: $50,000
Project Duration: 3 years
Budget:

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

[1] $10,000 to USDA – ARS, Wapato (Unruh and Horton); $10,000 to WSU (Beers); $20,000 OSU (Hilton, Medford and Reidl, Hood River); $10,000 to UC, Berkeley (Mills)

CONTINUING PROJECT **YEAR 2/2**
WTFRC Project # ARS **Organization Project #** 5352-22000-013-22T

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

PI: Eugene Miliczky

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

Co-PI: David Horton, USDA-ARS, Wapato, WA
Tom Unruh, USDA-ARS, Wapato, WA

Objectives: 2001: 1) Sample a broad range of plant species in extra-orchard habitats adjacent to apple and pear orchards in Washington and Oregon, and identify plants that may be important to natural enemies of apple and pear pests as alternative feeding, mating, oviposition, or overwintering sites. 2) Identify insects occurring on plant species in extra-orchard habitats that may provide alternative prey or hosts for natural enemies of orchard pests. 3) Correlate the seasonal occurrence of important natural enemies in extra-orchard habitats with their occurrence within the orchard. 4) Determine if natural enemy densities within an orchard decrease as distance from extra-orchard habitat increases.

2002: 1) Based on 2001 results, conduct more intensive sampling (weekly or bi-weekly) of extra-orchard host plants that appear to have strong associations with natural enemies of orchard pests to more clearly define the relationship. Some plants will be sampled throughout the season (ex. sagebrush, bitterbrush, oak). Other plants will be sampled during a more limited window of time such as before, during, and after flowering (ex. rabbitbrush, tall buckwheat, deerbrush). Sampling to begin in late March/early April and continue through October. 2) At eight to ten sites in south-central Washington survey native and introduced plant species (especially herbaceous forms) for leafrollers. Survey sites will include riparian and other types of extra-orchard habitat. Some sites will be the same as those used in objective 1. Leafrollers will be collected and reared for species identification and to obtain parasitoids. Sites will be visited monthly (more frequently if necessary). 3) Sampling during 2001 showed that several native plant species (willow, golden currant, curly dock, bitterbrush, scotch broom) were host to psyllids. At the same sites used for objectives 1 and 2 psyllids will be collected and reared to obtain parasitoids. We hope to determine if known parasitoids of pear psylla attack any of the native psyllids. 4) Minute pirate bugs (Orius) and flower thrips (a principal prey item) were abundant on several extra-orchard plant species in 2001. Predator and prey seemed to be particularly abundant during flowering. At sites used for objectives 1, 2, and 3 sample for Orius and thrips to better define phenologies of these insects. 5) 2001 samples from several extra-orchard plant species contained mirid bugs similar to Campylomma, many of which were immature and therefore difficult to identify. The bugs were sometimes abundant. Samples of these insects will be obtained from appropriate host plants and reared to the adult stage for identification to determine if any are Campylomma.

The original (2001) proposal gave monitoring movement of natural enemies between orchard and extra-orchard habitat as a possible objective for year 2. This has not been included in the 2002 proposal. However, additional objectives have been identified based on 2001 results. Natural enemy movement may be the subject of a future proposal.

Significant findings: All within-orchard and extra-orchard collections have been processed and insect and spider specimens identified. Data analysis will be conducted this winter but several preliminary statements can be made at this time.

- Some extra-orchard plant species (sagebrush, bitterbrush, oak, pine) host beneficial arthropods during much of the season including lacewings, ladybeetles, predatory bugs, and various spiders. Even late in the season, when conditions are dry, sagebrush and bitterbrush often support fair numbers of beneficial arthropods including species found in adjacent orchards.

- Some extra-orchard plant species support large numbers of beneficial arthropods (Orius and certain spiders for example) for a limited time during the season, particularly around the time of flowering. Examples include balsamroot, tall buckwheat, and rabbitbrush.

- Some beneficial arthropods occur in orchards **and** on certain plant species in extra-orchard habitat. These include insects such as Deraeocoris, Orius, and lacewings and the lynx spider Oxyopes and the jumping spider Sassacus.

- Some beneficial arthropods are common within the orchard but are rare or absent in certain extra-orchard habitats. Pelegrina aeneola, a jumping spider common in some organic orchards, is rarely found in sagebrush steppe. It does occur on trees and shrubs in wooded areas. The related Pelegrina helenae and P. clemata are common in sagebrush steppe but were rarely found in adjacent orchards. Among insects, we found assassin bugs and ambush bugs regularly on sagebrush and associated plants but rarely in adjacent orchards.

- Some natural enemy species appear to exploit a succession of host plants during the season, presumably in search of suitable prey. Minute pirate bugs (Orius), for example, occurred on several species during the season including yarrow, tall buckwheat, rabbitbrush, and sagebrush. Orius were abundant during flowering when large numbers of thrips, a principal prey item, were also abundant.

- Natural enemy densities in some orchards decreased as distance from extra-orchard habitat increased. This is consistent with the idea that extra-orchard habitat may serve as a source of colonists for the orchard.

- Numerous records were obtained documenting the occurrence of orchard pests (stinkbugs, Lygus, thrips, possibly grape mealybug) on extra-orchard host plants.

Methods: 2001: Orchards and extra-orchard habitats were sampled monthly from May to October. Orchards were divided into two or three sections at greater distances from extra-orchard habitat. Smaller orchards were divided into 0' to 200' and 200' to 400' sections. Larger orchards included a section beyond 400'. Twenty-six beat tray samples were taken per section each month. All beneficial or potentially beneficial insects and spiders were collected, exclusive of predatory mites. Pest species were noted on a presence/absence basis. Purpose of within orchard samples was to look for variation in natural enemy density with increasing distance from extra-orchard habitat and for comparison with the fauna on extra-orchard host plants.

Fifty-five species of native and introduced plants were sampled (9 trees, 18 shrubs, 28 forbs). Most sampling was with a beat tray. Ten to 20 trays were taken per species depending on abundance, lushness of foliage, state of bloom, and number of arthropods present. Plants were sampled a variable number of times. All beneficial arthropods and a representative sample of potential prey and host species were collected.

Three-inch wide, cardboard bands were placed around trees in ten orchards during September and October to study overwintering natural enemies. Bands will be collected in December or January. Bands were placed at different distances from extra-orchard habitat to look for differences in the overwintering fauna with increasing distance from the extra-orchard habitat. Cardboard bands were placed in or around several species of trees and shrubs in extra-orchard habitat at six orchards and will be examined for overwintering insects and spiders in December or January.

2002: Objective 1 – Beat tray samples will be taken at weekly or bi-weekly intervals throughout the season or during some portion of it, depending on the plant species and quantity/quality of arthropod material present. Ten to 20 beat trays will be taken per species and all beneficial arthropods and a sample of prey/host species will be collected and identified. Some specimens may be reared to obtain parasitoids, etc. Study sites will be those used in 2001 with possible addition of new locations. Objective 2 – Potential leafroller host plants will be sampled with a beat tray and/or visual search once a month or more frequently if appropriate. Live specimens will be collected for rearing in the laboratory on native plant material or other appropriate substrate. Adult specimens, parasitoids, and host plants will be identified. Objective 3 – Methods will be similar to those used in objective 2. Objective 4 – Plant species found to harbor high numbers of minute pirate bugs and flower thrips during 2001 will be sampled (beat tray) before, during, and after bloom. Additional plant species may be sampled if appropriate. Four to six sites will be sampled. Objective 5 – Suspected Campylomma nymphs will be collected from plant species identified in 2001 and reared to the adult stage for identification.

Results and discussion: Eight apple and ten pear orchards were studied. All were under reduced OP or soft insecticide programs – mating disruption or certified organic. Two orchards are located near Hood River, Oregon, three are near Peshastin, Washington, one is near Wallula, Washington, one is near Mattawa, Washington, one is in Kittitas County, Washington, and the remaining ten are in Yakima County, Washington. Each orchard has one edge adjacent to a sizable tract of uncultivated, relatively undisturbed land (extra-orchard habitat) dominated by native plant species. Four orchards border wooded areas containing mixed hardwoods, conifers, shrubs, and herbaceous plants, two border riparian areas with mixed vegetation along rivers, and the other 12 are adjacent to sagebrush steppe. The remaining three sides of each orchard border other agricultural land, usually other orchard.

Arthropod samples were processed as they were collected and at this time most specimens have been identified at least to family level. Some 250 insects (beneficial and non-beneficial species) and 330 spiders have been or are being reared for species identification or to obtain parasitoids.

Raw collection data indicate that total beneficial arthropod numbers may vary inversely with distance from extra-orchard habitat in some orchards, as was true for a small, organic pear block (Table 1, Orchard 1) bordering sagebrush steppe near Zillah, WA. Such a trend was evident in all six collections although it was most notable late in the season (September and October). Other orchards did not show a similar trend, as was the case for an organic apple block near Wallula, WA that also bordered sagebrush steppe (Table 1 Orchard 2). Numbers of total beneficial insects, total spiders, and the sum of insects plus spiders were similar at all three distances in this orchard on all six collection dates.

Individual species or groups of species may show a distance related trend whereas others may not. This appeared to be the case for a mating disruption pear orchard near Granger, WA that bordered riparian habitat along the Yakima River (Table 2). Total spiders and spiders in the genus Tetragnatha showed decreasing abundance with distance from extra-orchard habitat in this orchard, especially comparing the 0-200' and the 200-400' sections. Total beneficial insects did not show such a trend. Tetragnatha spiders are web spinners and are probably general predators of flying insects. They are common in habitats near water and it seems possible that these spiders were moving out of extra-orchard habitat along the river and into the orchard. Tetragnatha are uncommon in orchards adjacent to sagebrush steppe as this and previous years' collections indicated.

Worth noting among the three orchards discussed above are differences in the abundance of the main groups of beneficial arthropods. Spiders as a group comprised 74% of the beneficial arthropods in the Wallula orchard and 78% in the Granger orchard whereas beneficial insects as a group comprised 82% of the beneficial arthropods in the Zillah orchard, with Deraeocoris the most abundant taxon.

Data from the collections on plant species in extra-orchard habitats will be analyzed in greater detail this winter but several general statements regarding work in 2001 are given under significant findings (above).

The rationale for research on extra-orchard habitats was that they might serve as sources of beneficial arthropods important to orchard biocontrol and that these organisms could move into an orchard to augment populations already present or repopulate the orchard following loss due to insecticide treatment or other man-made or natural disturbance. It was also thought that extra-orchard habitat and host plants might provide alternate resources for some natural enemy species during portions of their life cycles – resources such as prey, hosts, mating and egg laying sites, and places to overwinter. Preliminary examination of the 2001 data indicates that extra-orchard habitat may be important for some of these reasons. The finding, in some orchards, that natural enemy densities decreased as distance from extra-orchard habitat increased supports the idea that these organisms may move into an orchard from outside. Movement of this kind may occur in some species and not others, but when it occurs proximity of extra-orchard habitat likely enhances speed of colonization or repopulation of an orchard. Some extra-orchard plant species were found to support a variety of natural enemy species including species that contribute to orchard biocontrol. This was true even in sagebrush steppe, a habitat markedly different in many ways from adjacent orchards. Several species of spiders and minute pirate bugs, Orius spp., were common on a variety of plant species typical of the sagebrush steppe and are also known to contribute to orchard biocontrol. Orius were utilizing prey, thrips, found on several plant species in sagebrush habitats.

BUDGET
Identification of extra-orchard host plants and habitats for key natural enemies of pome fruit pests suitable for manipulation or conservation
Eugene Miliczky
Project duration: 2001-2002
Current year: 2002
Original budget request:

Year	Year 1 (2001)	**Year 2 (2002)**
Total	$26,620	**$28,510**

Current year breakdown:

Item	Year 1 (2001)	**Year 2 (2002)**
Salaries[1]	$20,264	**$21,791**
Benefits[2]	$ 6,356	**$ 6,719**
Supplies[3]	$ 0	**$ 500**
Travel[4]	$ 0	**$ 500**
Totals	$26,620	**$29,510**

[1]Post-Doc, Eugene Miliczky, 0.40 FTE (12 mos.)
[2]Benefits, Eugene Miliczky, 0.40 FTE (12 mos.)
[3]Supplies – vials and alcohol for specimen storage, cardboard, etc. Funds for supplies were not requested in the original proposal.
[4]Travel to and from experimental sites. Funds for travel were not requested in the original proposal.
Other sources of funding: Partial (approximately 60%) support for the salary of Dr. Miliczky has been obtained with an IFAFS (Initiative for Future Agriculture and Food Systems) grant to Jay Brunner et al. Funds are requested to meet the remaining costs for a postdoctoral position for Dr. Miliczky.

Table 1. Monthly collections of beneficial arthropods at different distances from extra-orchard habitat in an organic pear orchard (orchard 1) and an organic apple orchard (orchard 2) during 2001. Total number of beneficial insects, total number of spiders, and total number of insects plus spiders, respectively, are given for each collection (26 beat trays at each distance each month).

Orchard 1

	Distance from extra-orchard habitat	
	0 – 200'	200' – 400'
May	3 – 2 – 5	1 – 1 – 2
June	8 – 6 – 14	3 – 3 – 6
July	3 – 4 – 7	2 – 2 – 4
August	10 – 3 – 13	4 – 6 – 10
September	99 – 13 – 112	60 – 7 – 67
October	51 – 10 – 61	35 – 5 – 40
Totals	174 – 38 – 212	105 – 24 – 129

Orchard 2

	Distance from extra-orchard habitat		
	0 – 200'	200' – 400'	> 400'
May	25 – 23 – 48	9 – 19 – 28	13 – 23 – 36
June	12 – 52 – 64	9 – 56 – 62	4 – 48 – 52
July	9 – 55 – 64	4 – 55 – 59	7 – 46 – 53
August	26 – 47 – 73	15 – 40 – 55	18 – 59 – 77
September	16 – 45 – 61	34 – 34 – 68	40 – 51 – 91
October	5 – 19 – 24	8 – 31 – 39	9 – 33 – 42
Totals	93 – 241 – 334	76 – 235 – 311	91 – 260 – 351

Table 2. Monthly collections of beneficial arthropods at different distances from extra-orchard habitat in a mating disruption pear orchard near Granger, WA during 2001. Total number of beneficial insects, total number of spiders (total number of Tetragnatha spiders), and total number of insects plus spiders, respectively, are given for each collection (26 beat trays at each distance each month).

	Distance from extra-orchard habitat		
	0 – 200'	200' – 400'	> 400'
May	8 – 20(1) – 28	8 – 12(0) – 20	2 – 8(0) – 10
June	3 – 15(1) – 18	1 – 4(0) – 5	2 – 5(0) – 7
July	9 – 43(6) – 52	10 – 24(0) – 34	1 – 7(0) – 8
August	2 – 39(4) – 41	7 – 25(0) – 32	11 – 24(0) – 35
September	18 – 51(12) – 69	21 – 31(3) – 52	11 – 27(2) – 38
October	11 – 75(16) – 86	6 – 45(6) – 51	10 – 40(3) – 50
Totals	51 – 243(40) – 294	53 – 141(9) – 194	37 – 111(5) – 148

CONTINUING PROJECT **YEAR 3/3**
WTFRC Project # AE-01-55 **WSU Project # 3326**

Project title: RAYNOX for suppression of insects in apple and pear

PI: Larry Schrader, Horticulturist
Organization: WSU Tree Fruit Research and Extension Center, Wenatchee, WA
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Co-PIs: Jay F. Brunner, Elizabeth H. Beers and John Dunley
Affiliation: WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Objectives:
1. Establish the optimal timing, rates and application frequency for RAYNOX against selected pests of apple and pear.
2. Determine whether RAYNOX has negative effects on natural enemies.
3. Investigate the mechanism(s) by which RAYNOX deters certain pests.
4. Study efficacy of RAYNOX as a stabilizer or protectant for insecticides.

Significant findings:

From 2000:
- RAYNOX significantly suppressed first and second generation codling moth (CM) in apple but did not provide adequate control to stand alone for controlling CM.
- In a rate and timing trial on second generation CM, all rates of RAYNOX provided about 50% control of CM. All RAYNOX treatments were as good as Confirm 2F + Orchex 796 oil.
- In a direct choice bioassay, apples were dipped in RAYNOX and then neonate CM larvae were added. Only 10.6% of CM neonates were found on the apples treated with RAYNOX. Entries by CM into fruit were significantly lower with RAYNOX as compared to the control.
- With leaf disk bioassays, choice tests showed that RAYNOX deterred colonization of Pandemis leafroller (PLR) and lacanobia fruitworm (Ls) larvae. For PLR, only 22% chose RAYNOX-treated disks. For Ls, 17% chose RAYNOX-treated disks.
- RAYNOX was effective against white apple leafhopper (first and second generation). RAYNOX was significantly better than untreated controls for first generation. Results for second generation were less definitive. RAYNOX and Sevin were not statistically different.
- A single application of RAYNOX in early August provided a commercially acceptable level of mite suppression.
- With pears, RAYNOX shows promise for control of psylla and CM.

From 2001:
- RAYNOX was compared at three rates in field trials to Orchex 796 and Assail 70WP for control of CM in apple. RAYNOX at all rates [5%, 10% and 20% (v/v)] significantly reduced first generation CM injury versus untreated control (60% injury in control). However, Orchex and Assail were more effective than RAYNOX.
- At harvest, only the high rate (20%) of RAYNOX had less fruit injury than the untreated control (92% injury in control) and was similar to the Orchex treatment. The Assail treatment had the least CM injury (data not shown). This confirms our earlier conclusion that RAYNOX will not serve as a stand-alone treatment for CM but may be used to augment IPM programs.

- Laboratory bioassays with leafrollers were used to test efficacy of RAYNOX as a spreader/sticker as well as a UV light inhibitor. The efficacy of Deliver (*Bacillus thuringiensis*) mixed with RAYNOX was not significantly different from Deliver alone with PLR or obliquebanded leafroller (OBLR) (see Table 1). This indicates that the Bt product was still accessed by the pest.
- Leaf disk bioassays were used to evaluate RAYNOX as a spreader/sticker as well as a UV light inhibitor with a leafroller granulosis virus (Virosoft OB). Virosoft OB was evaluated alone and in combination with RAYNOX for residue effects on PLR and OBLR. Virosoft OB shows promise as an insecticide for both leafroller larvae. The efficacy of Virosoft OB embedded in RAYNOX was similar to Virosoft OB alone (see Table 2), indicating that the virus product was still accessed by the pest. Both PLR and OBLR bioassays were conducted in late summer so the full potential of RAYNOX as a pesticide protectant was probably not realized and will be further evaluated in 2002.
- Western tentiform leafminers were suppressed about 50% in the bottom half of trees when RAYNOX, Surround and Intrepid were applied to trees before exposing trees to leafminer adults. RAYNOX suppressed the mines per leaf more than 80% in the top half of the trees and was as effective as the other products.
- Neither RAYNOX nor Surround was effective in significantly reducing seasonal nymph populations of *Campylomma verbasci* or in reducing fruit damage by Campylomma. Early season applications of Actara and Carzol were most effective.
- With pears, three prebloom applications of RAYNOX were usually as effective as full-season applications. RAYNOX and Surround were equally effective in suppressing pear psylla (see Table 3). RAYNOX should be used only prebloom to avoid russeting problems.

Methods:
Objective 1: Field trials with several cultivars of apples will be conducted with the following target insects: codling moth, leafrollers, leafhoppers, spider mites, aphids, leafminers, fruit worms and cherry fruit fly. Two years of data collection are needed to develop a basis for making recommendations on pest control for inclusion in EB-0419, the Crop Protection Guide for Tree Fruits in Washington. Initial tests will be conducted with high pressure handgun spray equipment using a spray volume equivalent to a dilute spray application. The results obtained will allow determination of an activity profile for RAYNOX on the target insects. Treatments will be replicated with single trees or small blocks of trees for initial trials. Larger trials will be used for studies of those insects for which RAYNOX looked promising in 2000 or 2001 trials. Up to three rates of RAYNOX will be evaluated for each target insect to determine a rate effect. Time and frequency of applications will be studied. For entomological evaluations of pests on foliage, populations of insects such as mites, aphids, leafhoppers, pear psylla and leafminers will be evaluated pre-treatment and at intervals in the post-treatment period to determine efficacy. For pests such as the codling moth that injure fruit, the level of injury to fruit will be evaluated at appropriate intervals during the growing season in each treatment by checking at least 25 fruits per tree or replicate.

Objective 2: The effects on natural enemies will be evaluated in all trials by sampling for their presence. The effects of RAYNOX on certain natural enemies, predatory mites, parasites of leafminer and leafroller, and general predators such as lady beetles and lacewings will be evaluated with bioassays. Various natural enemies will be exposed to direct sprays of RAYNOX. Their mortality or tendency to leave treated areas will be examined after a period of time determined from preliminary tests.

Objective 3: The mechanism of action of RAYNOX appears to be different in some respects from an alternative particle film technology. Surround (kaolin) masks an insect's ability to detect its host plant, irritates or repels pests, or actually causes direct mortality of pests. While RAYNOX may mask host plant cues used by some pests, it may also have direct toxic effects on some life stages. The direct and indirect effects of RAYNOX on selected pests will be evaluated in laboratory bioassays against leafroller, lacanobia fruitworm, codling moth, leafhopper and leafminer. Various stages will be exposed directly to RAYNOX at different concentrations to determine dose-mortality relationships. The behavioral effects of RAYNOX residues will be evaluated by treating plant or fruit surfaces to determine suppression of oviposition activities.

Objective 4: Various insecticides (e.g., IGRs and Bt) will be incorporated with varying concentrations of RAYNOX and applied to leaves or fruit to determine if the product is stabilized by RAYNOX and whether the product is still accessed by the pest. Alternatively, the insecticide can be applied first and then followed by an application of RAYNOX to determine if RAYNOX protects the insecticides from rain, irrigation water and UV-B radiation. Field-aged studies of RAYNOX-protected insecticide residues will be compared with unprotected residues to determine the utility of this product as a surfactant. Standard bioassays developed to test insecticides for leafrollers and lacanobia fruitworm will be used in these tests. Experiments with Virosoft OB mixed with RAYNOX need repeating because, even after 14 days of exposure in the field, mortality of larvae placed on the leaves was over 60%. RAYNOX and Virosoft OB also need to be tested earlier in the season when there are young leaves, and the length of the experiment needs to be increased to see if RAYNOX can stabilize the Virosoft OB.

Results and discussion:

I. Bioassays with RAYNOX and Deliver on mortality of Pandemis leafroller (PLR) (*Pandemis pyrusana* Kearfott) and obliquebanded leafroller (OBLR) (*Choristoneura rosaceana* Harris)—Brunner and Doerr—2001

Using a leaf-disk bioassay, *Bacillus thuringiensis* var. Kurstaki (Deliver, Certis USA Crop Protection) and a novel particle film technology (RAYNOX, Washington State University) were evaluated alone and in combination for residue effects on PLR and OBLR neonate larvae. RAYNOX was added to the Deliver treatment to test its efficacy as a spreader/sticker as well as a UV light inhibitor. The test was conducted on 15-yr-old spur-type Delicious on dwarfing roots. The treatments were applied on 6 Sep with a handgun sprayer at 300 psi to the point of drip, simulating a dilute spray of approximately 400 gal per acre. Ten leaves were collected from the interior canopy of each tree at 1, 4, 7, and 14 days post-treatment (DAT). Two disks (2.3 cm diameter) were taken from each leaf. Four disks were placed in a petri dish (Falcon 1006, 50x9 mm), keeping the leaves from each replication separate. Five 1- to 2-day-old leafroller larvae were placed on the leaves. Five petri dishes were prepared for each tree and each leafroller species (75 larvae per treatment). Petri dishes were examined after 7 days and larval survival recorded.

Significant mortality was noted in the Deliver treatment against both PLR and OBLR neonate larvae at 1 DAT (Table 1). The level of mortality caused by the Deliver treatment in this bioassay was moderate relative to that seen in field-aged bioassays of other Bt products. Low mortality levels from the Deliver treatment were noted at 4 DAT and 7 DAT against both species. The 4 DAT evaluation against OBLR and the 7 DAT evaluation against PLR were not significantly different than the untreated control. No significant mortality was noted against either species at 14 DAT. The RAYNOX-only treatment resulted in a significant but low level of mortality against PLR neonate larvae and no significant mortality against OBLR neonate larvae at 1 DAT. No significant mortality was noted against either species at 4 DAT or 7 DAT. The RAYNOX-only treatment was not evaluated at 14 DAT. The addition of RAYNOX to Deliver did not significantly increase efficacy or residual activity of the Bt product. There was no apparent difference in susceptibility of either leafroller species to Deliver or RAYNOX.

Table 1. Effect of RAYNOX and Deliver on mortality of leafrollers.

Treatment	Rate/ 100 gal	Avg corr. % mortality- PLR 1 DAT[1]	4 DAT	7 DAT	14 DAT
Deliver	113.5 g	60.9bc	16.7b	13.0ab	4.1a
Deliver + RAYNOX	113.5 g 10% v:v	82.6c	22.2b	24.6b	28.0a
RAYNOX	10% v:v	36.2b	1.4a	0.0a	---

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1] DAT = Days After Treatment.

Treatment	Rate/ 100 gal	Avg corr. % mortality- OBLR 1 DAT[1]	4 DAT	7 DAT	14 DAT
Deliver	113.5 g	57.5b	8.8a	16.7b	4.5a
Deliver + RAYNOX	113.5 g 10% v:v	38.4b	20.6a	22.2b	4.5a
RAYNOX	10% v:v	10.9a	4.4a	1.4a	---

Means in the same column followed by the same letter not significantly different (p=0.05, Student's Paired
t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1] DAT = Days After Treatment.

II. Bioassays with Virosoft OB (leafroller granulosis virus) and RAYNOX on Pandemis leafroller (PLR); *Pandemis pyrusana* Kearfott and obliquebanded leafroller (OBLR); *Choristoneura rosaceana* (Harris)—Brunner and Doerr—2001

Using a leaf-disk bioassay, a leafroller granulosis virus (Virosoft OB, BioTepp, Inc., Charlesbourg, Qc, Canada) and a novel particle film technology (RAYNOX, Washington State University) were evaluated alone and in combination for residual effects on PLR and OBLR neonate larvae. RAYNOX was added to the Virosoft OB treatment to test its efficacy as a spreader/sticker as well as a UV light inhibitor. The methods were as described above for the Bt tests. Petri dishes were examined up to 14 days and larval survival recorded.

Very little neonate larval mortality was noted after 7 days exposure to the virus treatments, and thus only the 14-day exposure data are presented (Table 2). Significant mortality was noted with both PLR and OBLR neonate larvae at each evaluation date with the exception of the 7 DAT bioassay against OBLR. However, significant OBLR neonate larval mortality was again noted at 14 DAT. It appeared that the relative efficacy of Virosoft OB declined at the 7 DAT evaluation but did not continue to decline through the 14 DAT evaluation. The RAYNOX-only treatment resulted in a significant but low level of mortality against PLR neonate larvae and no significant mortality against OBLR neonate larvae at 1 DAT. The RAYNOX-only treatment was not evaluated at 14 DAT. The addition of RAYNOX to Virosoft OB did not significantly increase efficacy or residual activity of the Bt product, but RAYNOX did not affect access of the pest to Virosoft OB. There was no apparent difference in susceptibility of either leafroller species to Virosoft OB or RAYNOX. These bioassays indicate that the Virosoft OB formulation is a promising insecticide for the management of both PLR and OBLR larvae. It appeared that at least 10 days were needed before larval mortality could be detected. Further residual tests should continue until significant mortality was no longer noted in the Virosoft OB treatment. This test was conducted relatively late in the growing season and thus the growth of new foliage was minimal and UV exposure was less than what would be observed when the product would be used for pest management.

Table 2. Effect of RAYNOX and Virosoft on mortality of leafrollers.

Treatment	Rate/ 100 gal	Avg corr. % mortality- PLR (14 d) 1 DAT[1]	4 DAT	7 DAT	14 DAT
Virosoft OB	23.7 fl oz	78.6c	86.6b	50.0b	75.9b
Virosoft OB + RAYNOX	23.7 fl oz 10% v:v	92.9c	93.3b	63.0b	62.5b
RAYNOX	10% v:v	36.2b	1.4a	0.0a	---

Means within the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1] DAT = Days After Treatment.

Treatment	Rate/ 100 gal	Avg corrected % mortality—OBLR (14d) 1 DAT[1]	4 DAT	7 DAT	14 DAT
Virosoft OB	23.7 fl oz	70.8b	90.5b	17.4ab	65.0b
Virosoft OB + RAYNOX	23.7 fl oz 10% v:v	81.3b	88.1b	50.0b	55.0b
RAYNOX	10% v:v	10.9a	4.4a	1.4a	---

Means within the same column followed by the same letter not significantly different (p=0.05, Student's Paired t-test).
Any mean followed by the letter 'a' not significantly different than the untreated control.
[1] DAT = Days After Treatment.

III. CONTROL OF PEAR PSYLLA WITH PARTICLE FILM MATERIALS AND CONVENTIONAL COMPOUNDS, 2001—Dunley, Greenfield and Bennett

These tests were performed to compare different timings and combinations of particle film technologies [Surround (Kaolin), RAYNOX] and conventional treatments to give seasonal control of pear psylla on pear. Treatments were applied to deliver 200 gpa on prebloom applications and 100 gpa on postbloom applications. All insect counts were made on the middle tree of the middle row of each plot or, in the case of the RAYNOX treatments, on each of the treated trees. The conventional prebloom treatments were followed either by conventional postbloom sprays or applications of either RAYNOX or Surround. Conventional postbloom treatments were preceded by conventional prebloom treatments or prebloom applications of either RAYNOX or Surround. Pear psylla adult counts were made by using a beating tray and counting the number of adults on four trays. Pear psylla egg and nymph counts up through 19 Apr were made by collecting five spurs per plot and examining them under magnification and counting the number of eggs and nymphs. Beginning 28 Apr, 25 leaves were collected from each plot and brushed with a standard mite-brushing machine onto a glass plate. Plates were examined under magnification and the number of eggs and nymphs counted and recorded.

In general, the particle film technologies were successful in controlling pear psylla nymphs (the damaging stage) when compared to a conventional pear psylla management program. Prebloom applications of Surround or RAYNOX followed by a conventional spray program appear to provide adequate control of pear psylla eggs (data not shown) and nymphs (Table 3). The half rate of RAYNOX followed by a conventional program provided similar control to the other prebloom PFT treatments. The seasonal Surround program also maintained pear psylla at less than damaging levels, as did the conventional treatment. The postbloom treatments, with conventional prebloom treatments did not perform as well as those treatments that had PFT prebloom. It appears that the prebloom timing of PFT applications is important in reducing pear psylla for the postbloom period.

Table 3. Mean pear psylla nymphs in samples collected from particle film technology treatments.

	Nymphs/spur		Nymphs/leaf						
	20-Apr	27-Apr	7-May	16-May	30-May	11-Jun	22-Jun	12-Jul	27-Jul
1. Check	3.22a	13.40ab	0.88ab	1.40a	0.72a	0.38a	1.12a	7.08a	2.34a
2. Surround + Conventional	0.15b	0.80b	0.16b	0.14c	0.12b	0.10a	0.12b	2.20b	1.50a
3. Conventional + Surround	0.50b	3.55ab	0.58ab	0.94ab	0.28b	0.16a	0.14b	1.88b	1.22a
4. Surround	0.05b	3.35ab	0.08b	0.06c	0.02b	0.10a	0.10b	1.38b	1.06a
5. Conventional	0.45b	18.60a	1.24a	0.34bc	0.32b	0.18a	0.32b	2.56b	1.68a
6. RAYNOX + Conventional	0.10b	0.95b	0.00b	0.12c	0.16b	0.28a	0.24b	2.68b	1.70a
7. Conventional + RAYNOX	0.25b	16.80a	1.02ab	0.68bc	0.18b	0.30a	0.36b	1.90b	1.92a
8 RAYNOX	0.00b	1.10b	0.12b	0.02c	0.06b	0.14a	0.46b	2.68b	1.82a
9. RAYNOX rate + Conventional	0.15b	9.20ab	0.16b	0.28bc	0.00b	0.02a	0.34b	3.32b	1.80a

Means within the same column followed by the same letter are not significantly different (Student-Newman-Keuls *P*=0.05).

Budget:
RAYNOX for suppression of insects in apple and pear
Larry Schrader,
Project duration: 2000-2002

Year	Year 1 (2000)	Year 2 (2001)	**Year 3 (2002)**
Total	20,000	20,000	**20,000**

Current year breakdown

Item	Year 1 (2000)	Year 2 (2001)	**Year 3 (2002)**
Salaries	0		
Benefits (%)	0		
Wages	13,800	13,800	**13,800**
Benefits (16 %)[1]	2,200	2,200	**2,200**
Equipment	0	0	**0**
Supplies	3,000	3,000	**3,000**
Travel[2]	1,000	1,000	**1,000**
Miscellaneous	0	0	**0**
Total	20,000	20,000	**20,000**

[1]Fringe benefits are 16% for time-slip employees; [2]Travel related to data collection for project.
Other support: We anticipate $23,500 for 2002 from the Washington State Commission on Pesticide Registration if the $20,000 request presented above for 2002 to the Tree Fruit Research Commission is approved.

FINAL REPORT
WTFRC Project # AE-01-50 **WSU Project # 8096**

Project title: Control of apple and pear pests with Surround

PI: Jay F. Brunner
Organization: WSU Tree Fruit Research and Extension Center

Co-PIs and affiliation: Betsy Beers and John Dunley, Tree Fruit Research and Extension Center, Wenatchee, WA

Objectives:
1. Determine the optimum timing, rates and application frequency of Surround® against selected apple pests.
2. Determine the optimum timing, rates and application frequency of Surround® against selected pear pests.
3. Determine the negative effects of Surround® on natural enemies.
4. Determine if use of Surround® will increase damage thresholds for spider mites on pear, thus increasing the potential for biological control.

Significant findings:
- Surround WP reduced **codling moth** injury by approximately 50% in field trials. Concerns regarding the effect of Surround on integrated mite control still exist. Neonate codling moth larvae were deterred from entering the fruit by Surround WP residues in a laboratory bioassay, and in whole-fruit treatments the number of entries in Surround WP treated fruit were about 60% of the untreated fruit.
- In leaf disk bioassays 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.
- Field trials with Surround WP against **lacanobia fruitworm** showed it to reduce foliage feeding and protect fruit when applied prior to egg hatch.
- Surround applied prior to oviposition of the **western tentiform leafminer** can reduce mines by 50%. However, there was a strong effect of Surround applications on ***Pnigalio flavipes*****,** the key biological control agent of leafminer. It may be possible to avoid application activity periods of ***Pnigalio flavipes*** thus reducing the negative aspects of Surround on this beneficial insect.
- Surround has no apparent effect on **campylomma** nymphs or the prevention of damage by this pest on apple. This could be good news for pear where campylomma is considered a valuable early season predator of pear psylla.
- Surround effects on **spider mite**s were variable, having no effect in one test and reducing densities in another. Surround treatments also reduced predatory mite densities in field tests. There is evidence and concern that certain uses of Surround in apple orchards will stimulate spider mite populations through the suppression of **predatory mites**.
- Surround is a highly effective control for **white apple leafhopper**. One or, in the second generation, two applications provide a high level of suppression of this pest.

- Surround effects on **stink bugs** were variable, but in general it appears not to deter fruit injury in late summer from adults immigrating to orchards from native habitats.
- 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**.
- **Recommendations in EB-0419 –** One output of this research is the recommendations for pest control published in the Crop Protection Guide for Tree Fruits in Washington. Recommendations exist for codling moth, leafhoppers, lacanobia fruitworm and pear psylla.

Methods:
Methods have been provided in previous reports and due to the large number of studies summarized here it would be confusing to report all the methodologies used for various trials. Brief descriptions of methods are offered within the Result and Discussion section for each pest.

Results and discussion:

Codling moth apple dip bioassay: Surround was evaluated in direct choice tests for its ability to deter codling moth neonate larvae from penetrating a treated apple. Treatments were applied to apple at equivalent label rates for dilute applications. Apples treated with Surround were dipped, allowed to dry, then dipped again for a total of two applications. CM entries were evaluated at 10 days after treatment. Surround significantly deterred neonate CM from entering the treated half of the fruit. However, CM larvae found "holes" in the residues and were able to enter at these locations. The reduction in entries is about what is observed in field trials where multiple applications are made.

Surround bioassay

	Avg no. CM entries	
Test	Surround	Untreated
1-sprayed	1.54	1.96
2-dipped 3X	1.20	2.44
3-"painted"	0.86	2.07
3-controls	3.20	4.70
4-Sylwet	0.85	1.59
4-controls	2.50	4.60

Surround bioassay.

	CM entries at 10 days	
Evaluation	Surround	Untreated
Choice test		
Avg % on treated half (SE)	20.9 (1.2)	
No choice test		
Avg number of entries (SE)	3.3 (0.4)a	5.3 (0.3)b

Means in the same ROW followed by the same letter not significantly different (p=0.05, Student's paired t-test).

Codling moth field trials: Surround was evaluated for its ability to control CM when applied at different timing intervals (1999) or in a full season program (2000) targeting both first and second generation larvae. The experimental design was single-tree plots replicated four times in a randomized complete block. In 1999 trials, there was no advantage to applying treatments covering both oviposition or hatch periods, both having provided about 70% suppression of injury. In 2000 different timing regimes were again evaluated, oviposition versus hatch. There was no difference in the level of suppression between the treatments, and they provided suppression of fruit damage, 50%, similar to tebufenozide (Confirm) but not as good as azinphosmethyl (Guthion).

1999 field trials.

Treatment	Rate (form./25 gal)	Timing/appl.#[1]	No. per 50 fruits Stings	Entries	% total injury
Surround	25 lbs	Oviposition/3	0.8a	3.0bc	7.6b
Surround	25 lbs	Hatch/3	0.8a	4.0b	9.6b
Surround	25 lbs	Ovip.+hatch/6	0.8a	2.0bc	5.6b
Untreated	NONE		0.8a	12.2a	26.0a

Means in the same column followed by the same letter not significantly different (p=0.05, Duncan's new MRT).
[1] Application dates for "oviposition timing" were 19, 27 Jul and 4 Aug and for the "hatch timing" were 12, 18 and 25 Aug. Applications for the "oviposition+hatch timing" were on all six dates. Treatments were applied only against the second generation.

2000 field trials.

		Avg % CM injury[1]					
		5 Jul			31 Aug		
Treatment	Rate (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.	0.0 c	0.0 b	0.0c	2.0 bc	1.3 d	3.3 d
Confirm 2SC + Orchex 796	21 fl oz 0.25% v/v	0.8 abc	1.0 b	1.8 bc	3.5 abc	8.5 bc	12.0 bc
Untreated		1.3 a	4.3 a	5.5 a	3.8 ab	25.5 a	29.3 a

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

Leafrollers

Apple leaf disk bioassay: Surround was evaluated in direct choice tests for its ability to deter leafroller neonate larvae from colonizing treated apple leaf disks. The treatment concentration was equivalent to recommended label rates for dilute applications. An untreated control was prepared using water plus the wetting agent only. Surround treatments deterred colonization in choice tests. Only 9% of pandemis leafroller larvae were found on the Surround WP treated disk over the untreated disk.

Field-aged residues: Using a leaf-disk bioassay, Surround® WP was evaluated for residual effects on pandemis leafroller neonate larvae. The test was designed as a direct choice test between treated and untreated leaf disks. The treatments were applied with a handgun sprayer simulating a dilute spray of approximately 300 gal/acre. Surround was applied twice, allowing a drying time between successive applications. Results were similar to those discovered in the leaf disk bioassays. Surround deterred pandemis leafroller from colonizing treated leaf disks for 7 days but the effect did not last through 14 days.

Field-aged residues – pandemis leafroller.

	Avg percentages		
	1 DAT	7 DAT	14 DAT
Choice test – 5 day exposure			
% on Surround (SE)	33.3 (3.3)	30.0 (10.0)	50.0 (0.0)
Controls – 10 day exposure			
% mortality on Surround	37.8b	42.2b	11.1a

Means in the same column followed by the same letter not significantly different (p=0.05, Student's paired t-test). Any mean followed by the letter 'a' not significantly different than the untreated control.

Lacanobia fruitworm

Apple leaf disk bioassay: Surround was evaluated in direct choice tests for its ability to deter lacanobia fruitworm neonate larvae from colonizing treated apple leaf disks. The treatment concentration was equivalent to recommended label rates for dilute applications. Surround treatments deterred colonization in choice tests. Only 6% were found on the Surround WP treated disk. Where there was no choice there was 100% mortality of lacanobia fruitworm larvae.

Field-aged residues: Using a leaf-disk bioassay, Surround WP was evaluated for residual effects on lacanobia fruitworm neonate larvae using methods described under leafrollers above. Surround deterred lacanobia fruitworm from colonizing treated leaf disks, and the effect lasted at least 14 days. Surround caused mortality significantly higher than the untreated control through 14 days for lacanobia fruitworm.

Field-aged residues – lacanobia fruitworm.

	Avg percentages		
	1 DAT	7 DAT	14 DAT
Choice test – 5 day exposure			
% on Surround (SE)	13.3 (3.3)	30.0 (15.3)	33.3 (8.8)
Controls – 10 day exposure			
% mortality on Surround	46.1b	28.1b	34.5b

Means in the same column followed by the same letter not significantly different (p=0.05, Student's paired t-test). Any mean followed by the letter 'a' not significantly different than the untreated control.

Field tests with Surround: One-third acre plots were replicated three times at a rate of 50 lbs/100 gal water plus 1 pt M-03/100 gal. Surround provided suppression of lacanobia fruitworm larval feeding and larval populations and appeared to protect fruit from injury.

Surround vs. *Lacanobia subjuncta* field trial, 1999.

			Posttreatment averages			
			19 Jul		9 Aug	
Treatment	Rate/a	Timing	lvs/20 trays	% inf. shoots	% fruit injury [1]	% inf. shoots
Surround	50 lbs.	Oviposition	3.3b	6.8ab	0.0a	30.0b
Surround	50 lbs.	Hatch	1.0a	2.8a	0.0a	11.3ab
Surround	50 lbs.	Ovi + hatch	0.3a	1.2a	0.0a	6.7a
Untreated			4.0b	16.8b	0.3a	99.7c

Means in the same column followed by the same letter not significantly different (Fisher's Protected LSD, p=0.05).

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. However, in subsequent greenhouse bioassays Surround caused about 50% suppression of mines when applied prior to adult oviposition. In a field test, different strategies were evaluated 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 when early season Surround treatments were adjusted to avoid the period of parasitoid activity; however, no treatment differences were apparent by the second generation.

Stink bugs: Surround was applied to a number of experimental blocks in areas of historically high stink bug pressure. Each replicate consisted of 0.5 acres of orchard, with Surround-treated and untreated control blocks immediately adjacent to one another and bordering rangeland. Surround applications commenced in late July, and there was a total of five applications applied, at 2-week intervals. Results from these trials were highly variable. In some orchards, stink bug damage was slightly reduced in the Surround treatments; in one site injury was higher in the Surround-treated plot, and in the rest of the sites there was no difference between Surround-treated and untreated plots.

Leafhopper: In first-year tests, both the single and triple applications of Surround provided suppression of leafhopper nymphs (with better control in the triple application), but the residual population was higher than with conventional materials. In the second year of testing, 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: In the first test against mites, treatments were applied with a multi-tank PTO airblast sprayer calibrated to deliver 100 gpa. The treatments were applied on 4 Aug, 1999. In this test Surround and Orchex provided very little suppression of mites. In a subsequent test Surround gave reasonable mite suppression where there was a high initial mite population (e.g. 20-60 mites/leaf). While the activity was slower than conventional miticides, populations were below 1 mite/leaf by nine days after application. The predatory mite population in this test was high initially (0.2-0.9 mites/leaf). The Surround application significantly reduced the predatory mite numbers in relation to the check (48 h posttreatment). 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.

Campylomma: This material was tested against the spring generation of nymphs that cause damage to apple fruitlets around bloom. Surround had no apparent effect on nymphs, nor did it prevent fruit damage from occurring.

Pear psylla: Surround has become one of the most important pear psylla controls in north-central Washington. Surround treatments combined with conventional psylla management programs have shown that this product is better used prebloom compared to the postbloom period. Results from work on pear psylla are reported in a separate proposal by Dr. Dunley and will be presented at the pear research review.

Budget:
Control of apple and pear pests with Surround
Jay F. Brunner
Proposed project duration: 3 years and this represents a final report
Current year request: 0

Year	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**Total 1999-2001**
Total	19,460	22,135	22,348	**63,943**

Item	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**Totals 1999-2001**
Salaries[1]	8,391	9,353	9,444	27,188
Benefits (30%)	2,949	2,806	2,928	8,683
Wages	7,000	8,600	8,600	24,200
Benefits (16%)	1,120	1,376	1,376	3,872
Equipment	0	0	0	0
Supplies	0	0	0	0
Travel	0	0	0	0
Miscellaneous	0	0	0	0
Total	19,460	22,135	22,348	**63,943**

FINAL REPORT
WTFRC Project # AE-01-46 **WSU Project # 5802**

Project title: Effect of herbivory and water stress on pome fruit photosynthesis and productivity

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

Co-PIs and affiliations: 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
Steve Welter, Entomology, UC Berkeley
Steve Drake, Post-harvest, USDA-ARS, Wenatchee
Jim McFerson, WA-TFREC, Wenatchee

Funding history: Funded 2000-01 ($28,746), 2001-02 ($20,000)

Objectives:
1. Determine if water stress significantly increases the negative effect of mite feeding damage on four cultivars of apple. (2000-2002).
2. Determine diurnal pattern of CO_2 assimilation on mite-damaged and/or water stressed trees. (2000).

Significant findings:
Findings from the 2000 season indicate that water stress caused a 37% decrease in net photosynthesis (Pn) after six weeks of reduced irrigation levels, although this effect was temporary (Pn rates recovered after moisture stress was alleviated). Mite levels were low in 2000 and did not have a significant effect on Pn. Data from the 2001 season have not been completely analyzed, although preliminary analysis indicates no reduction in Pn due to either mites or water stress.

Methods:
1. Create mite-infested and mite-free trees in a uniform block of 5^{th} leaf trees containing plots with the cultivars ‘Oregon Spur,’ ‘Golden Delicious’ and ‘Fuji BC 2’ and ‘Royal Gala.’ For each level of mite damage, create two 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^2). 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 and obtain average leaf area and use to obtain a measure of m^2 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 storability of fruit.
2. Measure whole-canopy photosynthesis at 2-hour intervals during the photophase three times during the period of maximum water stress and mite infestation (July/August).

Results and discussion (2001):
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 entire block was sprayed several times throughout the growing season with esfenvalerate to eliminate predatory mite populations. Mites were counted ca. weekly by removing 10 leaves/tree, brushing the leaves with a Leedom mite-brushing machine and counting the mites that fell onto the revolving glass plate. Five to 10 leaves/tree were collected and kept cool during transportation and storage. The mites were brushed from the leaves with a Leedom mite-brushing machine and collected on a revolving sticky glass plate. The composite sample on the plate was counted using a stereoscopic microscope. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), *Tetranychus* urticae Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor (the eggs of TSM and MCD could not be distinguished and were recorded as a group); western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); a stigmaeid predatory mite, *Zetzellia mali* Ewing; and motile stages of apple rust mite (ARM), *Aculus schlechtendali* (Nalepa). 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).

The population was composed primarily of *T. urticae* with a lesser proportion of *P. ulmi.* Despite inoculations, tetranychid mite populations remained low throughout the season (Fig. 1); functionally, no mite stress occurred on the trees. Predatory mites, however, were relatively numerous (Fig. 2) and were doubtless in part responsible for the ongoing suppression of phytophagous mites. This level of *T. occidentalis* in the presence of nearly continual residues of esfenvalerate is unprecedented, and further investigation is necessary to determine the tolerance status of this predator.

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 of the four replicates, with sensors located at four 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 the amount of soil moisture and movement of irrigation pulses through the soil profile.

The high-water plots were watered weekly (usually 8-hour sets) (Table 1), and the low-water plots were watered ca. every third week, or three times during the stress period (July-August). There was good separation between the high- and low-water plots over the course of the water stress period (July-Aug) (Fig. 3).

Table 1. Irrigation schedule, 2001.

Date	Time	Hours	Water treatment level High (1, 3)	Low (2,4)
7/12/01	9:00am-5:00pm	8	x	x
7/16/01	9:00am-5:00pm	8	x	
7/23/01	9:00am-5:00pm	8	x	
7/30/01	9:00am-5:00pm	8	x	x
8/7/01	9:00am-5:00pm	8	x	
8/15/01	9:00am-9:00am	24	x	
8/21/01	8:30am-4:30pm	8	x	
9/4/01	9:00am-5:00pm	8	x	x

Whole-canopy photosynthesis: (WCP) was measured on 'Oregon Spur' trees the week of Aug 27. On each sampling date, one replicate (one tree/treatment) was measured approximately between 10 am and 3 pm. Total leaf area was estimated for each tree at the same time. Leaf area estimates were made by systematically counting all the leaves on the tree and removing every 100th leaf. A composite measurement of these leaves was made with a Li-Cor leaf area meter.

No differences were found either among treatments or either of the two factor levels (mites, water) (Tables 2, 3). The lack of differences in the mite factor levels is expected, given that mite stress in 2001 was almost nonexistent. The differences in soil moisture, however, did not translate to a reduction in photosynthesis.

Table 2. Net assimilation measurements, 28 Aug - 2 Sep, 2001 - treatment means.

Mites	Water	n	Net assimilation umoles/m2/sec	
High	High	4	22.92	a
High	Low	4	22.37	a
Low	High	4	22.95	a
Low	Low	4	20.77	a

Table 3. Net assimilation measurements, 28 Aug - 2 Sep, 2001 - factorial means.

Mite	n	Net assimilation umoles/m2/sec	
High	8	22.64	a
Low	8	21.86	a

Water	n	Net assimilation umoles/m2/sec	
High	8	22.93	a
Low	8	21.57	a

Fruit measurements: Forty fruits/tree were sampled for quality assessments, 20 for each of two storage periods. These measurements will be completed in February 2002.

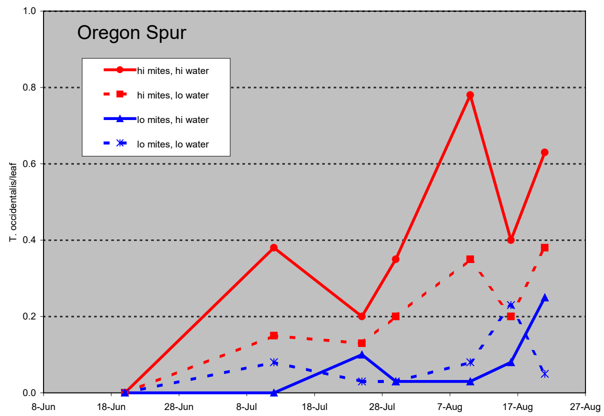


Fig. 1. Tetranychid mite populations, 2001

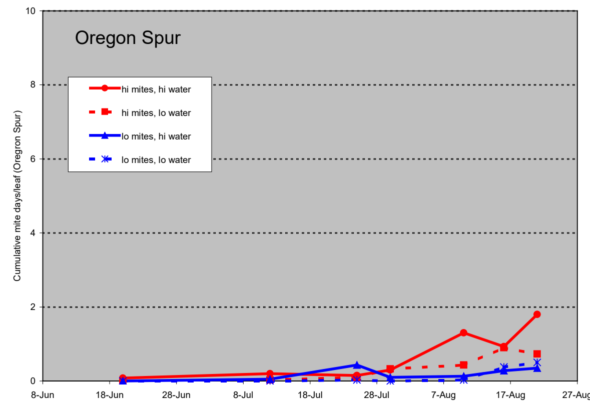


Fig. 2. Predatory mite populations, 2001

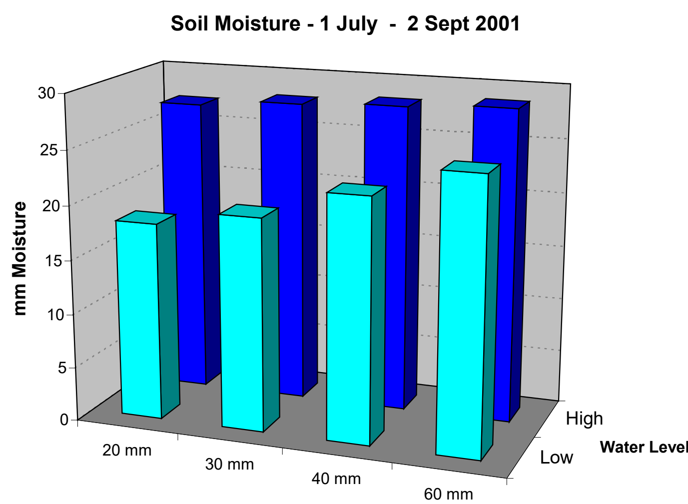


Fig. 3. Soil moisture levels, 2001

CONTINUING PROJECT **YEAR 2/3**
PROJECT NO.: 13C-3343-3123

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

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

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

OBJECTIVES: *Lygus*

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

OBJECTIVES new for 2002: Thrips

1. Screen candidate compounds for their ability to suppress thrips populations that are infesting apple orchards. Candidate compounds include spinosyns, insect growth regulators, synthetic pyrethroids, and neonicotinyls.
2. Evaluate several sampling techniques to determine thrips abundance in apple.
3. Develop a relationship between thrips feeding after fruit set and fruit injury.
4. Evaluate edge effects of migration of thrips from adjacent riparian habits into apple orchards.

OBJECTIVES: Riparian Buffers

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

SIGNIFICANT FINDINGS- *LYGUS*

Phenology Model.. A phenology model currently used in California proved effective at predicting the first generation hatch of *Lygus* in spring in Eastern Washington. However, the model lost predictive accuracy as the season progressed.

Orchard Sampling. In 2001 several *Lygus* abundance sampling techniques were investigated. Techniques tested included sweep net samples of the orchard floor, beat sampling of trees, and colored sticky cards. Sweep net sampling of the orchard floor was the only technique tested that caught any observable numbers of *Lygus*. Tree beating was ineffective as was the use of colored sticky cards in measuring abundance of *Lygus.*

***Lygus* Damage.** Branch cage studies in 2001 have helped quantify proportional *Lygus* abundance to fruit damage. *Lygus* feeding in April resulted in greater proportional amount of fruit injury then *Lygus* feeding in feeding in July.

SIGNIFICANT FINDINGS-THRIPS

Flight monitoring. Thrips flight activities were monitored in apple orchards with yellow and blue sticky card traps. Blue cards are proving to be significantly (P<0.01) more effective then yellow cards at catching western flower in apple orchards.

SIGNIFICANT FINDINGS-RIPARIN BUFFERS
We have developed considerable evidence that riparian areas are confirmed sources of hemipteran and thrips pests. These include both stinkbugs, (Jay Brunner, personal com), and *Lygus* bugs and flower thrips (Walsh unpl. data).

METHODS- *Lygus*
1. Lygus sampling. We will compare several sampling techniques to assess Lygus populations in the riparian sites and nearby orchard floors in early spring to assess when adults become active and to determine when first generation egg hatch takes place. Sampling techniques that will be tested include sweepnet, colored sticky cards, and whole plant quadrants.
2. Lygus damage thresholds. Sleeve cages were sewn in 2001 that covered 1 meter lengths of apple branch. Fruit was thinned so that constant ratios of Lygus to apples can be maintained. Adult *Lygus* were introduced into the sleeve cages at ratios of 0.25, 0.17, 0.125, 0.083, 0.056, 0.033, and 0.015 Lygus per fruit. Each cage treatment was replicated 4 times on Fuji fruit set in April and mid-season in July. Cages were left on the trees for 2 weeks and when they were removed the branches were treated with acephate. A damage assessment was taken just prior to commercial harvest. We would like to repeat these studies in 2002.
3. Phenology model. Data collected in section below "Riparian habitats" will be used for this sub-study. We will use Washington Public Access Weather Systems data and calculate degree days with a lower developmental cutoff of 50, 51, 52, 53, and 54 degrees Fahrenheit respectively. We will then apply these calculations to our field observations to determine if we can acquire a model with better fit.

Methods- Thrips
1. Insecticide efficacy. An insecticide efficacy trial will be established in an apple orchard in the lower Yakima Valley to test the post fruit set efficacy of registered and candidate compounds on flower thrips. Registered compounds that will be screened include endosulfan, carzol, and spinosad. Candidate compounds will include lamda-cyhalothrin, pyriproxifen, thiamethoxam, and novoluron. Other candidate compounds will be considered and screened if sufficient interest is exhibited by researchers or industry. Insecticides will be applied by hand wand at 100 gallons per acre to 6 one tree replicates. Shake samples will be taken prior to and at timed intervals following insecticide application.
2 Insecticide residual. A primary constraint for efficacy studies on thrips on apples is inconsistency in pest distribution. One, eight, and 15 days following insecticide application we will cage 4 individual fruit per treatment in the orchard. Into these cages we will place approximately 20 immature thrips. The cages will be removed after 1 week and the individual fruit will be treated with an effective insecticide. These individual treated fruit will be collected several days prior to commercial harvest. The fruit will be evaluated and graded for thrips damage.
4. We have tested several techniques for evaluating thrips population abundance in stone fruits. Techniques tested have included several colors of sticky card traps, a beating sample, a water shake sample, and an alcohol shake sample. We will adapt these techniques to provide recommendations for sampling thrips abundance in apple orchards.

Methods- Riparian habitats.
1. In 2001 we identified apple orchards in the Yakima Valley that had "protected waterways" running in, next to, or near their orchards. In early spring 2000 we established 3 field survey sites of 180' by 180' along protected waterways and monitored; a) the ambient plant species, and b) the abundance of *Lygus*, thrips and other insects present at each respective riparian site and at the adjacent orchard edge every 2 weeks from March through November. In spring 2002 we plan on splitting 2 of these sites; treating one half of each site with a herbicide and leave the other half of each site in the pair untreated. We will then seed the herbicide treated site with seeds

recommended by the Natural Resource Conservation Service for use in Conservation Reserve Enhancement Program Buffers. Additionally we will plant specific native shrubs in clusters along the protected waterway. We would plan on submitting future proposals to the WTFRC to continue monitoring Lygus and thrips in these sites in subsequent years. Cost sharing is likely for this project. Companion proposals have been submitted to the WSCPR and NSFIPM for continuation of this project.

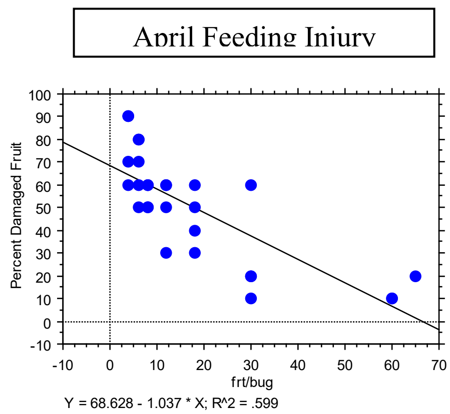
RESULTS/ DISCUSSION LYGUS-2001

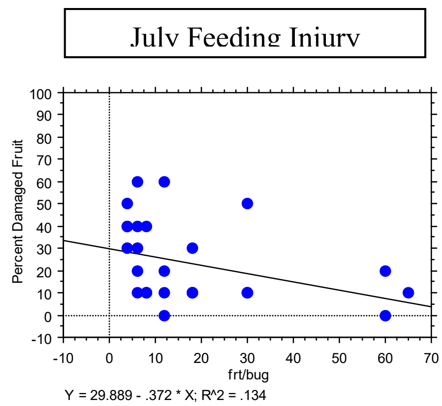
Lygus have an extensive host range of both native and exotic plants. In extensive field surveys conducted during summer of 2001 we have observed a greater abundance of *Lygus* in riparian field sites directly adjacent to apple orchards with established stands of exotic flowering weedy plants then in sites comprised mainly of bunch grasses or native shrubbery.

Lygus overwinter as adults in plants and plant debris. Russian, thistle, Kochia, smotherweed, mullein, horseweed, sweetclover, wild mustards, ragweed, and sagebrush are among many plants that will serve as good overwintering hosts for *Lygus*. Overwintering *Lygus* adults became active as temperatures warmed in spring 2001. Mating was observed soon after emergence and mated females begin laying eggs several days hence. A phenology model Walsh helped develop (Pickel et al. 1990) proved effective at predicting the first generation hatch of *Lygus* in spring in Eastern Washington. However, the model loses predictive accuracy as spring progresses (Walsh 2001 field results). With further study we may be able to fine-tune this model for use in Eastern Washington. However, the model provided little predictive value for determining flights of Lygus in early spring.

In 2001 several *Lygus* abundance sampling techniques were investigated during spring. Techniques tested included sweep net samples of the orchard floor, beat sampling of trees, and colored sticky cards. Sweep net sampling of the orchard floor was the only technique tested that caught any observable numbers of *Lygus*. Tree beating was ineffective as was the use of colored sticky cards.

Lygus feeding has been likened to chemical injury. *Lygus* feeding damage in apple orchards is a significant concern after fruit set, however feeding damage can result in fruit disfigurement during the fruit growing season. Branch cage studies in 2001 have helped quantify proportional *Lygus* abundance to fruit damage. Two sets of sleeve ages were placed on branches of Fuji trees twice. Fruit was hand thinned within each cage and specific numbers of adult *Lygus* were added to each cage to produce specific ratios of fruit to *Lygus* bug in each respective cage. Ratios of fruit to Lygus per cage included 0, 4, 6, 8, 12, 18, 30, & 60 fruit per Lygus. Cages were left on for 2 weeks at each cycle in April and July and then removed each tree branch was then treated with acephate (Orthene) to prevent subsequent feeding injury from occurring. On August 30, 10 fruit were removed from each cage site and peeled with a paring knife. Lygus damage was noted if necrotic feeding spots were present below the fruit skin surface. Our estimates for fruit damage are much higher then typical consumer standards. A majority of *Lygus* feeding damage was not observable above the fruit skin surface. However, April feeding injury was greater then feeding damage in July. We plan on repeating these studies in 2002.





RESULTS/ DISCUSSION- Thrips 2001

Thrips are tiny insects that have four featherlike wings, each consisting of a thick supporting strut with fine hairs on the front and hind edges. Thrips go through six life stages: egg, first instar, second instar, prepupa, pupa, and adult. Thrips insert eggs into plant tissue. The first two instars and the adults feed by piercing and removing the contents of individual plant cells.

Western flower thrips can have several color forms and populations can vary substantially in abundance depending on the time of year. Western flower thrips usually feed in enclosed tissues such as flowers, buds, fruit or growing meristems. Adults also feed on pollen and spider mite eggs. The prepupa and pupal stages are completed in the soil litter beneath infested trees. Females will lay male eggs if unmated. Mating is required for the production of female eggs. Development times for completion of one generation of western flower thrips varies from 11 days at 77° to 87°F, to 44 days at 50° to 60°F.

Biological Control. A number of generalist predators feed on western flower thrips. Minute pirate bugs, big-eyed bugs, and several species of predatory mites have been identified as efficient predators. Unfortunately, populations of these generalist predators are disrupted and slow to recover following the application of disruptive insecticides.

Chemical control of *Lygus* and thrips has proven challenging for entomologists across a wide range of crops. For thrips control here in Washington State, snap pea growers rely on 2 applications of dimethoate, while onion growers apply lambda-cyhalothrin or methyl-parathion. Lygus control on a number of crops in recent years has been dependent on the use of synthetic pyrethroids. Both Lygus and flower thrips have a history of developing tolerance and then resistance to organophosphate and pyrethroid insecticides. Tree fruit growers historically applied formetanate hydrochloride and endosulfan. However, both of these chemistries will be subject to regulatory. Endosulfan may be at particular risk due to its lethal and sub-lethal effects on aquatic invertebrates. Hindering foods of endangered fish species falls within the bounds of the Endangered Species Act 4d rules.

Several new chemistries have been developed in the last decade that show some promise for thrips suppression. Spinosad (Success™), a reduced risk insecticide has demonstrated some thrips control activity and has recently been registered for use on stone fruits. Pyriproxifen (Knack™) has a registration pending on apples and several other crops. This insect growth regulator has some activity against thrips. Noveluron, another insect growth regulator will be a candidate compound in our trials. Neonicotinyls have demonstrated some ability to suppress thrips and Lygus. We would plan on testing several candidate compounds in this category of insecticides for their ability to suppress thrips and Lygus populations.

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

	Thrips per 3" by 5" sticky			
	Count	Mean	Std. Dev.	Std. Err.
bl stky cd	756	369.504	798.592	29.045
yl stky cd	886	226.445	447.793	15.044

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

	Thrips per 3" by 5" sticky			
	Count	Mean	Std. Dev.	Std. Err.
6/29/01, bl stky cd	31	224.839	280.040	50.297
6/29/01, yl stky cd	31	178.871	227.104	40.789
7/13/01, bl stky cd	31	440.968	320.108	57.493
7/13/01, yl stky cd	31	324.032	316.892	56.916
7/27/01, bl stky cd	13	359.615	382.777	106.163
7/27/01, yl stky cd	13	183.077	231.755	64.277

RESULTS/ DISCUSSION- Riparian Habitats-2001. Proposed regulations designed to mitigate issues arising from the Endangered Species and Clean Water Acts that promote rehabilitation of riparian areas could modify land use and pest management practices throughout Washington State. Native plant species are promoted in these rehabilitation/re-vegetation plans. Little consideration has been directed towards biological issues like identifying plant species that will not serve as hosts for arthropod or pathogenic pests of tree fruit and other crops. Furthermore, these proposed regulations provide few provisions to reduce competition from exotic weeds while reestablishing preferred native plants. We have developed considerable evidence that riparian areas are confirmed sources of hemipteran and thrips pests. These include both stinkbugs, (Jay Brunner, personal com), and *Lygus* bugs and flower thrips (Walsh unpl. data).

BUDGET:
***Lygus* Bug and Western Flower Thrips Ecology in Washington State Apple Orchards**
D.B.Walsh
Project duration: 2001-2003
Current year: 2002
Original budget request: 23,000

Year	Year 1 (2001)	Year 2 (2002)	Year 3(2003)
Total	23,000	36,000	36,000

Current year breakdown

Item	Year 1 (2001)	**Year 2 (2002)**	Year 3(2003)
Salaries	10688 Assoc. in Res. 0.375 FTE	22,230 Assoc. in Res. 0.75 FTE	23,119 Assoc. in Res. 0.75 FTE
Benefits (34%)	3,634	**7,558**	7,860
Wages	3,360	**3,360**	3,360
Benefits (%)	538	**538**	538
Equipment			
Supplies	4,180	**1,624**	433
Travel	600	**690**	690
Miscellaneous			
Total	23,000	**36,000**	36,000

OTHER SUPPORT OF PROJECT: Walsh drafted a companion proposal for submission to the WSCPR in 2000 for riparian buffer studies that was funded for $18,000. An additional proposal for $19,600 will be submitted to the WSCPR for consideration at their January 2002, funding meeting. An additional proposal has been submitted to the NSF Center for Integrated Pest Management for $18,085 in 2002 and $19,265 in 2002. This proposal has made the preliminary cut and a final funding decision will be made by Jan. 2002.

CONTINUING PROJECT **YEAR 2/3**

Project Title: Optimizing Ammonia with Traps to Manage Apple Maggot in Washington

PI: Wee L. Yee, Research Entomologist
Co-PI: Pete J. Landolt, Research Entomologist
Organization: USDA-ARS, Wapato, WA

Objectives:

Objectives in 2001 were to 1) Determine the effectiveness of 2 rates of ammonia hydroxide (28% ammonia) released from dry and wet traps; 2) Determine the effectiveness of sugar-derived baits alone and in combination with ammonia baits in dry traps; 3) Determine populations of apple maggots among 3 regions and habitat types in western Washington by using these dry and wet traps; 4) Relate trap captures to food abundance in the environment over the season; 5) Determine reproductive status of trapped flies over the season.

In 2002, objective 1 of 2001 will be repeated. New objectives in 2002 are aimed at further optimizing the use of ammonia (NH_3) with traps. These are to 1) determine optimal NH_3 concentrations, 2) determine optimal release rates, to 3) compare ammonium hydroxide with conventional, commercially available ammonia lures; and to 4) test effects of different ammonium hydroxide volumes on prolonging trap effectiveness.

Significant Findings:

- All rates of 28% NH_3 release, based on 0.05-(about 2 mg/hour), 0.16-, and 0.32-cm diameter holes, were equally effective in trapping apple maggots. Baited traps usually captured more flies than unbaited traps. Best traps were baited spheres, followed by yellow rectangles and domes. However, differences were not always significant. Males preferred spheres.
- Ethanol, acetic acid, and butanol compounds, shown to attract other fruit flies and insects, were not effective in attracting apple maggot, probably because it does not feed on rotting fruit.
- Fly populations in 3 areas of Washington - Puyallup (Pierce County), Fort Vancouver (F.V.) (Clark County), and St. Cloud Ranch (S.C.) (Skamania County) differed. Numbers were lowest in Puyallup, higher in F.V., and highest at S. C. At S.C., the high numbers of trees and host diversity probably contributed to high populations. Females at both Puyallup and F.V. responded to traps similarly. There was no change in trap preference over the season.
- Aphid honeydew and bird feces were rare at all sites, and did not appear to be primarily food sources.
- Over 80% of trapped females contained eggs, even in early July.
- Flies responded best to ammonia in the 14-28% concentration range when dispensed using 10 ml volumes in vials with 0.05-cm holes.

Methods for 2002:

1. Preliminary work in 2001 indicated 28% NH_3 released from vials with the 0.05 cm hole was more effective in capturing apple maggots than 1.75-7.0% NH_3. In 2002, this work will be repeated to validate 2001 results. Concentrations of 0, 1.75, 3.5, 7.0, 14, 20, and 28% ammonia in fifteen-ml polyethylene vials with 0.05 cm diameter holes will be tested using sticky yellow rectangle traps. Vials will be replaced every 2 days and trap positions will be randomized daily (same for objectives 2 and 3 below). Flies will be removed daily. There will be at least 3 replicates of each concentration treatment. Tests will be conducted over 4-day periods. All tests (objectives 1-4) will be conducted in the Puyallup and Vancouver areas simultaneously.
2. To determine if the highest NH_3 release rates are optimal for attraction or whether they repel flies, 28% NH_3 solutions will be loaded into 1, 3, and 6 fifteen-ml vials and hung above sticky yellow

rectangle traps. A control, with 6 vials only, will be included as a control. There will be at least 5 replicates of each. Tests will be run over 4-day periods. Laboratory tests will be conducted to determine the actual NH_3 release rates per trap that correspond to the different numbers of vials.

3. To compare ammonia hydroxide (AH) with conventional, commercially available ammonia lures, sticky yellow rectangles (YR) and sticky red spheres (RS) will be used. The optimal ammonia concentration or numbers of vials of AH from objective 1 will be used and compared with the conventional ammonia lures. These lures are ammonium carbonate (AC) in vials and ammonium acetate pre-baited (PB) in sticky adhesive. There will be 5 treatments: 1) AH-YR, 2) AH-RS, 3) AC-YR, 4) AC-RS, 5) PB-YR, 6) PB-YR, and 7) YR only, and 8) RS only. In AH treatments, lures will be replaced every 2 days. Several weekly tests will run over 4-day periods.
4. To test the effects of ammonia lure volume on ammonia release longevity and fly attraction, vials of different volumes using the 0.05 cm diameter openings will be used. Vials that are 15, 30, 60, 125, and 250 ml will be filled with 10, 20, 40, 84, and 168 ml of AH, respectively. An unbaited control will be included. Vials will be hung above sticky yellow rectangles and red spheres, for 12 total treatments. Flies will be collected once weekly from the traps over the season. There will be at least 3 replicates of each treatment. Unlike in objectives 1-3, the AH will not be replaced. The effectiveness of the different volumes will be followed for 2 months, with the hypothesis that the higher volumes will be more effective over the long term.

Results and Discussion of 2001 Study

There was no consistent difference in fly captures using 3 NH_3 release rates (hole sizes) from traps at either site, indicating the low release rate, corresponding to 2 mg/hour, may be sufficient. (NH_3 release rates corresponding to all hole sizes are being determined.) However, because higher numbers of vials seemed to work better, more work needs to be done in 2002 to solve this apparent inconsistency. All release rates were more effective than the controls (Fig. 1 and Fig. 2). Among trap types, females and males showed clear differences in responses at Puyallup. Fewest females were captured in the dome traps, but approximately equal numbers were captured on yellow panels and red spheres, with red spheres being slightly superior. Males were overwhelmingly more attracted to red spheres, especially when baited with NH_3. However, this was not seen in Vancouver (Fig. 2).

The higher NH_3 concentrations captured more female flies than the lower concentrations at both sites, with a leveling of response at 14-20%. The best relationships obtained were not linear, but cubic (Fig. 3A and B), suggesting a leveling off of response before the highest concentration. Males were less responsive to NH_3 (Fig. 3C and D).

Only one other study has determined the effects of ammonia release rates on apple maggot responses in the field. Jones (1988) determined that higher release rates of ammonium carbonate increased captures of flies in Utah. However, the rates were determined based on dry weight loss (not taking into account the breakdown of carbonate), so the actual amounts of ammonia released were not determined. The effects of aqueous ammonia concentrations on fly responses were also unknown. Early work suggested that 1 or 2% household ammonia was more effective than dry ammonium carbonate (Hodson 1948), but there had been no mention of the effects of different ammonia concentrations in any later literature.

The results suggest the red sphere is superior to the yellow rectangle in most cases. Early results (which need to be confirmed) suggest 14-28% NH_3 concentrations are sufficient to maximize apple maggot captures. To further increase detection power of traps and to reduce fly numbers through trapping, the NH_3 concentration, its release rate, and its release longevity need to be optimized. Once this is achieved, trapping can be used to more effectively detect and manage the fly.

Budget:
Optimizing Ammonia with Traps to Manage Apple Maggot in Washington
Wee L. Yee,
Project duration: 2001-2003
Current year: 2002
Original budget request:

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

Current year breakdown:

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

[1]Two GS-5 employees ($11.32/hour), full-time, 6-month appointments, and benefits
[2] Pesticide-treated spheres, sticky yellow traps, sticky spheres, vials, chemicals, miscellaneous supplies
[3]Travel to field sites, fuel costs

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Jones, V. P. 1988. Longevity of apple maggot (Diptera: Tephritidae) lures under laboratory and field conditions in Utah. Environ. Entomol. 17: 704-708.

Jones, V. P. and D. W. Davis. 1989. Evaluation of traps for apple maggot (Diptera: Tephritidae) populations associated with cherry and hawthorn in Utah. Environ. Entomol. 18: 521-525.

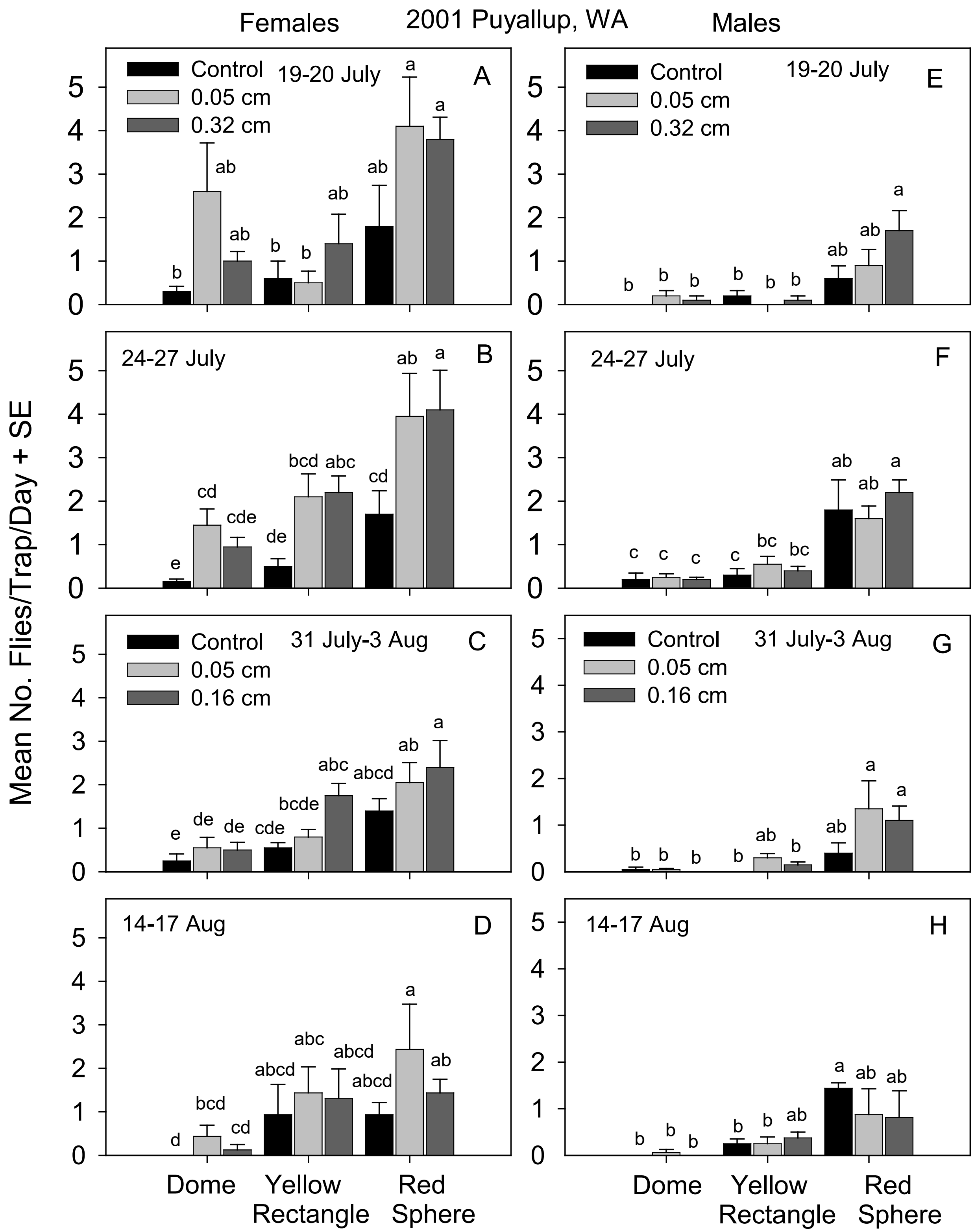
Prokopy, R. J. and K. I. Hauschild. 1979. Comparative effectiveness of sticky red spheres and pherocon apple maggot standard traps for monitoring apple maggot flies in commercial orchards. Environ. Entomol. 8: 696-700.

Reissig, W. H. 1975. Evaluation of traps for apple maggot in unsprayed and commercial apple orchards. J. Econ. Entomol. 68: 445-448.

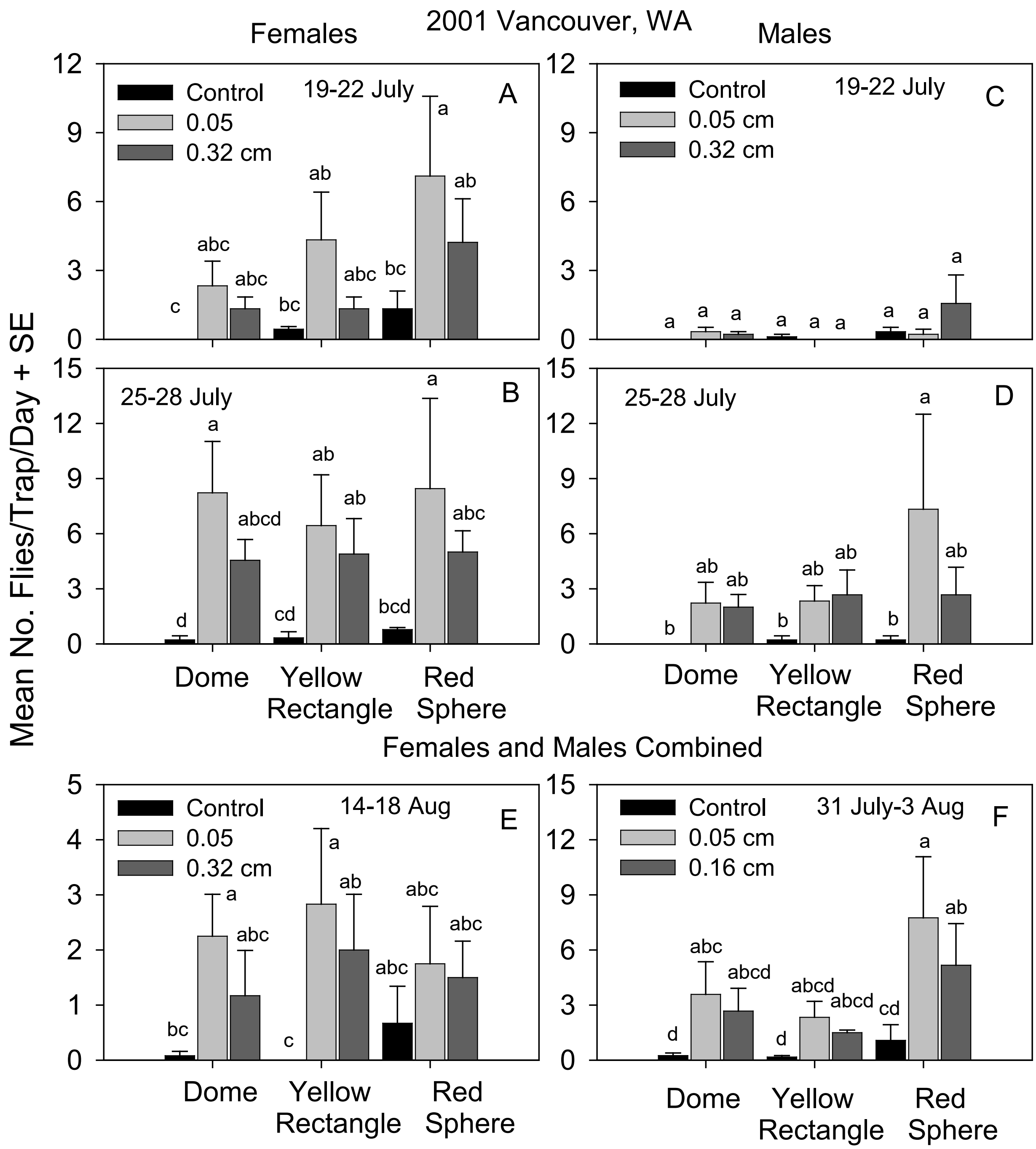
Fig. 1. Effects of NH_3 release rate (hole size) and trap type on apple maggot captures in Puyallup, Washington. A-D, females, E-H, males. Means with the same letters are not significantly different ($P > 0.05$).

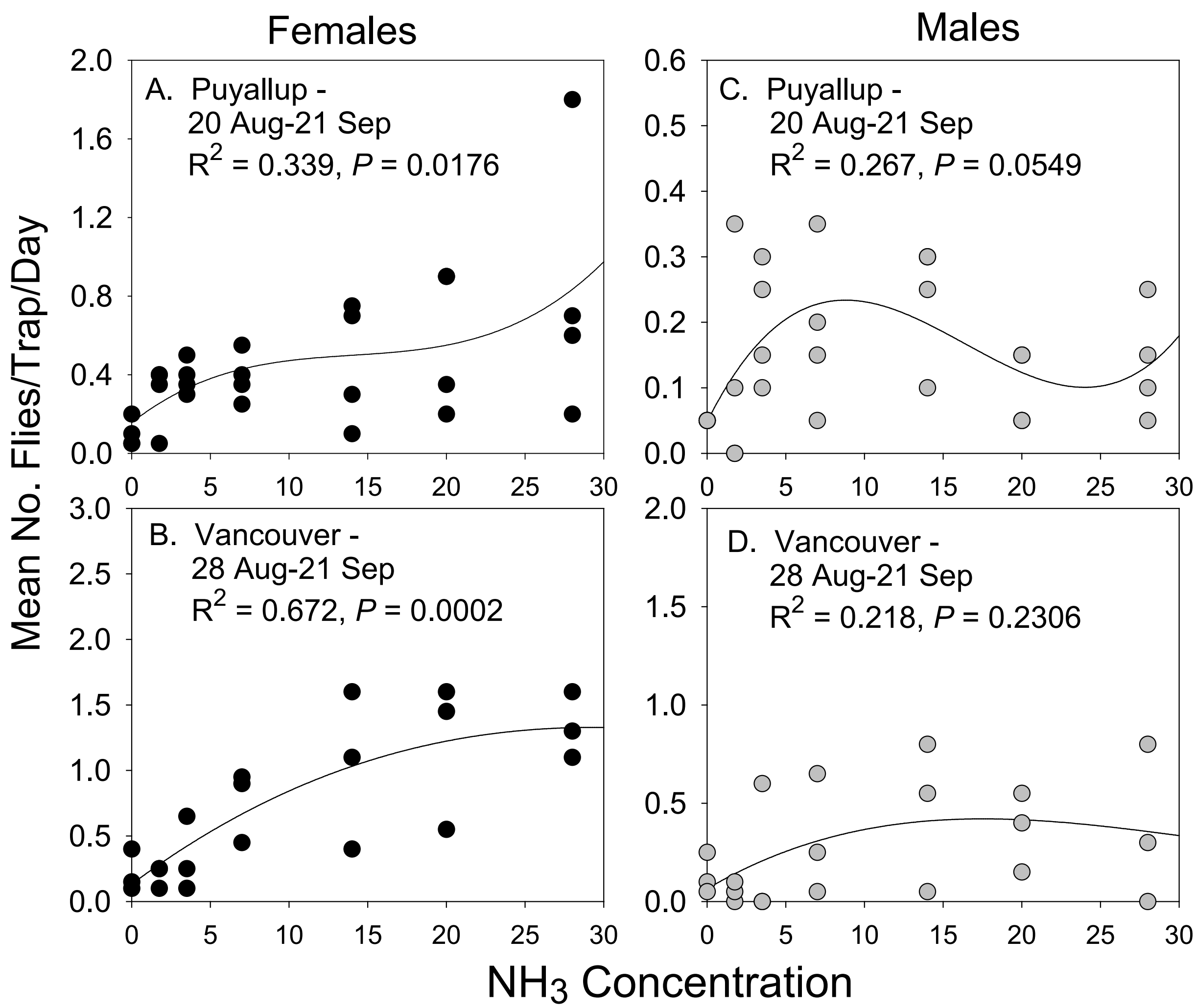
Fig. 2. Effects of NH_3 release rate (hole size) and trap type on apple maggot captures in Vancouver, Washington. A and B, females, C and D, males. E and F, both sexes combined. Means with the same letters are not significantly different ($P > 0.05$).

Fig. 3. Responses of (A and B) female and (C and D) male apple maggot in Puyallup and in Vancouver, Washington, to yellow panels baited with one vial (0.05 cm hole size) loaded with NH_3 at different concentrations.



Females
2001 Puyallup, WA
Males
Mean No. Flies/Trap/Day + SE
Control
0.05 cm
0.32 cm
0.16 cm
19-20 July
24-27 July
31 July-3 Aug
14-17 Aug
A
B
C
D
E
F
G
H
Dome
Yellow Rectangle
Red Sphere


2001 Vancouver, WA
Females
Males
Mean No. Flies/Trap/Day + SE
Control
0.05
0.32 cm
19-22 July
A
0.05 cm
C
25-28 July
B
D
Dome
Yellow Rectangle
Red Sphere
Females and Males Combined
14-18 Aug
E
31 July-3 Aug
F
0.16 cm



Females
Males
A. Puyallup -
20 Aug-21 Sep
R2 = 0.339, P = 0.0176
B. Vancouver -
28 Aug-21 Sep
R2 = 0.672, P = 0.0002
C. Puyallup -
20 Aug-21 Sep
R2 = 0.267, P = 0.0549
D. Vancouver -
28 Aug-21 Sep
R2 = 0.218, P = 0.2306
Mean No. Flies/Trap/Day
NH3 Concentration

CONTINUING PROJECT **YEAR 2/3**

Project title: Development of an Attractive, Toxic Bait for the Apple Maggot, *Rhagoletis pomonella*

PI: Daniel S. Moreno

Organization: USDA, Agricultural Research Service, Kika de la Garza Subtropical Agricultural Research Center; Crop Quality and Fruit Insects Research Unit; 2413 East Highway 83; Weslaco, Texas 78596

Co-PI(s) and affiliations(s):
Robert L. Mangan, and Guy J. Hallman. USDA, Agricultural Research Service, Kika de la Garza Subtropical Agricultural Research Center; Crop Quality and Fruit Insects Research Unit; 2413 East Highway 83; Weslaco, Texas 78596

Cooperators:
W.H. Reissig, Professor Entomology, NY State Agricultural Experiment Station, Geneva, NY
J. D. Hansen, Research Entomologist, ARS-Yakima, WA
L. G. Neven, Research Entomologist, ARS-Yakima, WA

Objectives:
The objectives of this proposal are: (1) Rear apple maggot in sufficient numbers to conduct testing in the laboratory, greenhouse, or field cages. (2) Develop a bait-matrix suitable to the apple maggot that enhances toxicity of various classes of insecticides. (3) Develop a toxic-bait that can be used for ground or aerial sprays. (4) Explore the potential for the use of a bait station to control apple maggot in the field.

Significant findings:
- Estimated time spent in each stage of development for apple maggot.
- Defined the ovipositional rhythm of females in the laboratory.
- Estimated an LC_{50} for fipronil and abamectin in SolBait in the laboratory.

Methods:
Small numbers (≈300 pupae every two weeks) of apple maggot used in the studies were received from Dr. Harvey Reissig, Geneva, NY, who maintains a non-diapausing colony on apples in his laboratory. The pupae were sent to Weslaco, TX, where they completed their development in rearing rooms held at 26 ºC, 70% RH, and 14 h light. From these flies, a number of preliminary tests were conducted. These were egg collection on artificial substrates (7 tests), egg sterilization (8 tests), incubation (2 tests), ovipositional rhythm (2 tests), larval diet (36 tests with modified Mexican and West Indian fruit fly diets), Holiday plums (4 tests), insecticides (6 tests with fipronil and abamectin). The number of flies per replication were, by necessity, about 10.

Egg collection devices were artificial wax domes, Parafilm rings partially filled with a xanthan gum gel, and Parafilm-formed semi-spherical spheres (simulating fruit) filled with the same gel used in the Parafilm rings. The oviposition-rhythm study was conducted using the spheres for collecting eggs and started at 0600 when lights were turned on; spheres were changed every hour until 2000 h when lights were turned off. All eggs were counted under a microscope. Incubation of eggs consisted of a single temperature with eggs placed in a moist Petri dish, on moist filter paper in Petri dish, or on moist filter paper over a moist sponge inside Petri dish. Larval diets were the original and modifications of those published by Spishakoff and Hernandez Davila (1968) for the Mexican fruit fly and Moreno et al. (1997) for the West Indian fruit fly. Eggs were incubated first and when eclosion began, these were placed on top of the diet to complete their eclosion. Holiday plums were tested to see if female apple maggot would lay eggs in them, determine if larvae would develop in them, and determine if plums could last sufficiently long for larvae to complete their development. The idea was to find an additional host for the purposes of quickly augmenting the colony, in the laboratory, other than apples. The insecticides abamectin and fipronil were tested to determine their LC_{50s} against adult apple maggot. The insecticides were mixed in SolBait at various concentrations and fed to flies for 24 h; after this, the insecticide-laden SolBait was removed and replaced with rearing adult diet. Mortality counts were made every 24 h for three days.

Results and discussion:
For ease of handling, egg collection worked best when using the Parafilm-formed semi-spherical spheres. Flies preferred to lay eggs on this substrate versus wax domes or Parafilm rings. Thus, we have adopted the sphere as a means of collecting eggs. Eggs were sterilized with chlorox up to 1000 ppm without inhibiting larval eclosion. The temperature used for incubation worked fine but eclosion and handling of eggs worked best by using the combined moist filter paper on a moist sponge inside a Petri dish. None of the larval diets worked sufficiently well to say that we can use one to rear the colony. Three aspects could be the main barriers for larval survival in an artificial diet; larval developmental time, sensitivity to antimicrobials, and diet texture. We tried to work without antimicrobials initially because reportedly apple maggot larvae are very sensitive to those commonly used in diets, sodium benzoate and methyl parahydroxybenzoate (Nielsen 1969). However, without antimicrobials diets rapidly become contaminated with bacteria, yeast, or molds. Larval developmental time is longer for apple maggot than for West Indian or Mexican fruit flies, 15 days versus 8 and 12 days, respectively. Incorporation of methyl parahydroxybenzoate improved the condition of the diet but few larvae survived. A change in diet texture to bind water completely appears to be working better than other diets; however, we had sufficient larval survival to estimate a preliminary larval developmental time (Fig.1). Total developmental time is 62 days for all stages with about half of this time spent in the pupal stage. This is twice the time as that for West Indian and Mexican fruit flies. Results from the oviposition-rhythm study indicates that females lay most of their eggs in the afternoon, after 1400 h (Fig. 2); thus egg collections for our studies are confined to this period. Flies consumed fipronil- or abamectin-laden SolBait *ad libitum* judged by their mortality when exposed to these compounds for 24 h. The LC_{50} obtained for fipronil, 0.45 ppm (Fig. 3), is lower than that recorded for the Mexican fruit fly, 2.89 ppm. The LC_{50} obtained for abamectin, 0.052 (Fig. 4), is also lower than that recorded for the Mexican fruit fly, 0.17 ppm. The results for this two compounds indicates that we have a working base-bait and that apple maggot may be more sensitive to insecticides than the Mexican fruit fly. The Holiday plums are an acceptable alternative host to apples as twice as many larvae (30) were obtained per fruit compared to apples (15).

So far, the information obtained indicates that developing an artificial diet for apple maggot is feasible but not easy, a desirable bait formulation is obtainable, and controlling the apple maggot with novel-type compounds is achievable.

Literature Cited

Moreno, D.S., D.A. Ortega Zaleta, and R.L. Mangan. 1997. Development of artificial larval diets for West Indian fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 90: 427-434.

Spishakoff, L.M. and J.G. Hernandez Davila. 1968. Dried torula yeast as a substitute for brewer's yeast in the larval rearing medium for the Mexican fruit fly. J. Econ. Entomol. 61: 859-860.

Neilson, W.T.A. 1969. Rearing larvae of the apple maggot on an artificial diet. J. Econ. Entomol. 62: 1028-1031.

Budjet:
Project duration: 2002 – 2004
Current year: 2001
Development of an Attractive, Toxic Bait for the Apple Maggot, *Rhagoletis pomonella*
Daniel S. Moreno

- **Original Budget Request**

Year	Year 1 (2001)	Year 2 (2002)	Year 3 (2003)
Total $	30,000	31,500	33,075

- **Current year breakdown**

Item	Year 1 (2001)	**Year 2 (2002)**	Year 3 (2003)
Salaries[1]	0	**12,000**	12,000
Benefits (18%)	0	**2,200**	2,200
Wages[2]	0	**0**	6,000
Benefits (18%)	0	**0**	1,100
Equipment[3]		**2,500**	1,000
Supplies[4]	0	**3,000**	3,000
Travel[5]	0	**0**	4,000
Miscellaneous	0	**300**	700
Total $[6]	**20,000**	**0**	30,000

1. Assist in paying technician salary
2. Temporary help projected needed for field work.
3. Purchase a small incubator for eggs and digital camera for recording and reporting fly developmental events. The following purchase a viscometer.
4. Purchase additional cages and rearing supplies
5. Travel expense projected to work in apple growing areas.
6. **Money awarded for fiscal year 2001 was not used because the administrative mechanisms were not set in place to claim it. The progress reported herein was accomplished with USDA funds only. No monetary requests are made for fiscal year 2002 because the $20,000 already awarded will be used for that period as suggested in the budget. If everything works out this time, we will make a follow-up request for fiscal 2003.**

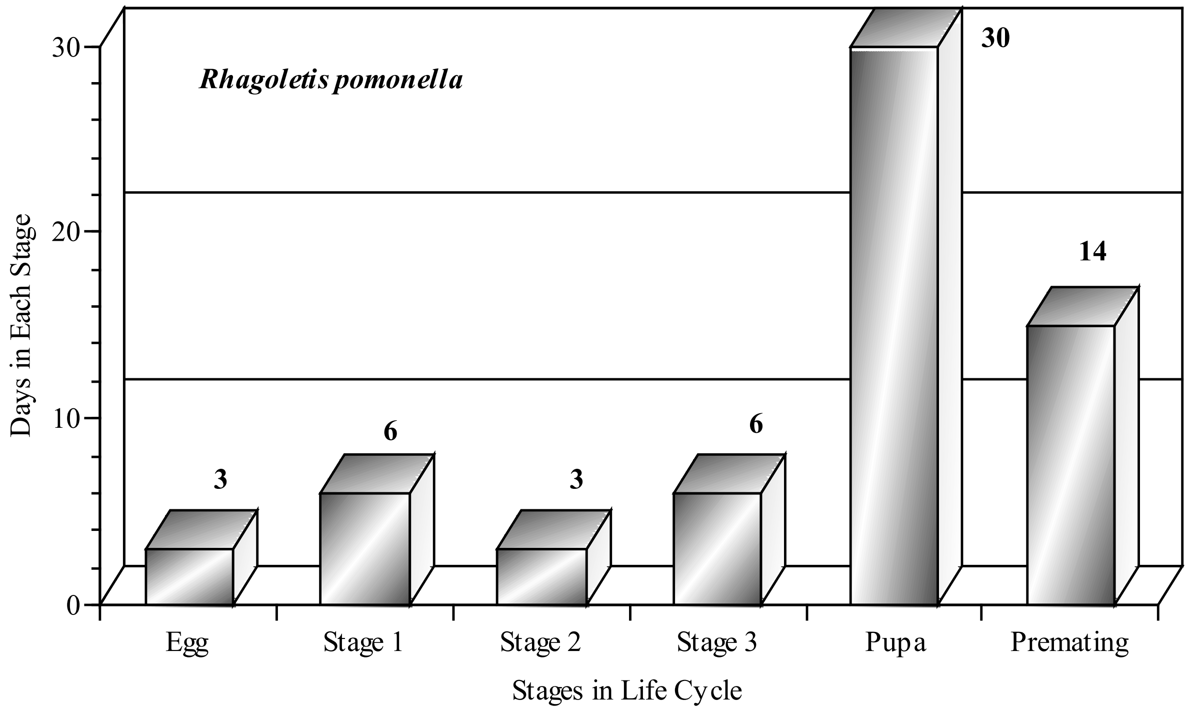


Fig. 1. Number of days spent in each stage of development for the apple maggot reared in artificial diet or red plums.

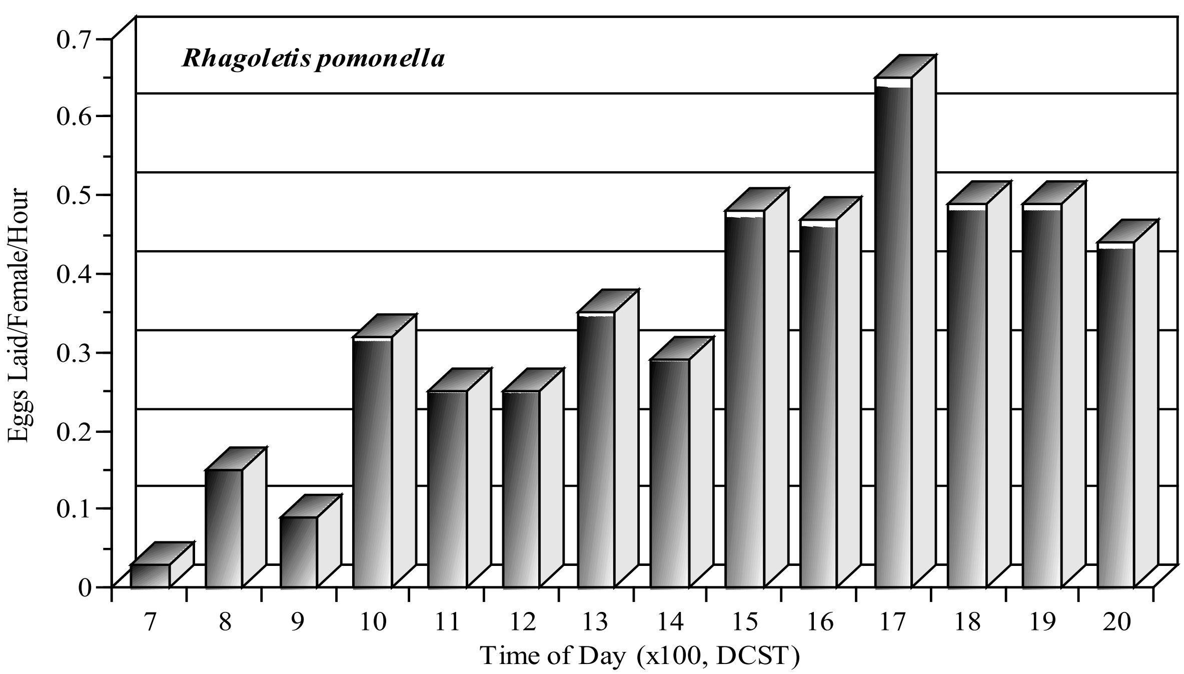


Fig. 2. Ovipositional rhythm of female apple maggot reared from apples, held at 26 °C, 65% RH, 14 h photoperiod, and fed an artificial adult diet.

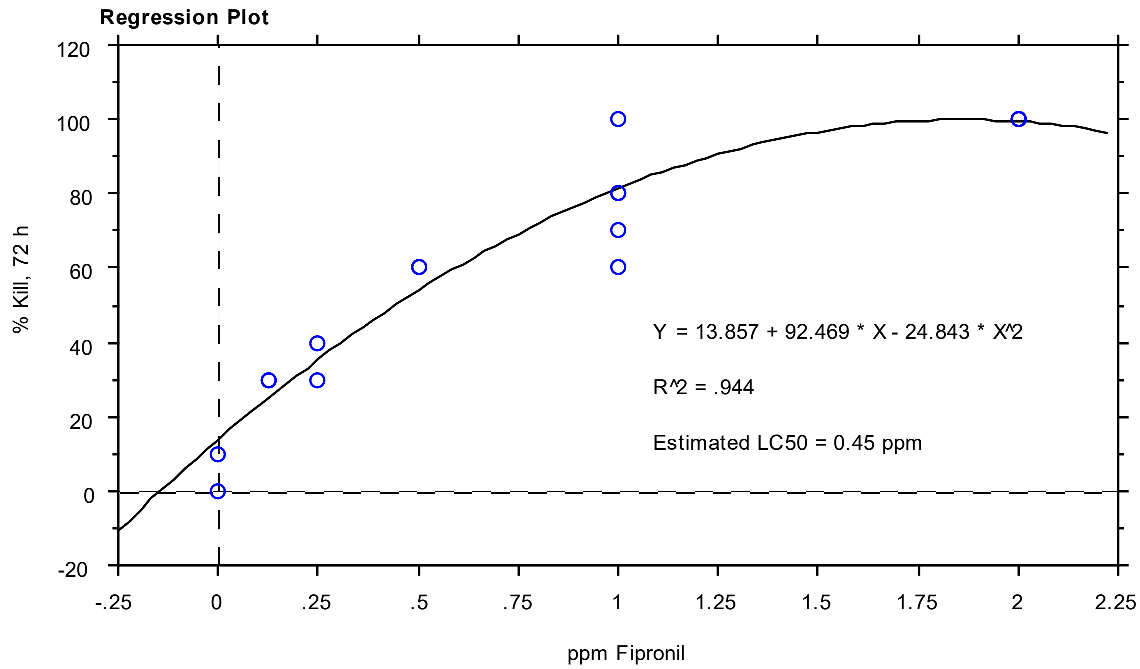


Fig. 3. Estimated concentration needed to kill 50% of apple maggot population (LC50) with fipronil in SolBait in laboratory tests.

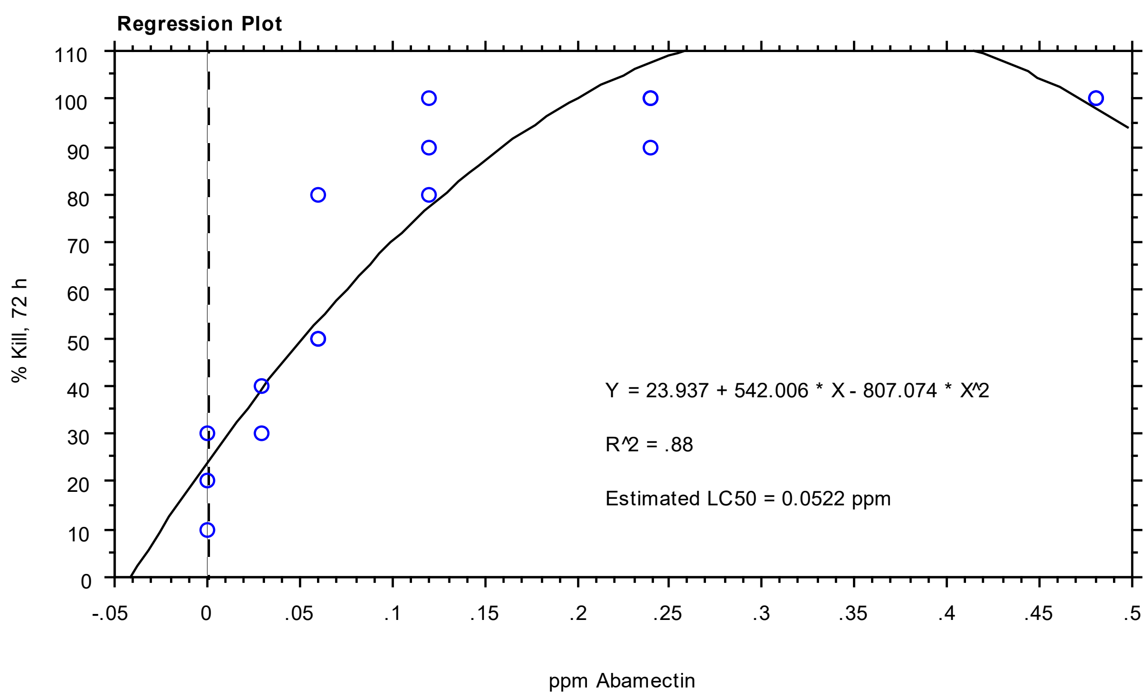


Fig. 4. Estimated concentration needed to kill 50% of apple maggot population (LC50) with abamectin in SolBait in laboratory tests.

FINAL REPORT -- <u>Confidential</u>

Title: An Alternative Management Strategy for Codling Moth: Autocidal Biological Control
PI: Lisa G. Neven, Research Entomologist, USDA-ARS, Wapato, WA
Co-PI: Holly J. Ferguson, Assistant Professor, Heritage College, Toppenish, WA

Objectives:
The primary objective of this project is to genetically engineer codling moth to contain a single point alteration of the genome to render the insect conditionally sterile under normal environmental conditions. Optimal system will have a selectable marker gene (EGFP) and a conditional lethal mutation which would allow for mass production under controlled environmental conditions, but would be lethal to offspring under normal environmental conditions [truncated *Notch* (N^{60G11})]. The truncated *Notch* (N^{60G11}) is a dominant mutation which is triggered at temperatures below 20°C and prevents embryogenesis. In order to obtain the primary objective, it was necessary to develop a reliable transformation technology for codling moth which included the development of a microinjection technique to successfully deliver DNA into the early embryo. It was also necessary to determine the optimal transposable element and vector construct to give stable transformation and integration into genomic DNA. Once the initial transgenic insects, expressing only the marker gene were obtained, it was necessary to determine the integration site in the moth genome and the exact sequence of surrounding the insertion site.

After successful transformation was determined and the system was optimized, a DNA vector with the conditionally lethal N^{60g11} mutation was used to create a strain of codling moth which could be reared above the critical temperature in the laboratory and perform temperature experiments with transformed codling moth, homozygous and heterozygous for the N^{60g11} mutation.

Significant Findings:
- Injected DNA containing green fluorescent protein (EGFP) resulted in transient expression of the fluorescent gene following injection in codling moth embryos. Both *piggyBac* and *hermes* transposons were used to insert the DNA into the chromosomes.
- The *piggyBac* transposable element was the most successful in inserting the marker gene with reliable excision of the plasmid.
- The *hermes* containing plasmid resulted in the generation of a number of eye mutants, but low expression of the EGFP.
- Successful transformations of Oriental Fruit Moth and Lesser Apple worm were obtained using the *piggyBac*/EGFP vector.
- Evidence using polymerase chain reaction (PCR, amplified DNA) indicates that the green fluorescent gene was present in codling moths raised from eggs that were injected with an EGFP construct and in the offspring of those moths, indicating inheritance of the injected gene.
- Evidence using blotting analyses (unamplified DNA) indicates that the green fluorescent gene was present in Generation 5 of five codling moth lines which originated from DNA-injected individuals.
- Two lines of EGFP expression codling moth resulted in positive PCR for 25 generations and positive DNA blots up to generation 13.
- Sequencing of the DNA surrounding the insertion site of EGFP lines using inverse PCR is now under way.
- Comparisons of oviposition, egg hatch, and survivorship of the EGFP lines with wild type show not deleterious effects of the EGFP insertion into the genome.
- A draft of the confined field release (caged tree studies) has been filed with USDA-APHIS Biotechnology permitting office. A final draft will be filed in January 2002.
- Successful transformation of codling moth with the EGFP/N^{60g11} was obtained this summer. There

are 4 lines obtained from different plasmid constructs. Expression of the EGFP is very high in two lines and are PCR positive. Lines are currently up to generations 4 and 6.

- The EGFP/N^{60g11} lines have been split into two equal parts. One half will be used to continue the lines and the other half is in the temperature sensitivity experiments.

RESULTS:
Expression of the injected green fluorescent protein gene has been noted for codling moth embryos using both the *hermes* and *piggyBac* element with the fluorescent gene driven by a moth gene promoter (Tables 1 and 2). This transient gene expression lasts until the larva hatches. Helper DNA does not affect transient expression but becomes important in the insertion and inheritance of the marker DNA. The percent expression using these two vectors was similar, but the hatch rate was considerably higher when using the vector containing *hermes* (Table 1). Thus, both *piggyBac* and *hermes* DNA containing a silkworm gene promoter for GFP function well in codling moth eggs.

The *piggyBac* transposable element was used to transform both Oriental Fruit Moth and Lesser Appleworm (demonstrating that this technology, Autocidal Biological Control, coule also be developed for these pest and perhaps other Tortricid pests.

Based on polymerase chain reaction analyses (amplified DNA) performed during the past year, the GFP gene was present in moths raised from eggs injected with GFP (Generation 0) and in subsequent generations (Table 4). GFP-positive moths in Generations 11 and 12 indicate that the GFP gene was inherited in codling moth. Furthermore, blotting analyses, in which the DNA is NOT amplified revealed the presence of the GFP gene in Generation 5 and 8 pooled DNA samples of several moths (Table 4). The DNA originally injected was *piggyBac*/silkworm promoter/GFP. As evident from the table, the results may be variable between the two types of DNA analyses and between generations of a single line. The two outstanding lines (Lines 9 and 7) are being analyzed more closely with DNA sequencing analysis to determine where the inserted DNA landed in the chromosome. Three other lines were positive for marker DNA in Generation 5, but not always positive in preceding generations.

The transformation of codling moth with the EGFP/N^{60g11} is the first successful transformation of a Lepidopteran with a conditional lethal mutation. This puts the codling moth program well ahead of the pink bollworm program. Demonstration of the temperature sensitivity of this transgene to render these lines conditionally sterile under normal environmental conditions is still needed. If these tests are successful, an application for confined field releases will be made. Both lines of transgenic codling moth will be used in the development of an Environmental Impact Statement, the first required by USDA-APHIS for any transgenic organism. There is interest in both USDA-ARS and USDA-APHIS to complete this aspect of the program, and to also complete the first environmental impact study (EIS) for a transgenic organism. The final draft of the confined field release application will be sent to USDA-APHIS in January 2002, and posted on the Federal Register at that time. The caged field trials will be conducted over the 2002-2003 seasons (two field seasons). We will be filing for a Biotechnology Risk Assessment grant (USDA-CSREES-NRI) to complete the information for the EIS.

Conclusions:
The successful transformation of codling moth with both marker and conditionally sterile constructs has fulfilled the primary objective of this project. Research will continue to demonstrate the temperature sensitivity of the conditional sterile construct. The development of a system to transform other Lepidopteran pests may lead to the generation of more candidates for Autodical Biological Control, a 21st Century approach to pest eradication.

Table 1. Codling moth injection data with the DNA vector containing *hermes*, a silkworm gene promoter, and green fluorescent protein gene*.

	# injected	# green embryos (% of injected)	# hatched (% of injected)	# hatched that were green (% of hatched)
Without helper	377	234 (62.1)	179 (47.5)	147 (82.1)
With helper*	260	155 (59.6)	82 (31.5)	56 (68.3)
Total	637	389 (61.1)	261 (41.0)	203 (77.8)

*DNA from S. Thibault, Exelixis Pharmaceuticals, South San Francisco, CA.

Table 2. Codling moth injection data with the DNA vector containing *piggyBac*, a silkworm gene promoter, and enhanced green fluorescent protein gene*. Table contains both 1998 and 1999 data.

	# injected	#green embryos (% of injected)	# hatched (% of injected)	# hatched that were green (% of hatched)
without helper	804	464 (57.7)	191 (23.8)	138 (72.3)
With helper**	1373	857 (62.4)	396 (28.8)	299 (75.5)
Total	2177	1321 (60.7)	587 (27.0)	437 (74.4)

*DNA from S. Thibault, UC Riverside).
**Helper plasmids from S. Thibault, UC Riverside, and Al Handler, Gainesville, FL.

Table 3. Injection data for oriental fruit moth and lesser appleworm compared to codling moth using the DNA vector containing *piggyBac*, the silkworm gene promoter, and enhanced green fluorescent protein gene.

Moth	# injected	#green embryos (% of injected)	# hatched (% of injected)	# hatched that were green (% of hatched)
OFM	96	56 (58.3)	17 (17.7)	17 (100)
LAW	28	17 (60.7)	9 (32.1)	9 (100)
CM	1522	962 (63.2)	449 (29.5)	346 (77.1)

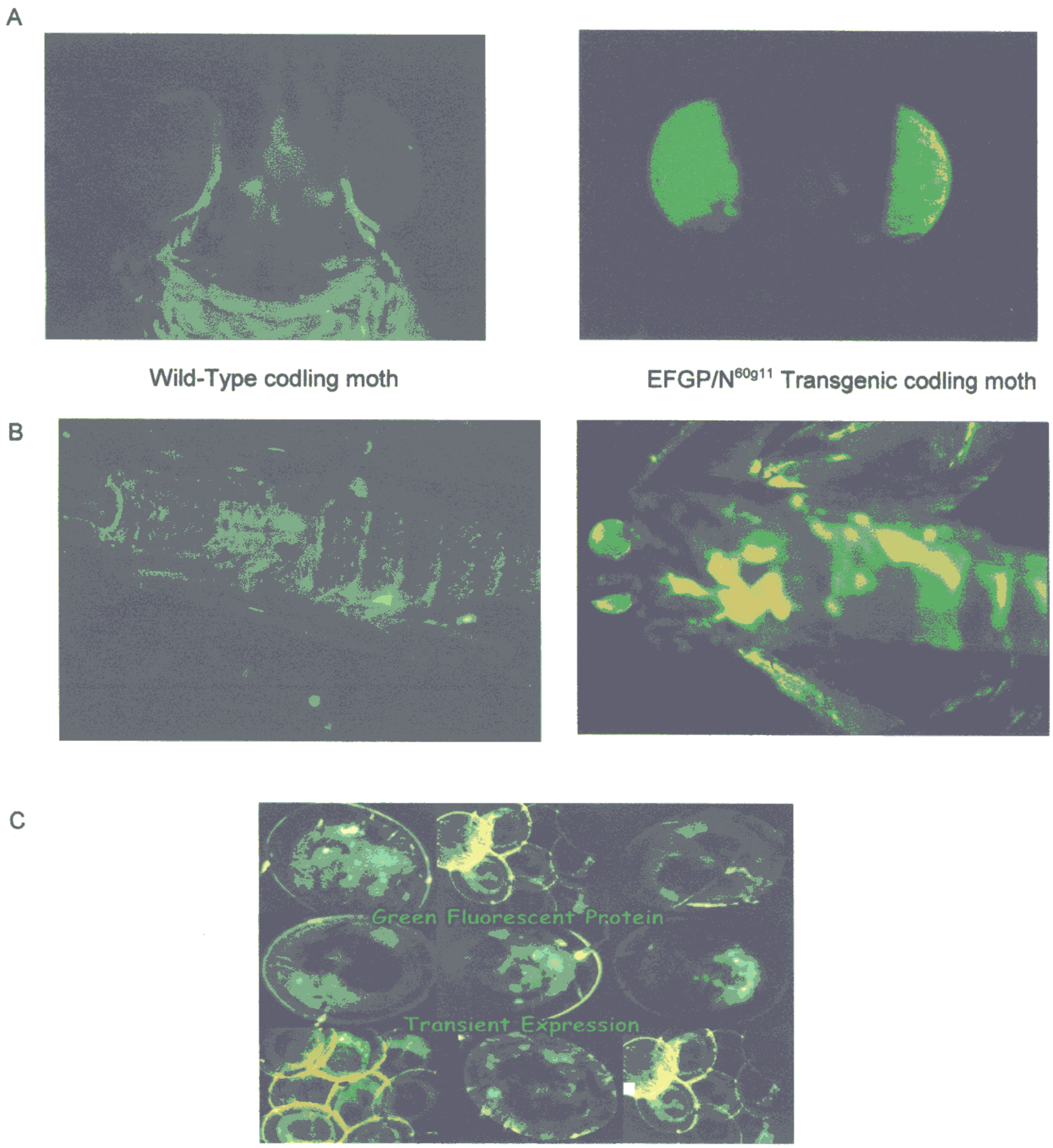
OFM = Oriental fruit moth, LAW = Lesser appleworm, CM = codling moth

Table 4. Summary of results from polymerase chain reaction (PCR) analyses (amplified DNA) and blotting analyses (unamplified DNA) on adult codling moths. DNA originally injected contained *piggyBac*, a silkworm gene promoter, and EGFP. The analyses probed for the presence of a portion of the EGFP gene that was part of the vector injected into Generation 0 embryos.

Analysis	Generation	# Sublines Positive for GFP / #Screened (Positive Sublines)
PCR	5	0/3
PCR	6	4/4 (39,43,77,82)
PCR	7	1/5 (66)
PCR	8	1/7 (39)
PCR	9	2/8 (77)
PCR	10	6/6 (82, 25, 66, 77)
PCR	11	1/3 (66, 82)
PCR	12	0/2
PCR	13	1/2 (39)
PCR	14	1/1 (43)
Southern	5	9/20*
Southern	8	16/12**

[1]Pooled DNA samples from each line for Southern blots.
Line 7 è Sublines 66,74,77,82
Line 9 è Sublines 25,28,30,39,43
*(Sublines 25,30,24,36,50,46,74,28,42)

Figure 1. Expression of EGFP in the eyes (A), body (B), and eggs (C) of transgenic codling moths. The moth on the left of A and B is the wild-type and has dull eyes and body. The moth on the right of A and B is the EGFP/N[60g11] transgenic moth and has bright green eyes and body (under the scales). The eggs in C show the bright green transient expression 24 hours following injection with the EGFP plasmid. These photographs were taken using a dissecting microscope with special filters designed to visualize EFGP activity.



FINAL REPORT
WTFRC Project #AE-01-51 **WSU Project #** 4094

Project title: Monitoring and control of stink bugs

PI: Jay F. Brunner
WSU Tree Fruit Research and Extension Center, Wenatchee, WA

Co-PIs and affiliations: Christian Krupke, Research Associate, Tree Fruit Research and Extension Center, Wenatchee, WA;
Peter Landolt, USDA-ARS Yakima

Objectives:

- Examine candidate insecticides for stink bug control in-orchard.
- Examine effects of pyrethroid insecticides on non-target organisms (mites).
- Re-evaluate the potential of pheromone based aggregate-and-kill strategies to manage stink bugs before mating and oviposition in late spring/early summer with larger blocks.
- Determine daily phenology of stink bug activity.
- Re-examine attractive radius of pheromone and continue dose/response studies to find optimum release rate for stink bug lures.
- Determine the potential for increasing the attractiveness of pheromone lures with the addition of feeding stimulants/arrestants.

Significant findings:
1999

- Removal of native vegetation from borders did not reduce damage by stink bugs in border rows.
- Baiting native vegetation with aggregation pheromone adjacent to orchard borders did not reduce fruit damage by stink bugs in border trees.
- Orthene appeared to offer the best suppression of stink bugs in borders.
- Three dispensers containing the major component of stink bug pheromone were evaluated for attractiveness to stink bugs. All lures attracted aggregations of stink bugs to mullein plants, but the same lures placed inside different traps were not effective.
- Fruit in “trap trees” placed in orchard borders had more damage than fruit in adjacent trees or control trees without lures.
- Additional pheromone components provided by UC Riverside did not increase aggregations of the consperse stink bug.
- The impact of Surround (kaolin) treatments to orchard borders in reducing fruit injury was highly variable. Fruit damage was significantly higher above 6 feet than below, and there was a strong border effect (70% of damage in the first two rows) in almost all orchards where studies were conducted.
- Apple alone does not provide a sufficient nutrient source to mature stink bugs.
- Feeding damage from stink bugs is evident within 24-48 hours following feeding.

2000

- Mark-recapture studies demonstrated that the pheromone is fairly short range, with an active space of 10-25 m. These studies also provided estimates of the numbers of bugs present in areas bordering orchards.
- Pheromone release devices were tested at a range of release rates. These studies, performed in cooperation with Dr. Peter Landolt, demonstrated that it is possible to attract stink bugs at rates

far below what we are currently using. Lower rates may also enhance trap catch by providing a more “realistic” stimulus to induce bugs to enter traps.

- 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.
- A blend of “green” chemicals common to a variety of host plants, including alfalfa, was field tested for attractiveness. The tested blends did not increase attraction of stink bugs to baited plants.
- A survey of parasites impacting stink bugs in central Washington indicated that two species of parasitic fly (Tachinidae; *Gymnoclytia occidentalis* and *Gymnosoma filiola*) and two species of parasitic wasp (Scelionidae; *Trissolcus utahensis*, *Trissolcus euschisti*, and *Telenomus podisi*) were identified. Parasitism and predation rates were assessed throughout the season, with predation being more important.
- Surveys conducted in the Columbia Basin determined that the stink bug complex in this area differed when compared to the complex in north-central Washington. Far lower numbers of *E. conspersus* were observed which may partially explain the lower level of stink bug damage experienced by the majority of Yakima area growers.
- Analysis of the surfaces of the beaks, or stylets, of *E. conspersus* collected in a variety of orchards from Orondo to Manson consistently revealed the presence of a single species of fungus. This fungus was isolated in pure culture and identified. If this fungus is pathogenic to apple, it may be a significant factor in the visible appearance of damage.
- Stink bug eggs were caged on apple limbs and they were unable to complete development on apple alone.
- Male stink bugs are sexually mature upon emergence from overwintering sites (early April), but females mature approximately two weeks after emergence. This information can be used to time mating, egg laying and subsequent control measures.

2001

- A number of insecticides were assayed in 2001 and the pyrethroid Danitol emerged as the clearly superior compound for stink bug control. However, this compound caused a reduction in populations of predatory mites and therefore has the potential to “flare” pest mite species. These data will be collected in spring 2002 to determine any carry-over effects of late summer and fall Danitol applications.
- Previous border baiting and spraying efforts succeeded in attracting large numbers of bugs to mullein plants, but the insecticide used (imidacloprid) had poor efficacy. This year’s results using a more effective insecticide (Carzol) were far more encouraging, as damage in treated areas was significantly reduced compared with check plots.
- Pheromone release rate studies revealed an optimum release rate, and this information may be used to determine the best release device for commercialization of the pheromone.
- A number of experiments involving videotaping of stink bugs on baited and unbaited host plants were conducted to further quantify stink bug behavior. Much of the stink bug movement to pheromone sources and mating occurs at night. These data are valuable from a management perspective (timing of sprays), as well as monitoring (timing of examination of baited plants).
- We identified a novel stink bug feeding stimulant. This compound induced stink bugs to feed and to remain in contact with treated areas longer than untreated areas. Combination of this compound in solution with the insecticide Danitol did not result in a significant decrease in the attractiveness of the compound. These data open the possibility of using this compound to enhance insecticide efficacy or possibly incorporating it into bait stations.

Results and Discussion - 2001

Chemical screening: The insecticides listed in Table 1 were assayed for activity against *E. conspersus.* These compounds represent a variety of old and new chemistries and were chosen based on a variety of factors including availability, re-entry interval and effects on non-target organisms. Table 1 shows acute toxicity of the compounds using a Potter tower assay. Compounds that demonstrated efficacy in Potter tower tests were applied to mullein leaves, which were then allowed to age in the field for 48 h before stink bugs were exposed to the leaves in a Petri dish. Results of this leaf disc bioassay are shown in Table 2. In both testing methods the pyrethroid insecticide Danitol was the most effective, followed by Thiodan and Swat (phosphamidon). Carzol and Thiodan are currently the recommended in-orchard treatments for stink bug control; however, Thiodan has a long PHI and restrictions on the number of applications per year, and Carzol can only be applied once after bloom and then under prescription use regulations. Both of these products could be phased out in the future due to FQPA action. Danitol is labeled for apple and appears to provide growers with an effective alternative for control of stink bugs.

Table 1. Corrected percent mortality of stink bugs exposed to insecticides applied in Potter tower bioassay.

Treatment	Rate (ppm)	Rate (Form./100 gal)	Corrected % mortality 24 h.	48 h.	96 h.	1 week
Assail 70WP	60	1 oz.	4	4	4	46
Avaunt 30WDG	34	1.5 oz.	0	0	0	0
Carzol 92SP	413	6 oz.	12	35	63	74
Danitol 2.4 EC	106	4.8 fl. oz.	98	98	98	98
Swat 8 EC	300	4 fl. oz.	90	95	97	100
Thiodan 50WP	149	1 lb.	12	42	59	91
Warrior T 1SC	7	1 oz.	64	96	96	96

Table 2. Corrected percent mortality of stink bugs exposed to insecticides applied to mullein leaf discs.

Treatment	Rate (ppm)	Rate (Form./100 gal)	Corrected % mortality 24 h.	48 h.	72 h.	120 h.
Carzol 92SP	413	6 oz.	12	12.5	16	16
Danitol 2.4 EC	106	4.8 fl. oz.	76	84	89	92
Swat 8 EC	300	4 fl. oz.	32	50	100	100
Thiodan 50WP	149	1 lb.	84	84	100	100
Warrior T 1SC	7	1 oz.	16	21	21	23

Danitol – In-orchard efficacy: Danitol sprays were applied on August 13, 2001, to all orchards in the study (n=7). This date was chosen because the second, or summer, generation of stink bugs had reached adulthood and the onset of fruit damage had been noted. The purpose of our study was to determine the in-orchard effects of Danitol in reducing stink bug injury and to assess the impact of treatments on spider mites and predatory mites. The results, summarized in Fig. 1, were quite encouraging from a management standpoint, reducing damage by over 80% on average (72-90%). It should be noted that these are extremely high-pressure orchards - in many cases 100% of the fruit on the border row were damaged. The reduction in fruit damage was achieved with only a single Danitol application, and the timing of this spray could likely have been improved (i.e., earlier in season). It is also important to note that the sprays were applied at, or shortly after, dusk – the period of highest stink bug activity.

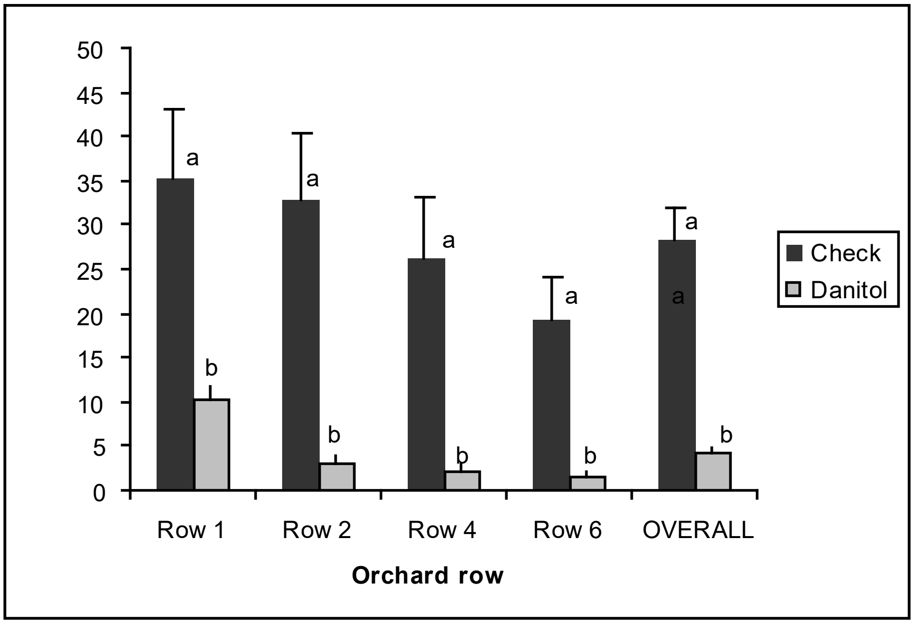


Figure 1. Stink bug fruit damage at harvest following a single in-orchard application of Danitol at label rate (20 fl. oz/acre) on August 13, 2001 (n=7 orchards). Bars with same letter superscript in each category are not significantly different ($P<0.05$).

Danitol – Non-target effects: The potential of Danitol as a powerful tool against stink bug populations in orchard led us to investigate its effects on non-target mites. Pyrethroid insecticides, such as Danitol, have been shown to cause increases in populations of pest mite species. We therefore conducted pre- and post-counts of European red mite (ERM), twospotted spider mite, apple rust mite, and beneficial mite populations (*Typhlodromus* and *Zetzellia* species) in six commercial orchards where Danitol had been applied at the label rate of 21 fl. oz./acre. The results are summarized in Table 3. Significant differences in mite densities are denoted by alphabetic superscripts where means within each column followed by different letters are significantly different.

Densities of both spider mites and beneficial mites were impacted by the Danitol treatment. However, it is important to note that beneficial mite populations often have a lag time in recovering from exposure to pyrethroids, and the full effects of these insecticide treatments may not be seen until next spring. We will be conducting surveys in the spring of 2002 to determine whether beneficial mite populations show long-term effects of Danitol exposure.

Table 3. Counts of pest and beneficial mites in orchards pre- and post-Danitol treatment (# of mites/50 leaves)

	Spider mites*			Beneficials*		Ratio
Date	Egg	Motile	Rust	Egg	Motile	ERM/beneficials
Pre-count - 8/13/01						
Treated	12.1[a]	2.3[a]	10.1[a]	0.28[a]	0.96[a]	2.75:1[a]
Check	18.5[a]	2.7[a]	6.8[a]	0.64[a]	1.24[a]	1.84:1[a]
24-h post - 8/21/01						
Treated	6.1[a]	0.5[a]	42.04[a]	0.02[a]	0.04[a]	9:1[a]
Check	13.6[a]	8.8[b]	71.84[a]	0.04[a]	0.36[b]	18:1[b]
8-wk post - 10/11/01						
Treated	2.2[a]	0.8[a]	55.28[a]	0	0[a]	N/A
Check	10.8[a]	5.9[b]	97.24[b]	0	0.92[b]	5.17:1

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

Bait-and-spray trials: Experiments conducted in 2000 revealed that stink bugs could be concentrated on orchard border plants (mullein) in large numbers using synthetic pheromone. However, our previous attempts to attract-and-kill the insects used Provado, which proved to be ineffective for stink bug control. Trials in 2001 used a more effective toxicant (Carzol). The protocol, the same as that used in 2000, was as follows: Beginning in mid-May (onset of mating/egg-laying for *E. conspersus*), aggregation pheromone lures were placed at 20-foot intervals on host plants (mullein). These baited plants were clearly flagged and remained constant throughout the season. Flagged plants were sprayed to drip using a handgun at weekly intervals throughout the entire egg-laying period (ending in early July). Four blocks were used in the study. Blocks were 400 feet in total length and were paired with 400-foot untreated sections of border.

Large numbers of bugs were attracted to treated plants during this period, and it was not uncommon to see 80 or even 100 bugs on a single mullein plant. Dead bugs were frequently noted beneath treated plants following insecticide treatments. Extensive sampling was conducted during the season following sprays (Fig. 1) and at harvest (Fig. 2), and these results are shown below.

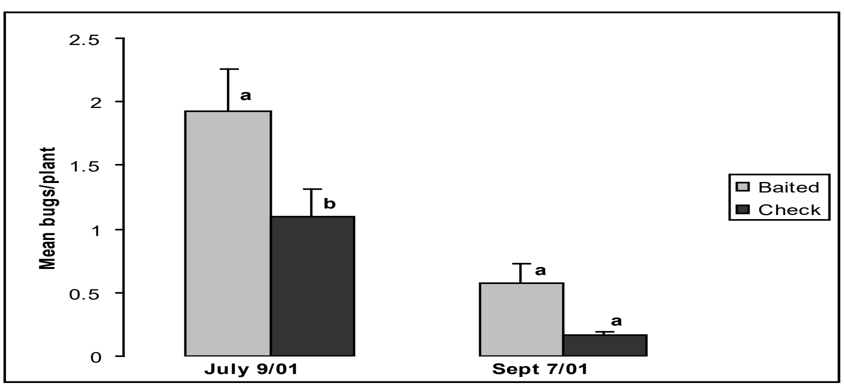


Figure 2. Mean bugs/plant at bait-and-spray and control (check) host plants in treatment areas at mid- and late season. Bars with same letter superscript at each date are not significantly different (P<0.05).

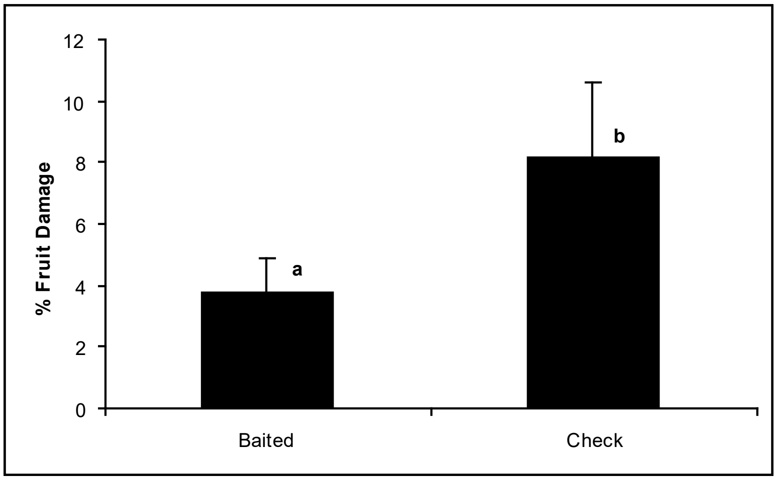


Figure 3. Mean stink bug damage to fruit at harvest on border rows associated with pheromone-baited vs. control border treatments (n=4). Bars with same letter superscript are not significantly different (P<0.05).

Stink bug chronology: Our observations of stink bug behavior have been restricted primarily to daylight hours when these insects were visibly active and moving among host plants, feeding, mating and laying eggs. It was assumed therefore that these insects were primarily active during daylight hours. However, an experiment filming bugs for 24 h in the field revealed that much of stink bug activity, particularly reproductive activity and response to pheromone, increased dramatically at dusk and continued through the hours of darkness. Bug counts at dawn were approximately half of those at their peak.

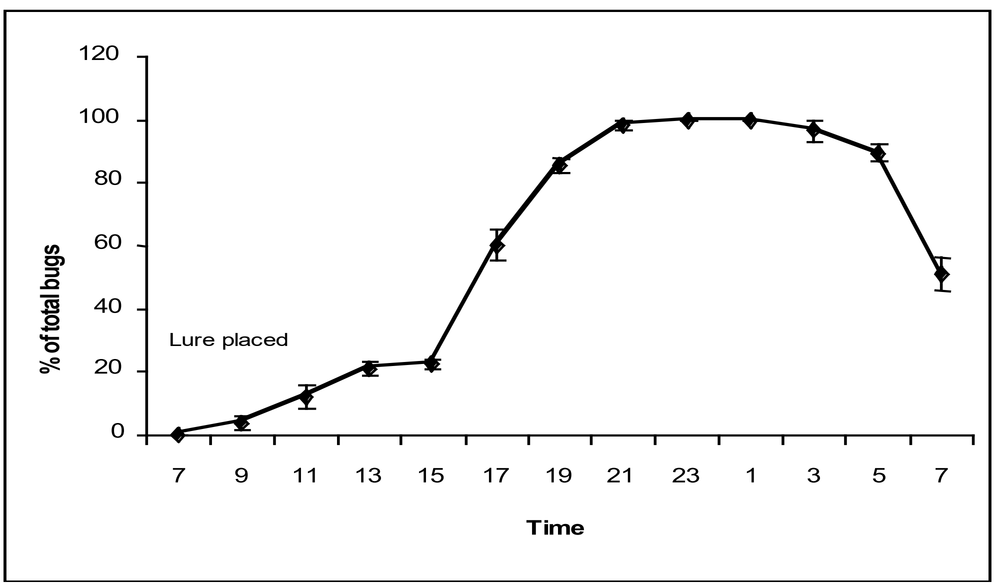


Figure 4. Twenty-four hour chronology of stink bug movement to baited host plants; lure was placed at 7:00 AM and removed 24 h later. Numbers expressed as a percentage of the total numbers of bugs visiting the plant in each case (n=3).

This suggests that our counts at baited lures may in fact be significantly lower than the peak catch of stink bugs. This also suggests that optimal spray timings may be in dusk, instead of the current "default" spray timing of morning or mid-afternoon. Additional research in 2002 will investigate this behavior further as it relates to activities in the orchard, as our observations were conducted on mullein plants on the orchard border.

Active space of pheromone lures: We continued efforts undertaken in 2001 to determine the active space of pheromone lures for both monitoring and control efforts using mark-recapture studies. For each experiment, bugs were released at sites 5, 10 and 25 m from pheromone point sources. A known number of bugs was released at each location (150 bugs/location), with an equal number of males and females at each location. Bugs were marked on the thorax with nail polish to denote the different release distances. Surveys were conducted for eight days following release of bugs, with counts at the release area, the baited plant and various random plants in between. These counts revealed that bugs usually tended to move to the nearest mullein plant to their release area and begin feeding there. The majority of the bugs recaptured were from the 5 m release site (Table 4), suggesting that this pheromone is a short-range communication tool for these insects. This information confirms our observations of attraction in the field and will serve to give us an attractive radius for future trapping and attract-and-kill experiments.

Table 4. Results of mark-recapture study of stink bug adults, showing numbers of bugs recaptured at baited mullein plant from each of three release distances.

	Overwintered generation adults	
Release distance	Total recaptured	% of total
5 m	32	68
10 m	10	21
25 m	5	11

Dose/response experiments: Although the current 'WSU lure,' developed in 1999 and used extensively since, has been shown to reliably attract stink bugs in the field, it is not a practical long-term solution. The plastic caps are tedious to load, leaky, and require large amounts of material. Growers and consultants require an improved system of getting the pheromone to the field. In order to develop a commercial lure for stink bug monitoring and control, we continued studies initiated in 2000 with a variety of release rates. The rates were modified by placing equal volumes of pheromone inside polypropylene vials, then placing upon these vials lids with a variety of hole sizes in them. The hole size dictates the release rate of the pheromone (i.e., larger hole=higher release rate). The treatments ranged from included hole diameters of 0.02", 0.04", 0.063", 0.125", 0.25", and an undrilled vial (check). These lures were prepared by Dr. Peter Landolt at USDA-ARS Yakima.

We were able to find a release rate that did clearly out-perform the others tested (Fig. 5), and this release rate indicates that we are able to attract bugs with far less pheromone than we had previously been using. This translates into lower costs for manufacture of a commercial lure and greater flexibility in release devices, including the possibility of rubber septa.

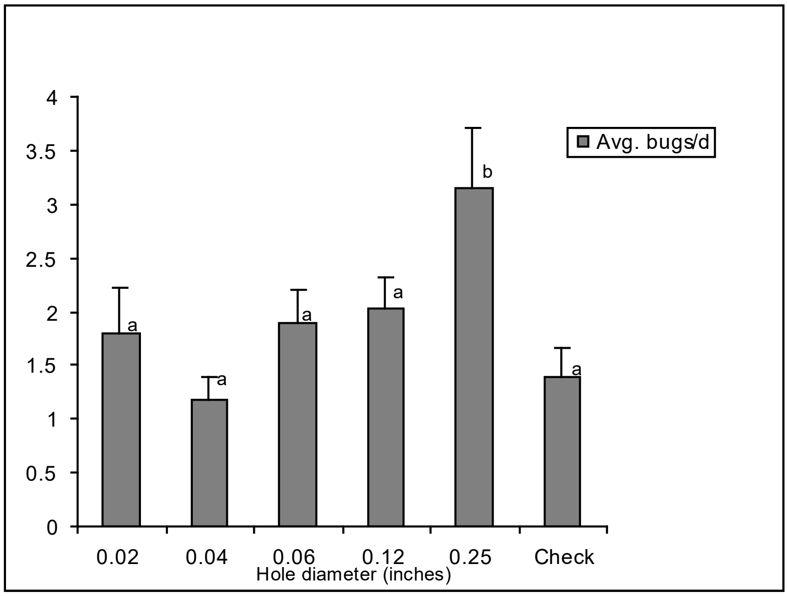


Figure 5. Mean bugs/day attracted to plants baited with synthetic pheromone at a range of release rates, determined by various hole sizes (n=25). Bars with same letter superscript are not significantly different (P<0.05).

Feeding attractant experiments: Experiments initiated early in 2001 revealed that a commercially available fertilizer product was very attractive to stink bugs in an aqueous solution. Where stink bugs were presented a choice between feeding on dental wicks soaked with either water only or a solution of the fertilizer in water, the fertilizer was shown not only to induce feeding but also stimulated bugs to feed longer than a water-only treatment (Fig. 6). This finding raises some possibilities in the realm of managing stink bugs using a bait-and-kill system, where a combination of the attractive pheromone, the fertilizer solution, and a killing agent (i.e., insecticide) could be used on bug-infested borders.

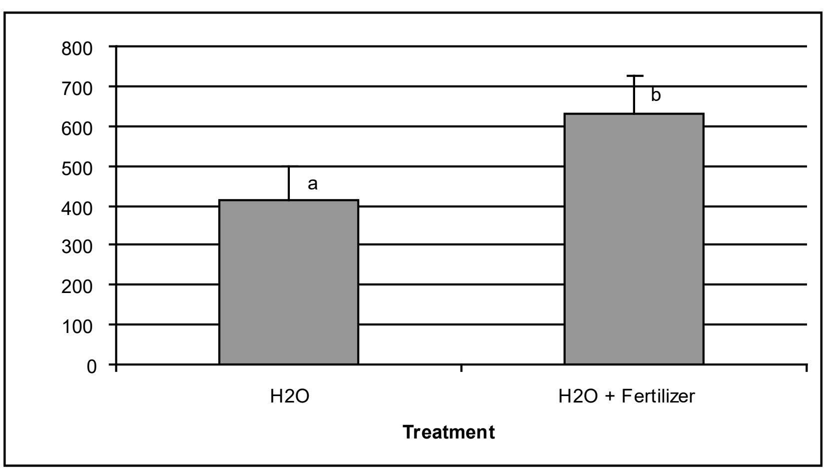


Figure 6. Time spent feeding by stink bugs in arena with 20-minute (1200 s.) cutoff point. Bars with same letter superscript are not significantly different (P<0.05)

Feeding attractant + insecticide experiments: Following the experiments described above, we initiated studies combining the attractive fertilizer with our most efficacious contact insecticide, the pyrethroid Danitol. The objective of the experiment was twofold: 1) to determine the minimal lethal dose of insecticide needed in solution; and 2) to determine whether there was an inhibitory or repellent effect upon bug feeding following addition of the insecticide. As in the previous experiment, the insects were presented with a choice test, and the time spent feeding (Fig. 7) and times until death (Fig. 8) were recorded. These data were recorded from a range of concentrations of Danitol with a check of fertilizer only. For reference, the field rate of Danitol results in a concentration of approximately 100 ppm.

The results of this experiment demonstrate that, while bugs did tend to feed upon the Danitol-treated wicks for less time than the control wicks, the exposure was still enough to cause death. These results are encouraging and suggest that the idea of a bait station that incorporates the attractive pheromone in combination with an insecticide/feeding attractant delivery system may be worth pursuing further.

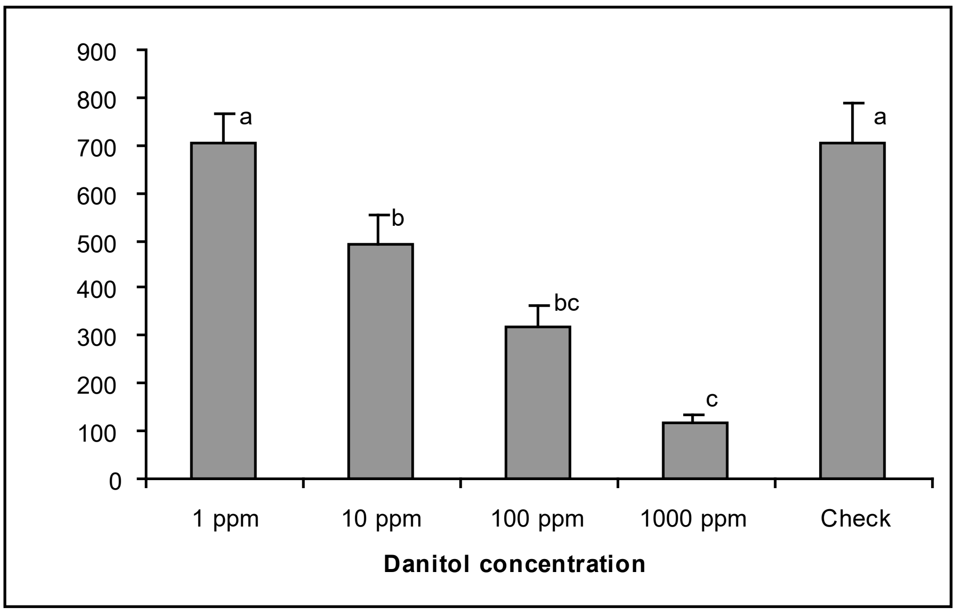


Figure 7. Time spent feeding upon cotton wicks soaked with fertilizer and varying concentrations of Danitol (n=30). Bars with different letter superscripts are not significantly different ($P<0.05$).

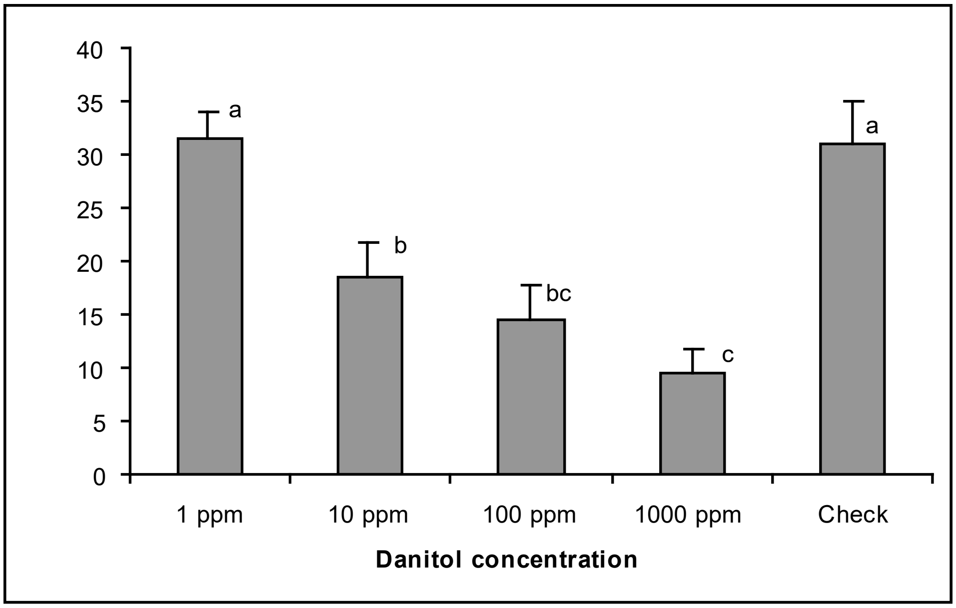


Figure 8. Time elapsed until death of bugs following feeding upon cotton wicks soaked with fertilizer and varying quantities of Danitol (n = 30). Bars with different letter superscripts are not significantly different (P<0.05).

Budget:
Proposed project duration: Termination of current project.
Funding 1999-2001: $154,335
Current year request: $0

FINAL REPORT
WTFRC Project #AE-01-47 **WSU Project # 6093**

Project title: Enhancing biological control of leafrollers through ground cover management and augmentation of alternate hosts for leafroller parasites.

PI: Jay F. Brunner
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David Granatstein, WSU-TFREC, Wenatchee
Nancy Decker, WSU-TFREC, Wenatchee

Cooperators: Tom Unruh and Robert Pfannenstiel, USDA-ARS

Objectives:
1. Determine the effects of cover crops in an apple orchard on the biological control of leafrollers, 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.

Significant findings:
- There was no difference in the level of leafroller parasitism between cover crop treatments. There was no establishment of *Xenotemna* as an alternate leafroller host in alfalfa due to high levels of general predator activity in this treatment. Low levels of parasitism by *Apanteles* sp. were noted in both cover crops, and natural infestations of *Colpoclypeus florus* were detected in both treatments without seeding but at low levels and only in the fall.
- Assessments of leafroller parasitism in 2001 in a nearby organic orchard suggested that treatments of Sevin for fruit thinning were highly detrimental to leafroller biological control in the WVC orchard.
- Leafroller densities increased from low levels in 1999 to very high levels following three years of NO chemical control. Fruit injury was high and there was no difference between plots.
- There were no significant advantages or disadvantages associated with an alfalfa cover crop compared to a grass cover crop relative to most pests. Predatory mites seemed higher and pest mites lower in alfalfa treatments, but spider mites were not a serious problem in either treatment.
- Lygus densities were high in the alfalfa cover crop compared to the grass, but this did not translate into higher injury levels to fruit.
- Horticulturally, there seemed to be no adverse effects from the alfalfa cover crop on tree growth, yields or fruit quality.

Methods:
Cover crop and biological control: Two cover crops were established in one-half acre replicated plots at the Wenatchee Valley College orchard. The cover crops were alfalfa and a standard orchard grass. A non-pest leafroller species, for example, *Xenotemna pallorana*, that can be propagated in the laboratory was introduced into both cover crop. Establishment of the non-pest leafroller species was determined through sampling cover crop plants. Aphid species known to support parasites of the apple aphid were introduced into both cover crops and establishment verified through sampling of cover crop plants.

Mating disruption was used for codling moth control, and leafroller control was maintained only with biological control. Non-pest leafrollers were seeded into the cover crop at regular intervals throughout the summer as a potential alternate host for parasites. Sentinel OBLR larvae were used to determine the establishment of *C. florus* and the activity of other parasites. Sentinel OBLR larvae were placed on trees throughout the different cover crop plots and on trees between the plots, collected after a period of exposure and evaluated for parasitism by *C. florus*. Parasitism of the non-pest leafroller was determined by sampling larvae of this species in the ground cover. The naturally occurring PLR populations in each plot were monitored on a regular interval to determine the level of parasitism in each ground cover treatment.

Population densities and species composition of aphids that establish on the alfalfa and grass cover crops were evaluated by sampling cover crop plants at regular intervals throughout the season. The relative percent parasitism of cover crop and apple tree aphid populations was evaluated by random samples along transects from throughout the cover crop and trees within cover crop treatments and on trees outside the cover crop treatment areas. Kinds of parasites attacking aphids in trees were determined through rearing sub-samples of aphids in the laboratory.

Other pest and beneficial arthropods occurring on the cover crops and in the orchard were monitored with standard methods developed in the SARE project. The level of fruit injury from codling moth, leafroller and other potential fruit pests were determined at harvest. Cover crops were maintained for three years to determine the long-term stability of leafroller biological control in orchards with different cover crop.

Cover crop and horticultural effects: The impact of a cover crop such as alfalfa on apple tree growth and fruit quality was evaluated by comparing shoot growth, yield, fruit size and fruit quality. Shoot growth assessments were taken at the end of the summer growth period from trees pre-selected in spring. Fruit size and quality were determined by collecting samples from pre-selected trees in each cover crop at harvest and making standard measurements. Yield was determined by measuring the number of fruit (bins) collected from each cover crop plot at harvest over time. All horticultural practices were maintained the same on trees within each cover crop plot.

Soil fertility and tree nutrition: Levels of nitrogen, and possibly other minor elements, were evaluated at regular intervals in different cover crop plots using standardized methods. Leaf nutrient levels were evaluated twice each year using standard methods to determine differences between cover crop plots.

Results and discussion:
During the spring of 1999, a 5th leaf Fuji apple (BC#2/M9, 800 TPA) orchard block at the WVC Grady Auvil Teaching and Demonstration Orchard was divided into six plots (for two treatments, alfalfa and grass, with three replications each). Each plot exceeded one acre in size and a core area for sampling in the center of each plot, approximately .25 acre in size, was delineated. A “star” configuration with a transect from the border of each plot to the approximate center was designated as the “sample area.” Thirty trees were selected within the star configuration and numbered 1-30. The orchard is farmed utilizing pheromone-based mating disruption for codling moth. Other than one delayed dormant superior oil treatment, no pesticides were applied.

Overview of progress and observations:

Leafrollers: During 1999, no pandemis larvae were detected in samples and hence no parasitism were observed. In 2000 a total of 29 of 900 buds (3%) examined was infested with pandemis leafroller larvae, and parasitism by *Apanteles* occurred in 12.5% of the larvae. In 2001 levels increased to 43% infested buds in both treatments, and parasitism by *Apanteles* was 5% in the grass treatment and 2.1% in the alfalfa treatment. During early May (petal fall stage), 100 leafroller-infested shoot tips were collected from each plot, and leafrollers were reared in the laboratory. During 1999 no parasitism was observed. In 2000 Pandemis leafroller parasitism by *Apanteles* was 3.3% in the alfalfa treatment and 5.0% in the grass treatment. In 2001, leafroller parasitism by *Apanteles* was 11% in alfalfa and 7% in the grass treatment.

Shoots high in the tree canopy were inspected (300 shoots per plot) to determine leafroller infestation levels from mid-May through late August. In 2000 infestation of leafrollers was 7% for both treatments in early spring but increased to an infestation level of 90% for both treatments by mid-July. Leafroller infestation appeared two months earlier in 2000 than in 1999, and leafroller counts were five times higher than in 1999. In 2001 early spring infestation was 42% for both treatments and increased to dense infestations of 95% by mid-July.

Sentinel leafroller larvae (OBLR) were placed in plots to assess the occurrence of parasites. In 1999, 2000 and 2001 a Braconid, *Apanteles sp.,* and Tachinids were detected in all plots from July through mid-September. Total parasitism of sentinel OBLR reached levels as high as 80% in 1999 and 86% in 2000. The presence of *Apanteles* was detected in both treatments and much earlier in 2000 than in 1999 when it was observed in alfalfa treatments only. *Colpoclypeus florus* was observed in both treatments from early September through early October in both years. During 1999 *C. florus* was detected only in the alfalfa treatment. This parasitoid occurred naturally in the orchard without seeding. In 2001 the sample trees in an organic block were included as part of this study in order to determine differences between parasitism by *C. florus* in untreated trees and the experimental plots where a spring application of Sevin had been used. Parasitism of sentinel OBLR by *C. florus* (33%) was observed in the organic block beginning in late August and increased to 89% by mid-September. Parasitism by *C. florus* in the grass treatment occurred by early September but remained below 5%. In the alfalfa treatment, parasitism by *C. florus* occurred by late August and reached 90% by early September. Tachinids were detected in all plots from early July through late August. Parasitism by *Apanteles* was detected 30 days earlier than in 2000; however, the percent parasitism remained the same with no differences between treatments.

During the summer of 2000, *Xenotemna pallorana* were released in the alfalfa cover crop on a weekly basis in an attempt to establish a host population for *C. florus*. Although *Xenotemna* was an adequate host for *C. florus* under laboratory conditions and preliminary field studies, we could not establish populations in the orchard. This was most likely due to high levels of natural mortality in the orchard by large numbers of general predators.

During the 2001 season the strawberry leafroller (*Ancylis comptana fragaria*) was substituted for *Xenotemna* as a possible host for *C. florus.* A colony of strawberry leafroller was established on 400 Quinault strawberry plants in the greenhouse and seeded with early instar *C. florus* larvae. When the larvae reached a mature stage, the plants were moved to the orchard and placed in the tree rows. Endemic pandemis larvae were collected every 10 days and brought to the lab for rearing out in order to determine if *C. florus* will move into the tree canopy to parasitize pandemis. One plot of each grass and alfalfa treatment served as the control. The first pandemis samples collected (early and mid-August) following the seeding of *C. florus* in the orchard indicated 0% parasitism by *C. florus*. One hundred percent of leafrollers collected were parasitized by either *Apanteles* or Tachinids. By late August and early September, only 6% of parasitism was by *C. florus* in both treatments. No parasitism by *C. florus* occurred in the control plots. Spring 2002 pandemis sampling will determine if *C. florus* was able to overwinter on the strawberry leafroller host and whether the parasitoid moves into the tree canopy in early spring. Additional research with the strawberry leafroller as an alternate host for *C. florus* is planned but under a different project directed by Tom Unruh and funded by IFAFS/RAMP.

It became apparent that cover crops did not appreciable enhance leafroller parasitism. This is likely due in great part to the use of Sevin as a fruit-thinning agent in both treatments. Sevin is known to be highly toxic to *C. florus* (Brunner et al. 2001), but its impact on other parasitoids is not as well understood.

Western tentiform leafminer and parasitism: In late June leaves were sampled for western tentiform leafminer (WTLM) to determine the density of this pest and to assess levels of parasitism. No leafminers were observed during the summer of 2001. In 2000, WTLM appeared 1.5 months earlier than in 1999, and parasitism by *Pnigalio flavipes* increased from 15% (1999) in both treatments, to over 70% in 2000. The average number of mines per leaf increased from <2 (1999) to 3-4 mines per leaf in 2000 but then declined again in 2001 to non-detectable levels in both treatments.

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 mid-June and early August revealed densities of <1 WALH per leaf. WALH counts made in August 2001 were five times greater in the grass compared to the alfalfa treatment, whereas in 2000 WALH counts were higher in the alfalfa treatment.

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 counted under magnification. The active stages and eggs of phytophagous and predaceous mite species were recorded. In 1999 and 2000 spider mites were higher in the grass compared to the alfalfa treatment while predatory mites were higher in the alfalfa treatment. In 2001 spider mites appeared first in late July, one month later than in 2000; predatory mites were three times higher in the grass compared to the alfalfa treatment by the late August peak, and no *Zetzellia mali* were observed.

Campylomma, lygus, thrips and stink bug: Another measure of arthropod presence and density within each cover crop treatment was made by four limb tap samples during the months of May and June. Campylomma densities were very low in 1999, increased by six times in 2000 although no fruit injury was noted, and were near zero in 2001, again with no fruit injury detected. Thrips densities decreased slightly from 2000 with no difference between treatments. In the three years of the study there were no lygus or stinkbug detected on beat trays nor was there any fruit injury noted by these pests. However, lygus was present and detected in sweep net sampling of the orchard cover crop. Beneficial insects observed included ladybeetle larvae and adults, parasitic wasps, arachnids, lacewing nymphs and adults, syrphid flies and deraeocoris.

Apple grain aphid (AGA), green apple aphid (GAA) and rosy apple aphid (RAA): Aphid density was 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 mid-July of 2001, densities of more than five infested leaves per shoot reached a peak of 66% in alfalfa and 45% in grass treatments, the same trend as in 2000 but with three times higher populations in both treatments. Aphid densities subsided to less than 1% by mid-August 2001 for both treatments. An abundance of lady beetles, damsel bugs, syrphid flies and brown and green lacewing was present from late May onward.

In 2001, active RAA 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 3% by late July and declined to 0% by early August, representing no change in RAA populations from 2000 counts. While RAA aphid densities seemed to be consistently higher in the alfalfa treatment, there was no impact on tree health or crop yield.

Aphid predator counts: Aphid predator counts were done from mid-July through mid-August. Aphid predators were more abundant in the alfalfa treatment with numbers peaking in early August of 2001. Predators observed include Damsel bug, deraeocoris, lacewings, ladybeetles, lygus, spiders, syrphid flies and minute pirate bug.

Overwintering arthropods: Overwintering arthropods were trapped inside one-inch wide cardboard strips wrapped around the trunk near the crown on each of 30 sample trees per plot during early September. Bands were collected in late October and various species counted. For 1999, the overwintering beneficial insect population was three times higher in the alfalfa treatments than in grass treatments. Pest arthropods collected were noctuids, lygus, Lygaeidae, winged green apple aphid, leafhopper and Arctiidae. Beneficial arthropods included Aphididae, Heerobiidae, Chalcidoidea, green lacewing, Stethorus, Deraeocoris sp., Nabid nymph, and Syrphidae. Miscellaneous arthropods included Collembola, weevil, Carabidae, Hymenoptera and Acarina. Total 1999 arthropod counts averaged 9.7 in grass treatments and 38.7 in alfalfa treatments. Data in 2000 indicated an increase of overwintering arthropods with no difference between treatments. Data from 2001 are not yet available.

Nutrition and leaf analysis: Pre-bloom ammonium nitrate 34-0-0 was applied to all treatment plots at .175 lbs. per tree or 45-50 lbs. of actual N per acre. No other fertilizers were applied. Leaf analysis in July of 2001 indicated that nitrogen levels in both treatments were 2.9%, which is slightly higher than 1999 and 2000 levels and exceeds optimum N ranges. Zinc levels in both treatments were 13 ppm in 2001, and this represents no change from 1999 and 2000 zinc levels.

Cover crop N content and biomass: 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 nitrogen. Cascade Analytical results for 2001 in the alfalfa treatment showed 5% total nitrogen, an excess N value which is slightly higher than in 2000. The nitrogen value for the grass treatment was 2.7%, which represents no change from 2000 N values.

Cover crop composition: During the second year of this project, various weeds invaded the primary cover crops of alfalfa and grass. In an attempt to reestablish a more homogenous cover, reseeding of the alfalfa and grass plots was done in early spring of 2001 and a broadleaf herbicide (2-4 D) was applied. By the end of the third year, both grass and alfalfa were well established and dominated the cover. Analysis of the resulting cover composition was done by recording species observed within 10 randomly chosen 1-meter areas per plot. By mid-July alfalfa and grass composed nearly 90% of the cover in their respective treatments.

Trunk growth measurements: During April of 1999, 10 groups of five trees per treatment plot were chosen based on uniform characteristics. Trunk circumference 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 were taken during April of 2000 and April of 2001. An average 2.2 cm growth was measured for the trees in both treatments.

Shoot growth assessment (vigor): The impact of grass vs. alfalfa treatments on apple tree growth is being evaluated by shoot growth assessments taken at the end of the grand growth period. Ten trees per plot were preselected, 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. Terminal shoot growth was greatest (14.3 cm) in the grass treatment, lateral shoot growth the same (6.3-6.9) for both treatments, and bourse shoot growth greater (10.1 cm) in the alfalfa treatment.

Fruit insect damage and horticultural effects - cull analysis: Data for 2001 have not yet been summarized.

Soils: General soil fertility is assessed each April and September. To assess soil, chemical and colloidal properties, samples will be subject to a complete nutrient analysis (N, P, K, S, Mg, Ca, Mn, B, Zn, Mo, Cu, Fe) and measure of pH and OM%. Separate samples were taken from the drive row and the tree row at a 12-inch depth using a 10-core composite for each treatment plot. Early spring 2001 soil N values were deficient in the alfalfa treatment and at optimum levels in the grass treatment. September 2001 soil N values remained the same for both treatments.

Budget:
Enhancing biological control of leafrollers through ground cover management and augmentation of alternate hosts for leafroller parasites.
Jay F. Brunner
Project duration: 3 years
Current year: 2001
Original budget request: $23,300

Year	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**Total 1999-01**
Total	$ 23,300	$ 26,202	$ 10,820	**$ 60,322**

Current year breakdown

Item	**Year 1 (1999)**	**Year 2 (2000)**	**Year 3 (2001)**	**Total 1999-01**
Salaries	0	18,540	7,500	26,040
Benefits (%)	0	5,562	1,820	7,382
Wages	15,000	0	0	15,000
Benefits (%)	2,400	0	0	2,400
Equipment	0	0	0	0
Supplies	5,000	1,000	1,000	7,000
Travel	900	1,100	500	2,500
Miscellaneous	0	0	0	0
Total	$ 23,300	$ 26,202	$ 10,820	**$ 60,322**

FINAL REPORT

Title: Development of Feeding Atttractants as Monitoring Lures for moth pests of apple.

PI: Peter J. Landolt, USDA-ARS, Wapato, WA

Co-PI(s): Jay Brunner and Mike Doerr, WSU, TFREC, Wenatchee

Objectives:

2001 Objectives

1. Use feeding attractant to monitor *Lacanobia*, *Pandemis* leafroller and codling moth through season.
2. Compare feeding attractant to pheromone lures for monitoring.
3. Relate moth capture to egg hatch and to larval densities.
4. Pursue improvements in feeding attractant lures.

Significant Findings:

1. A controlled release system was developed for the feeding attractants that include acetic acid and 3-methyl-1-butanol.
2. A dry trap (Universal Moth Trap) was found to be superior in capturing *Lacanobia* moths attracted to the feeding attractant.
3. A dry trap (large Delta) was found to be superior in capturing *Pandemis* leafroller moths attracted to the feeding attractant.
4. Most female *Lacanobia* moths are captured before they have laid many or none of their eggs.
5. A preliminary assessment of the data indicates that *Lacanobia* and *Pandemis* captures in feeding attractant traps correlate better with larval numbers that do captures in pheromone traps.
6. Best placement of traps baited with feeding attractant for *Lacanobia* is in the upper tree canopy.
7. In all tests, results for spotted cutworm and bertha armyworm were quite similar to results obtained for *Lacanobia*.

Methods:

Gravimetric (weight loss) studies were done on a series of polypropylene vial sizes, and with a range of vial lid hole diameters to determine release rates of acetic acid and 3-methyl-1-butanol from vial dispensers.

A long series of trapping experiments were conducted, using the feeding attractant dispensed from vials to evaluate trap designs, trap placement, vial hole diameters, lure component release rates, lure release rates, and additional fermentation chemicals.

Blocks of 3 to 5 acres of apples were monitored from April to October for *Pandemis* leafroller, *Lacanobia* fruitworm and codling moth. Monitoring was with sex pheromone traps and feeding attractant traps, with all traps checked twice per week. During both generations, plots were sampled for leafroller and *Lacanobia* larvae, and apple fruit infested with codling moth larvae. Leafroller larvae were sampled by visual searching for damage and for rolled leaves. Lacanobia larvae were sampled by limb knocking of larvae onto a sheet on the ground. Codling moth sampling was done by visual searching of apple fruit.

Results and Discussion:

Lure and Trap Optmization

An optimum feeding attractant monitoring system for *Lacanobia* fruitworm was developed. The recommended lure is a pair of 8 ml polypropylene vials, each with a hole in the lid of each that is 3 mm in diameter. One vial possesses 5 ml of acetic acid on cotton, the other vial possesses 5 ml of 3-methyl-1-butanol on cotton. The vials are suspended right-side-up in the bucket of a Universal Moth Trap with a piece of Vaportape to kill captured moths. Traps can be all green or multi-colored Universal Moth Traps, but should be placed in the upper canopy of orchard trees to capture maximum numbers of *Lacanobia* moths. A commercial prototypeof the lure has been field tested and performed comparable to our research lure. The recommended lure should last at least 4 weeks.

This system provides a strong lure and trap system for *Lacanobia*. It is also attractive to other moths however, primarily Noctuidae. This makes monitoring difficult because captured moths must be sorted and the *Lacanobia* moths recognized. This lure and trap was evaluated in a variety of habitats throughout the season to determine what types of moths are trapped (Landolt and Hammon in press). In apple orchards, moths captured are primarily *Lacanobia*, bertha armyworm, and spotted cutworm, while many other species are captured if traps are placed in natural habitats. For this reason, it is recommended that traps be placed well within orchard blocks, in order to minimize capture of non-target moths. Few insects in addition to noctuid moths are captured in these traps and the use of a dry trap makes moth identification much easier than in the previously tested wet trap (Agrisense Dome or Trappitt trap).

An optimum feeding attractant system for *Pandemis* leafroller was also developed. The recommended lure is a single 8 ml polypropylene vial with a hole in the lid that is 3 mm in diameter. The vial possesses 5 ml of acetic acid on cotton and is placed within a large Delta style sticky trap.

An optimum feeding attractant system was also developed for the codling moth, but appears to be too ineffective to be useful. It is comprised of a single 8 ml polypropylene vial with a hole in the lid that is 1 mm in diameter. The vial possesses 5 ml of acetic acid on cotton and is placed within a Pherocon Wing trap.

The development of a dispenser for acetic acid permitted the testing of dry trap designs. The original method of dispensing acetic acid was to place it in the drowning solution of a wet trap. Moths captured in these wet traps were very difficult to identify and were prone to rapid decomposition during hot weather. The dispenser led to the identification of effective dry trap designs for all three species of moths and made the use of the wet traps obsolete.

Comparison of Monitoring Methods

Results of season long monitoring of Lacanobia moths in orchards provided comparisons of pheromonal and feeding attractant monitoring systems. With both types of lures, the general phenology of *Lacanobia* was evident, with two distinct flights of moths. Numbers of moths captured were adequate with both lures at all sites to track moth phenology. However, there was considerable variance in the relationship between numbers of moths captured in feeding attractant traps and numbers of moths captured in pheromone traps. That is, in some blocks many more moths were captured in pheromone traps and in some blocks numbers of moths captured were comparable with the different lures. Although the data analysis is preliminary at this point in time, statistical analyses of trap catch results for *Lacanobia* moths indicates a much stronger correlation between numbers of moths captured in feeding attractant traps versus pheromone traps and numbers of larvae found in the sampling of tree foliage. These data will be combined with earlier sampling done for *Lacanobia* by Mark Hitchcox and work conducted by Jay Brunner's laboratory to see if this pattern is consistent.

Numbers of *Pandemis* leafroller moths captured in feeding attractant traps were consistently less than numbers in pheromone traps. However, these numbers were still sufficient to track the phenology of the moth through the season, with both types of lures. Additionally, as with *Lacanobia* moths, there was consistently a stronger correlation between numbers of *Pandemis* leafroller moths captured in feeding attractant traps versus pheromone traps and the numbers of leafroller larvae found in searches of orchard blocks.

<u>Improvement of Chemical Blends as Feeding Attractants</u>

A number of additional chemicals were tested in combination with acetic acid or added to the combination of acetic acid and 3-methyl-1-butanol. These included reassessing terpeneols, testing of several plant compounds reported in early literature to be attractive to codling moth, and developing and testing slow release formulations for carbon dioxide. These added chemicals did not consistently increase the capture of *Lacanobia, Pandemis*, or codling moth in traps baited with either acetic acid and 3-methyl-1-butanol or acetic acid.

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.

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

Landolt, P. J. and P. Hammond. Captures of non-target moths in traps baited with acetic acid and 3-methyl-1-butanol. J. Lepid. Soc. (In press)

Budget:

Development of Feeding Atttractants as Monitoring Lures for moth pests of apple.
Peter J. Landolt,
Project Duration: 1999-2001
Current Year: 2002

Year	Year 1 (1999)	Year 2 (2000)	Year 3 (2001)
Salary	15,350		17,000
Benefits	5,100		
Supplies	2,500		4,000
Travel	750		1,000
Total	22,750	23,700	22,000

Project cost 1999-2001: $48,450

FINAL REPORT:

Title: Identification of chemical attractants from apple foliage and fruit for codling moth.

PI: Peter J. Landolt, USDA-ARS, Wapato, WA

Objectives:

2001 Objectives

Test chemicals for responses of codling moth females
Test chemicals for responses of codling moth larvae
Study moth responses to host odor
Repeat GC-EAD work with walnut

Significant Findings:

1). Codling moth larvae are more strongly attracted to apple fruit that is infested and are not-attracted in laboratory assays to the odors of apple fruit that are undamaged.
2). Codling moth larvae are attracted to three different apple kairomones, but in all cases, amounts of the chemicals required for attraction are dramatically higher than that released by apple fruit.
3). Codling moth adults are more strongly attracted to apple fruit that is infested compared to un-infested fruit.
4). Trapping of male and female codling moth with kairomones was significantly improved in a preliminary trapping test using 2 and 3 component blends of compounds found in induced apple fruit, compared to compounds tested individually.
5). Codling moth adults are attracted to the odor of un-infested Bartlett pear fruit and similarly to the odor from cut Bartlett pear.

Methods:

Codling moth female responses to fruit, fruit odor, and fruit chemistry were evaluated both in a flight tunnel and in the field. Flight tunnel assays included responses to fruit, fruit odors piped into the tunnel, and to solvent washes of fruit. Larval responses to fruit odors and fruit chemistry were evaluated in Y-tube and parallel tube olfactometers.

Volatile collections were made to characterize the odorants released from uninfested and infested immature apple and pear fruit at 2-week intervals through the season. Most of these odorants were characterized, using GC-MS. GC-EAD was used to determine which of the numerous compounds found in apple and pear odor are detected by codling moth females.

One of the chemicals tested is not commercially available and was isolated from an essential oil of a different plant and was purified using both preparatory column and HPLC methods.

Results and Discussion:

Results of flight tunnel assays of female codling moth responses to fruit, fruit odors, fruit washes, and fruit kairomones yielded interesting biological results but no clear identification of the kairomonal attractant from infested immature apples. Codling moth adults were attracted to immature apples, but more so to apples that were infested by codling moth larvae. Moths were similarly attracted to odors of apples piped into the flight tunnel, indicating that the attraction is a response to volatile chemicals from the fruit. Female codling moths were also attracted to the odor of Bartlett pear fruit in the flight tunnel and similarly to the odor of damaged Bartlett pear fruit.

Results of trapping experiments that tested apple fruit kairomones were mixed, but did yield a blend of compounds that attracted more male and female codling moth than any of the single chemical treatments, which included the pear ester. As reported by others for the pear ester, there appeared to be more varied performance of kairomonal lures in the second generation in apple which obscured results when attempts were made to replicate tests through the season (that is, attraction results seen early in the season were sometimes not repeatable later in the season).

Field tests of synergism of codlemone and apple fruit kairomones did not yield any evidence of the enhancement of trap catch over that of codlemone alone. These tests were conducted during both codling moth flights, with similar results. However, this experiment was done with a limited set of apple fruit kairomones and will be pursued further with additional chemicals and over ranges of release rates. Some fruit kairomones significantly reduced male response (trap catch) to sex pheromone.

Laboratory olfactometer tests of neonate larval responses yielded a series of positive findings. In the first series of tests, it was determined that larvae were not significantly attracted to the odor of fresh-picked apple fruit, unless the apple fruit was infested. Larvae were attracted to the odor, however, of cold-stored thinning apples. Studies to isolate and identify the active ingredients in apple odor are still incomplete but indicate strong activity in two different liquid chromatography solvent fractions (one polar and one non-polar) obtained from volatile collections from infested, fresh-picked apples.

Testing of several chemicals known to be present or increased in the odors produced by infested apples yielded positive responses. E,E-alpha farnesene was identified in New Zealand (Sutherland and Hutchins 1972, Nature 239: 170) as an apple-produced attractant for codling moth larvae. We demonstrated upwind attraction of larvae to this compound at relatively high release rates. Such release rates occur from stored fruit but not immature fresh fruit. This compound may be part of a blend of compounds from apple that are attractive to larvae. Similar results were obtained with a second chemical identified in Europe as likely to be a codling moth attractant in apple. This chemical is not commercially available and was purified for use in assays from an essential oil. Again, attraction of larvae to this chemical was demonstrated, but at release rates that are high compared to our measurements of the compound from attractive apple fruit. Neonate codling moth larvae also responded to the pear ester, ethyl (E,Z)-2,4-decadienoate, at higher release rates. In addition, this response appeared to be limited to a turning response (Y-tube assay) and was not seen in a parallel tube olfactometer test designed to look at upwind forward movement. Experiments are underway to test for evidence of synergism among these chemicals as attractants for codling moth larvae.

Chemical analyses of volatile collections from apple and pear fruit clearly indicate a strong effect of codling moth larval infestation on the odor profile of these fruit. The number of chemicals produced and emitted is increased in response to codling moth damage and the total amount of volatiles emitted is increased greatly. This provides not only more odorants for the codling moth to detect and respond to, but provides a stronger signal that the insect may be able to more readily detect at some distance.

References:

Landolt, P. J., R. W. Hofstetter, and P. S. Chapman. 1998. Neonate codling moth larvae (Lepidoptera: Torticidae) orient anemotactically to odor of immature apple fruit. Pan-Pacific Entomol. 74: 140-149.

Landolt, P. J., J. A. Brumley, C. L. Smithhisler, L. L. Biddick, and R. W. Hofstetter. 2000. Apple fruit infested with codling moth are more attractive to neonate codling moth larvae and possess increased amounts of E,E-alpha farnesene. J. Chem. Ecol. 26: 1685-1699.

Reed, H. C. and P. J. Landolt. Attraction of mated female codling moths, *Cydia pomonella* L. (Lepidoptera: Tortricidae), to apples and apple odor in a flight tunnel. Florida Entomol. (In press).

Budget:
Identification of chemical attractants from apple foliage and fruit for codling moth.
Peter J. Landolt
Project Duration: 1999-2001
Current Year: 2002

Year	Year 1 (1999)	Year 2 (2000)	Year 3 (2001)
Salary		21,200	34,000
Benefits		6,700	
Supplies		6,000	5,000[2]
Travel		500	
Total	29,000	34,400	39,000

This work complemented funding from and efforts made under IFAFS and RAMP grants in 2001.

Total Project funding: $102,400

FINAL REPORT

Project Title: Maintenance of Guthion Registrations on Pome Fruits: Fruit Residue Reduction through Spray Timing Optimization

PI: Allan S. Felsot
Organization: Food & Environmental Quality Lab, Department of Entomology, WSU, Tri-Cities

Co-PI(s): Vince Hebert, FEQL, Department of Entomology, WSU-TC
Doug Walsh, FEQL, Department of Entomology, WSU-Prosser

Cooperators: Keith Klingele, Klingele Orchards, Prosser, WA
Keith Oliver, Olsen Brothers Ranches,, Prosser, WA

Objectives:

- Measure decline of Guthion (azinphos-methyl) apple residues in an orchard when applied one, two, or three times during the growing season.
- Measure Guthion apple residues in composited and single-serving fruit samples at harvest following one, two, or three applications.
- Measure Sevin (carbaryl) residues in composited and single-serving fruit samples at harvest.
- Develop an empirical phenological (degree-day) based fruit pesticide residue decline model that predicts the best application timing window to give the lowest possible residue concentration.
- Validate the fruit pesticide residue decline model by measuring Guthion residues in fruit harvested from commercial orchards receiving variable number of applications. Sevin residues would also be measured.
- Determine distribution of residues in pulp and on peel.
- Analytical Methods/GLPs/Quality Assurance development.

This project was initially funded for two years outside of the normal funding cycle in conjunction with a proposal submitted to the Washington State Commission for Pesticide Registration (WSCPR). In December 2000, a formal proposal was submitted to the WTFRC for consideration of continued funding, and a second proposal was also submitted to the WSCPR. Our goal has been to delineate how application practices affect the residues of insecticides on harvested apples. Furthermore, we sought to discover how application practices affect the magnitude of residues in individual apples, which are known as “single serving” samples. Ultimately, we wanted to build a kinetic model to predict residues at harvest given any application date throughout the growing season.
Because we were not on the normal funding cycle, we did not start the project until June 2000; we continued the project during the 2001 season. The project is somewhat unique in that the magnitude of residue studies (corresponding to objectives 1, 2, and 3) are being conducted under Good Laboratory Practice Standards, thereby requiring outside auditing of all of the procedures and data. We will be finishing all analyses of Guthion and Sevin residues on collected apples prior to June 2002. Work is ongoing for all listed objectives.

Significant Findings:

- Residues on apples collected within 24 hours of spraying did not significantly differ among spray treatments, which included one, two, or three Guthion cover sprays. Thus, for all practical purposes initial residues are the same when one-month intervals occur between sprays.
- Estimated half-lives (time to 50% loss of any given amount of residue) ranged from 17.8 to 19.7 days, but were not significantly related to number of sprays.

- Expression of residue data on a surface area basis (rather than a proportional weight basis, such as ppm or µg/g) only affected the relative magnitude of the residue after the first spray when the surface area:weight ratio ranged to 1.6.
- Average residues at harvest on composited apples and single serving apples were not significantly different. Furthermore, residues on single serving apples were at most three-fold above the average residues. However, only 7-8 apples out of 27 analyzed for each spray treatment were above the composite average.
- All residues, including those on the single serving apples were significantly below the Guthion tolerance of 1.5 ppm.

Methods:

During crop year 2000, a protocol for the field work was written for the conduct of the study consistent with GLPs. One, two or three applications of Guthion 50W (1 lb AI/acre) were made to a block of Rome apple trees on the Klingele Orchard (near Prosser). Sevin was applied by the grower. The experimental design was a random block with three replications consisting of 15 trees in each replicate plot. 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 were analyzed separately from apples collected on any other tree. The intention of this sampling technique was 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. Nine apples from the harvest day collections were composited in a food processor (called composite samples); magnitude of residue studies usually rely on composite samples. An additional 27 individual apples from each spray treatment (i.e., nine from each of the three replicates associated with a spray treatment) were processed as single servings. These apples represent exposures estimated for acute dietary risk assessments.

During crop year 2001, a GLP-compliant protocol was developed and approved for studying the effect of application number on Sevin residues. The study site was a block of Gala apples at the Olsen Brothers Ranches (near Prosser). Treatments were one spray during bloom, one spray after petal fall, and one spray three days prior to harvest. The treatments were arranged in a random block design with three blocks, and number of spray treatments were assigned randomly to each block. Apples were collected at various time intervals after application, and then they were collected at the manager's recommended harvest time.

In late September 2001, apples were collected along a transect of 50 trees in 11 commercial orchards. Apples were separated into 5 bags of 10 apples each, which werer stored at -20°C. Apples from four of the bags will be processed as composites, and apples from the remaining bag will be analyzed as single servings.

Analytical methods were developed and modified for Guthion residues in single serving samples and composites. Furthermore, analytical methods were developed and validated for distinguishing Guthion residues on peels and in pulp. Methods for Sevin analysis are currently under development. All field and analytical procedures were conducted under a GLP protocol, and field phases of the Guthion and Sevin studies were audited by an independent auditor.

Results and Discussion:

Residue Decline of Guthion After One, Two, or Three Applications. Residues recovered within 24 hours after Guthion application ranged from 1.36-1.47 parts per million (ppm or microgram per gram, µg/g) but did not differ significantly among treatments (Table 1). On a surface area basis, residues varied from 0.85 µg/cm2 (one spray) to 1.31 µg/cm2 (three sprays). This comparatively larger difference between initial residues on a surface area basis is due to the surface area to volume ratio differences as the fruit matures (Figure 1). The perspective of surface area coverage, as opposed to a weight based residue concentration, has implications for insect control because the codling moths and newly hatched larvae would be absorbing surface deposits. The lethal residue to 95% (LR95) of codling moth adults ranges from 0.31 to 0.72 µg/cm^2 (Howell and Maitlen 1987) for 96 hour and 48 hour exposures, respectively. Thus, average surface area deposits in our study were close to the baseline for efficient control. By 28 days after the first spray, residues on a surface area basis had declined to 0.19 µg/cm2, far below the level needed for adequate control. The residues recovered would not have been sufficient to control 95% of the codling moth larvae (LR95 = 1.17 µg/cm^2, Maitlen et al. 1985).

Table 1. Effect of number of sprays and pre-harvest interval on initial residues of Guthion, residues at harvest , and dissipation half-life.

Treatment	24-h Post Spray Residue (ppm)	Pre-Harvest Interval (Days)	Residue at Harvest (ppm)	Residue Dissipation Half-Life (days)
One Spray	1.362	99	0.033	17.8
Two Spray	1.573	71	0.110	18.5
Three Spray	1.469	41	0.360	19.7

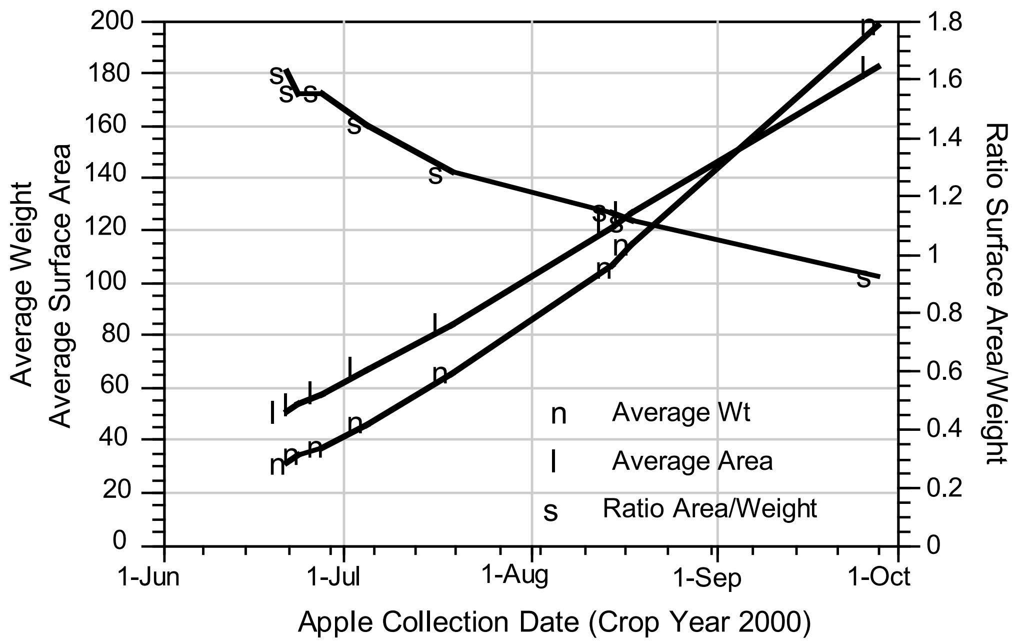

Figure 1. Relationship between average apple weight (grams) and surface area (cm^2) for apples collected on selected dates.

Half-life of Guthion was not affected by the number of sprays (Table 1), but was prolonged relative to the average 10 days reported for foliage (Maitlen et al. 1985).

Measure Guthion apple residues in composited and single-serving fruit samples at harvest following one, two, or three applications. Residues on apples at harvest following the three-spray treatment were at least five fold below the tolerance of 1.5 ppm. A reduction of the pre-harvest interval by about 28 days was associated with approximately a three-fold increase in residues.

Average residues from single serving samples (n=27) were not significantly different than average residues from composited apples (n=9) (Table 2), but variability was greater as evidenced from a comparison of standard deviations. However, the residue concentrations ranged at most three-fold above the average residues, which is on the low side of the range of 2-13 reported for several other single serving studies (Hamey and Harris 1999) (Figures 2-4).

Determine distribution of residues in pulp and on peel. Analysis of samples collected during September 2000 showed that nearly all of the residues reside on the peel. Diffusion of residues into the pulp seemed insignificant by comparison.

Table 2. Comparison of average residues for individually analyzed apples ("single serving apples") and composited apples collected at harvest.

Treatment	Single Serving Apples	Composite Apples
One Spray	0.03 ± 0.03	0.03 ± 0.01
Two Sprays	0.08 ± 0.07	0.11 ± 0.06
Three Sprays	0.25 ± 0.19	0.36 ± 0.10

Develop an empirical phenological (degree-day) based fruit pesticide residue decline model. The first steps toward model development have been completed with first-order kinetic analysis of the residue data collected thus far. The next step is to further refine the kinetic analysis with all of the residue data and then to tie the model to accumulation of degree days. Apples were collected from 11 grower orchards that represent variable management practices. These are in the process of analysis to determine if the kinetic model is predictive of the residue at harvest.

Measure Sevin residues in composited and single-serving fruit samples at harvest. Apples collected during crop year 2001 at the Olsen Brothers Ranch are being handled consistent with a GLP protocol. After method validation, both composites and single serving apples will be analyzed, similarly to the analysis conducted for Guthion. Also, apples that were collected several times throughout the growing season will be analyzed to determine half-life of Sevin residues.

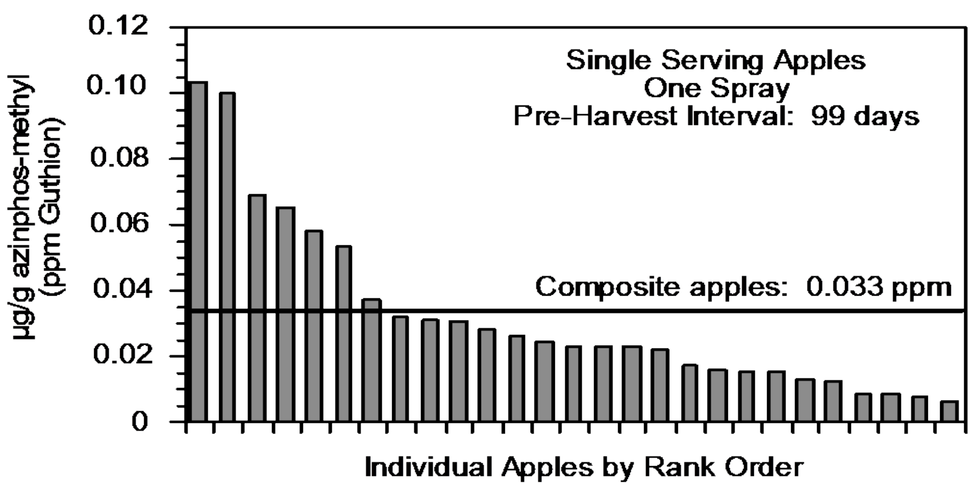

Figure 2. Rank order distribution of Guthion residues on individual apples collected 99 days after a single spray. The solid horizontal line represents the residue recovered from a composite of nine apples.

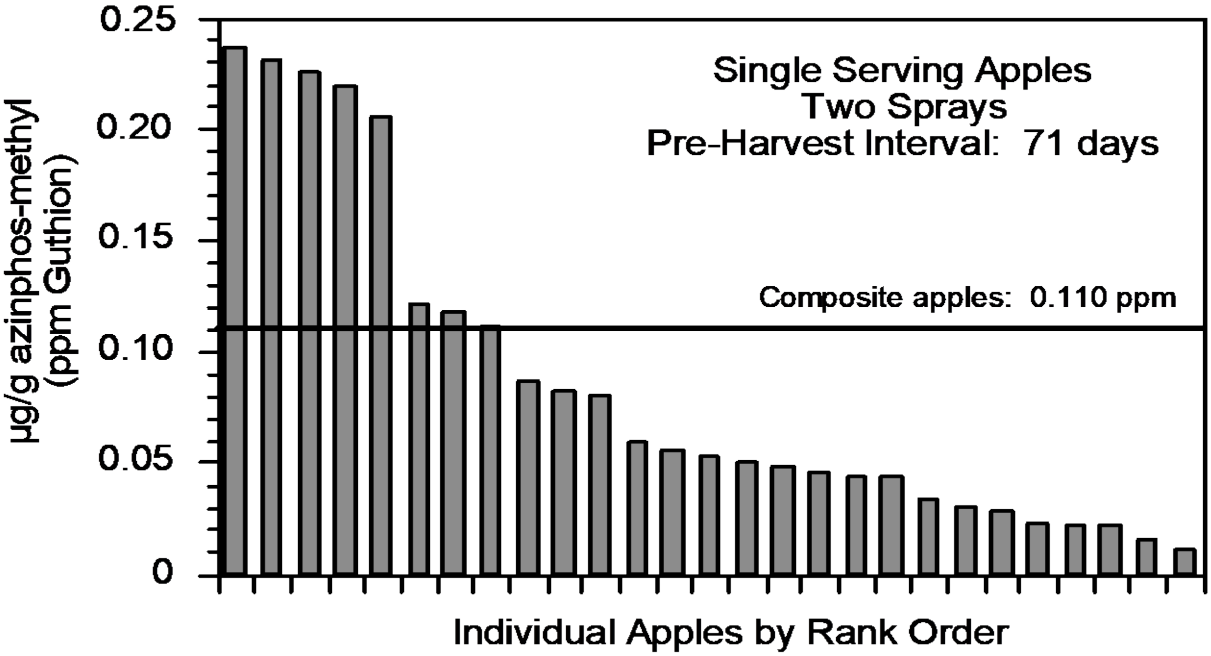

Figure 3. Rank order distribution of Guthion residues on individual apples 71 days after a second spray. The solid horizontal line represents the residue recovered from a composite of nine apples.

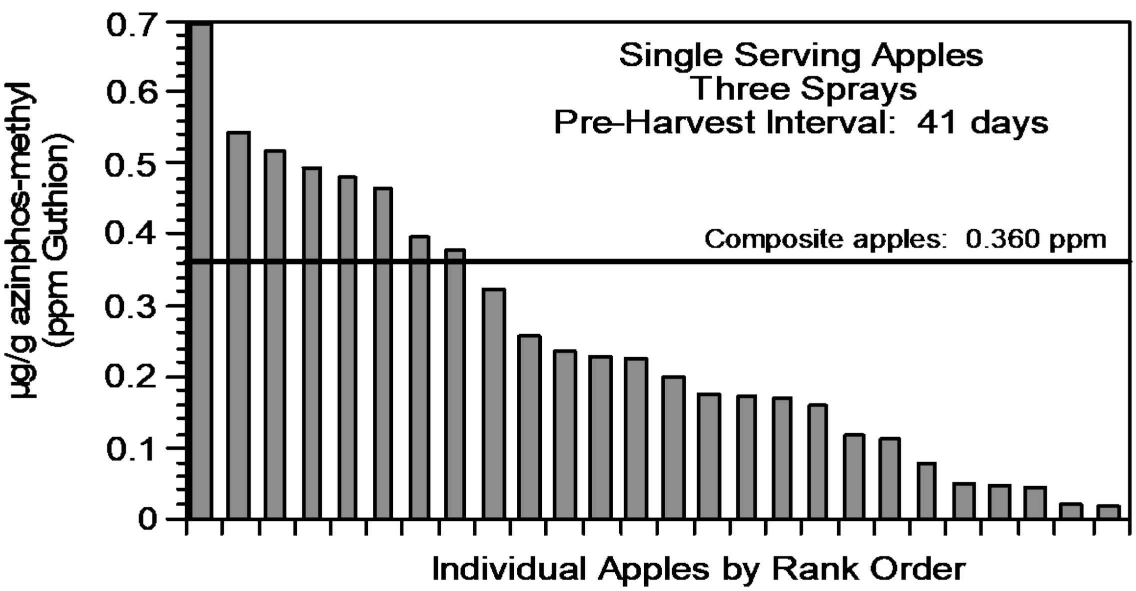

Figure 4. Rank order distribution of Guthion residues on individual apples 41 days after a third spray. The solid horizontal line represents the residue recovered from a composite of nine apples.

References Cited:

Hamey, P. Y. and C. A. Harris. 1999. The variation of pesticide residues in fruits and vegetables and the associated assessment of risk. Regulatory Toxicoogy and Pharmacology 30:S34-S41.

Howell, J. F. and Maitlen. 1987. Accelerated decay of residual azinphosmethyl and phosmet by sprinkler irrigation above tree and its effect on control of codling moth based on laboratory bioassays as estimated by laboratory simulation of insecticide deposits. J. Agric. Entomol. 4(4):281-288.

Maitlen, J. C., A. Cantu, and A. Palmer. 1985. Efficiency of pesticide application: azinphosmethyl on apples. Report to Cooperators 1985: USDA-ARS, Yakima Agricultural Research Laboratory 14 pp.

Budget:

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

Allan S. Felsot

Project Duration: 2 years (2000-2002)

Year	Year 1 (2000-2001)	Year 2 (2001-2002)
Total	$39,393	$32,572

Budget Breakdown

Expenditure	FY 2001		FY 2002	
	WTFRC	WSCPR	WTFRC	WSCPR
Salaries (Research Aide II, 0.75 FTE)	16,440	8,220	17,098	8,549
Employee Benefits for Aide	4,603	2,302	4,787	2,394
Technical Assistant I (Non-classified)	5,850	2925	0	0
Travel (to Orchards)	375	188	500	0
Equipment (GC supplies)	2,750		1,000	5,000
Other (Sample Analysis Cost)	8,375	4,188	9,188	3,063
Other (Study Audit)	1,000	1,875	0	2,000
Total	**$39,393**	**$19,697**	**$32,572**	**$21,005**

Project total: $71,965

FINAL REPORT

Title: Identification, biology and insecticide resistance of green apple aphid and spirea aphid.

PI: Tom Lowery
Agriculture & Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland,

Co-PIs: Robert Foottit, AAFC - Eastern Cereals and Oilseeds Research Centre, Ottawa, Ont.; Mike Smirle, AAFC- PARC, Summerland, B.C.; Elizabeth Peryea, WSU, Tree Fruit & Extension Center, Wenatchee, WA.

Objectives:

1) *Species Identification.*

a) Morphological Comparisons. Extensive samples of green apple aphid (GAA), *Aphis pomi* De Geer, and the morphologically similar spirea aphid (SA), *A. spiraecola* Patch, collected from apple orchards and ornamental hosts in B.C. and Washington State were examined and measured to determine if the species can be reliably separated based on morphological features. Inspection of slide-mounted specimens from eastern Canada, Utah, and New York were included for comparative purposes.

b) Molecular Diagnostics. A Visiting Research Fellow in Dr. Foottit's laboratory developed molecular markers based on DNA microsatellites for the separation of GAA and SA. Results from these tests were compared with traditional morphometric examinations.

2) *Comparative Biology*.

a) Sampling Field Populations. Extensive and intensive sampling of aphids collected from commercial apple orchards and from ornamental hosts throughout the year to determine species distribution and abundance. Samples collected from apple early in the season inspected for the presence of SA forms from overwintering eggs.

b) Development and Fecundity. Aphids of both species reared in the laboratory under a range of temperatures to determine developmental thresholds and differences in fecundity. SA and GAA reared on potted apple seedlings to determine population growth rates.

3) *Insecticide Resistance*.

a) Baseline Susceptibilities. Clones of SA and GAA from B.C. and WA tested for susceptibility to common aphicides, including imidacloprid (Admire®, Provado®), pirimicarb (Pirimor®) and dimethoate (Cygon®).

b) Quantification of Degradation Enzyme Activities. Comparison of detoxification enzyme activities and synergism of aphicides for resistant clones of SA or GAA.

Most of the proposed objectives have been met or are nearing completion. Additional early season samples from commercial apple orchards in WA will be collected in 2002. The large number of aphid samples are still being prepared and examined for the presence of SA from overwintering eggs (fundatriginae). Gugs Lushai, a Postdoctoral Fellow working in Dr. Foottit's laboratory, has used one of the four microsatellite markers in an attempt to separate SA and GAA and is completing studies with the remaining markers. They hope to have this component of the research completed during the coming calendar year.

Determination of baseline susceptibilities for a resistant and a susceptible clone of SA from commercial apple orchards are continuing for several additional insecticides. These bioassays will be completed by the end of the three year study period. Attempts will be made to synergize the toxicity of imidacloprid and pirimicarb to a clone of SA having low levels of resistance to these materials.

Upon completion of the above studies, results of this three year study will be provided in the form of scientific papers, articles in industry newsletters, and an additional report to the Research Commission. No additional funding is required for the completion of this research.

Significant Findings:

- SA and GAA cannot be reliably separated based on morphological characteristics. SA in particular is highly variable in color, size, and morphological features.
- Differences in one microsatellite gene sequence can be used to separate SA from GAA for aphids from most regions, but it was not diagnostic for aphids from southern B.C. and WA where the species occur together on apple. These preliminary results suggest that these two closely related species might interbreed on apple. Additional loci are being examined for their diagnostic ability.
- SA is widespread on apple, particularly in southern Washington. In late summer GAA is rarely found on apple in southern WA, it becomes increasingly common to the north, and was the only 'green apple aphid' found in commercial orchards north of Vernon, B.C. This north-south gradient in species abundance mirrors the increase in GAA populations recorded from orchards at higher elevations.
- SA are found on apple early in the season, indicating that they overwinter on this host, but we have not yet found the forms that develop from overwintering eggs. Large colonies consisting of all growth stages have been found on apple by the end of April, suggesting that they overwinter asexually on this host during mild winters.
- Laboratory studies have shown that GAA has a lower developmental threshold compared with SA. The latter species is more tolerant of high temperatures, likely explaining why SA predominates on apple in central and southern WA during summer.
- Based on probit analyses and LC_{50} values, susceptibility of SA to insecticides is more variable compared with GAA. SA are 4 to 5-fold less susceptible, on average, to imidacloprid and pirimicarb, but approximately 1.5 times more susceptible to dimethoate.
- Levels of detoxifying enzymes are higher for SA compared with GAA, which likely accounts for the lower susceptibility of SA to insecticides.
- Aphids appear to be an increasing problem on apple. The rosy apple aphid, *Dysaphis plantaginea* (Passerini), for example, can regularly be found damaging apple throughout the summer rather than migrating to alternate hosts.

Methods:

Species Identification. For traditional morphometric analyses, a large number of samples of 'green apple aphids' from commercial orchards and alternative hosts in southern B.C. and central and north central Washington were collected and preserved in ethanol and sent to Dr. Foottit for species identification. Morphological features were counted and measured for both species, and the results compared with specimens from other areas of North America.

Separation of species based on molecular diagnostic techniques required the preparation of suitable molecular markers from microsatellite flanking regions for clones of SA and GAA maintained in colony. A phage library was used to design primers that could be used in polymerase chain reaction (PCR) to amplify gene sequences that would allow for the rapid and accurate determination of species. DNA from individual aphids could then be extracted and the appropriate gene sequence amplified. The resulting fragment can then be compared with known gene sequences from SA and GAA

Comparative Biology. Aphid populations on apple were sampled throughout the growing season in organic and conventional orchards to provide information on the distribution and relative abundance of the two species. Sampling from a sufficient number of organic and conventional orchards will help determine the effect of insecticide sprays on populations of the two aphid species and determine if SA is better able to develop on apple during summer when the terminals are beginning to harden off. Material collected in spring was examined for the presence of specialized aphid morphs that develop from overwintering eggs (fundatriginae) to determine if SA has adapted to use apple as a primary host.

Both species of aphid were also reared in the laboratory on excised disks of apple leaves under several temperature regimes to provide information on aphid development, survival, and reproduction; factors that can influence aphid population growth and the degree of damage to the host trees.

Insecticide Resistance. Clones of SA and GAA from organic and conventional orchards in B.C. and WA were established in the laboratory on potted apple. Baseline susceptibilities to a number of insecticides were determined for these clones based on LC_{50} values derived from probit analyses. For the insecticide bioassays, 3rd instar nymphs were reared on leaf disks of apple dipped in one of several concentrations of the test material. Levels of detoxifying enzymes were determined for the clones used in the insecticide bioassays.

Results and Discussion:

Species Identification. Morphological examination of a large number of slide-mounted aphids from commercial orchards and alternate hosts over a period of two years did not allow for unequivocal separation of the two species. The morphometric study conducted by Dr. Foottit showed that a significant proportion of aphids of both species overlapped in length measurements or numbers and location of physical structures such as abdominal tubercles. Winged adults of GAA and SA can be distinguished by the veins in the forewings, the former species being distinctly pigmented. Reliably differentiating wingless aphids of these two species is important, as winged forms are often not produced until later in the season or when colonies become crowded.

In a previous study, physical characteristics used in Europe to distinguish the two species in the laboratory, such as the relative length of the last rostral segment and the number of hairs on the cauda (Blackman & Eastop, 1984), did not prove to be diagnostic for specimens collected in North America (Halbert & Voegtlin, 1992). Our results also demonstrate that apterae of the two species from western North America cannot be distinguished by morphological characteristics.

A molecular diagnostic technique based on microsatellite flanking region sequences revealed a number of important traits in the genotype diversity. Four broad clusters (Figure 1) indicated the presence of several genotypes across the continent. Eastern samples of GAA from Nova Scotia and Quebec are closely related to European types, while the similarity of samples within B.C. suggest a single source with subsequent genetic drift. The genetic similarity and grouping of SA and GAA from WA and southern B.C. into the prevalent genotype (cluster 4) suggests that the two species are very closely related and that they possibly interbreed on apple where their ranges overlap. Fertile hybrids could introduce genes for insecticide resistance from SA to populations of GAA. Examination of additional gene sequences and inclusion of more samples should help determine if these species hybridize.

Samples of GAA collected early and late in the growing season from Kelowna, Summerland and Cawston, B.C., indicate low genetic variability in the local population. As the season progresses, however, the dominant genotype observed early in the season declines; suggesting immigration from outside sources or selection for rare genotypes present early in the season. The significance of this genetic change is not known. Based on our studies, there is little variation between clones of GAA with respect to morphology or susceptibility to insecticides.

Comparative Biology. This study has provided important information required for the management of ‘green apple aphids’ on apple in B.C. and Washington. Extensive sampling has shown that SA is the predominant species in southern Washington. This distribution is consistent with laboratory results showing that SA is more tolerant of hot temperatures than GAA. Conversely, GAA predominates in cooler coastal areas, northern regions, and at higher elevations. Management programs should be designed to take these regional population differences into consideration. Additional samples from south central WA early in the growing season will determine seasonal differences in species abundance for that region.

The widespread distribution and abundance of SA in commercial orchards in WA is similar to findings in other regions. It has recently been shown, for example, that most pest apple aphids in the eastern U.S. are in fact SA (Pfeiffer *et al.* 1989, Mayer & Lunden, 1996). In greenhouse studies with potted Red Delicious apple, Kaakeh *et al.* (1993) found that GAA and SA reached similar densities and caused similar levels of damage. They concluded that the economic injury level would be about the same for these species. Their study was not designed to determine if SA is better able to remain on apple during the summer months. In addition to other factors such as insecticide applications and greater tolerance to heat, higher populations of SA might occur later in the summer on apple if this species can better survive on terminals that have largely ceased growing.

Large colonies of aphids of various ages were discovered on young apple trees as early as April in some locations, suggesting that GAA and SA can overwinter asexually on apple in some years. Samples collected early in the season from B.C. are still being examined for fundatriginae of SA.. The addition of samples that we plan to collect early in 2002 from WA where SA is the dominant species will help to determine if this species overwinters as eggs on apple.

The two species of 'green apple aphid' differ in biology. The GAA overwinters as eggs on apple and feeds throughout the summer on a restricted range of summer hosts, mainly apple, pear and hawthorn. The SA utilizes spirea as a primary host and migrates to a wide range of secondary hosts, including apple. In 1979, however, SA was found to overwinter successfully on citrus, and it has been suggested that this species has made another recent host shift to utilize apple as a primary host (Pfeiffer, 1991). Eggs of SA have not yet been found on apple, however, and it is possible that significant numbers overwinter as parthenogenetic forms. Additional migrants would be expected to arrive during summer from outside orchards.

Insecticide Resistance. Laboratory bioassays involving 12 aphid clones collected from commercial orchards in WA in the fall of 2000 showed that SA was approximately 5 to 10 times less susceptible to imidacloprid than GAA (Table 1). These results are comparable to those for aphids collected from B.C. orchards in 1999, where the average LC_{50} values were 0.17 and 0.87 ppm AI for GAA and SA, respectively (Table 2); which is almost identical to the overall averages obtained between 1997 and 2001 for 19 clones of GAA and 17 of SA. Although GAA from unsprayed or organic apple were more susceptible to imidacloprid in 1997, there were no consistent differences for aphids from conventionally managed orchards compared with those from organic orchards or unsprayed apple.

The decrease in susceptibility to imidacloprid recorded after 1997 (Table 2) could have resulted from increasing use of this product, but the small change in LC_{50} values does not indicate the development of significant levels of resistance. Within any particular year, LC_{50} values did not differ greatly between clones of each species and the relative difference between species remained more or less constant. The small year to year differences likely reflect changes in test conditions and materials.

Although high levels of resistance to imidacloprid have not yet been demonstrated for any species of aphid, Devine *et al.* (1996) reported that clones of green peach aphid, *Myzus persicae* (Sulzer), and tobacco aphid, *M. nicotianae* Blackman, resistant to nicotine also showed low levels of resistance to this aphicide. Sprays of this material might be ineffective against populations of SA that develop even low levels of resistance, as the margin of overkill is not particularly high for this species.

Pirimicarb was generally less toxic to SA than GAA and there were no consistent differences in susceptibility between clones from unsprayed or organic apple and those from conventional orchards (Tables 3 and 4). LC_{50} values for clones from B.C. were approximately half those of WA clones, however, which possibly correlates with the higher rates of detoxification enzyme activity recorded for GAA and SA clones from WA (Table 7). Higher esterase activity for aphids from WA was particularly evident when α-naphthyl-butyrate was used as the degradation substrate.

Previous studies have shown that SA is significantly more resistant to a wide range of carbamate and organophosphate insecticides, including azinphosmethyl (Hogmire *et al.*, 1990, 1992).

Contrary to these reports, we found that dimethoate is generally more toxic to SA than GAA (Tables 5 and 6). LC_{50} values for various clones of the two species did not always differ significantly, but these results suggest that sprays of dimethoate might be advisable in late summer where SA predominates. Higher levels of degradation enzyme activity for SA (Table 7) might increase the toxicity of dimethoate, or GAA might be less susceptible due to an unknown resistance mechanism.

Conclusions:

Spirea aphid (SA) is the predominant 'green apple aphid' species occurring in orchards in Washington State. To the north and in cooler coastal areas it is largely replaced by the morphologically similar green apple aphid (GAA). The distributions of these two species is best explained by differing climatic requirements; GAA has a lower developmental threshold, but SA is better able to tolerate warm conditions.

Winged GAA can be separated from winged SA based on the presence of darkened veins in the forewings of the former species, but apterae cannot be reliably distinguished based on morphological characteristics. Preliminary analyses of microsatellite gene sequences suggests that the two species might interbreed, but inclusion of aphids from additional sampling sites and examination of other gene loci might provide a diagnostic method that will accurately separate the two species.

In certain years, large colonies of asexually reproducing SA of various ages can be found on apple early in the spring, particularly in newly-planted orchards. We have not yet been able to state with certainty that SA does not overwinter on apple in the egg stage, and additional sampling is planned for early in 2002 in areas where this aphid is the predominant species on apple.

SA is significantly less susceptible than GAA to imidacloprid (Admire□, Provado□) and pirimicarb (Pirimior□), but not dimethoate (Cygon□). These differences possibly relate to higher rates of detoxification enzyme activity, specifically esterases, for SA. Insecticide bioassays are nearing completion for a number of additional insecticides. Differences in susceptibilities to insecticides between the two species is an important consideration for the management of aphids on apple. Efficacy trials should be conducted against the more resistant species, and aphid management programs should take into account the relative abundance of the two species throughout the season.

Budget:
Identification, biology and insecticide resistance of green apple aphid and spirea aphid.
Tom Lowery
Project Duration: 1 April, 1999 to 31 March 2002 (3 years).

	1999/2000	2000/2001	2001/2002
Salaries[1]	$20,250	$21,042	$19,792
Benefits (20%)	$4,050	$4,208	$3,958
Materials and Supplies	$6,200	$3,750	$1,750
Travel	$1,000	$1,000	$1,000
Publication costs	--	--	$1,500
Total[2]	$31,500	$30,500	$28,000
WTFRC Funds	**$15,750**	**$15,250**	**$14,000**
AAFC-MII Funds	$15,750	$15,250	$14,000

[1]Includes ~$10,000/yr to Dr. Foottit for contract slide mounting and technical salary; and technical assistance at PARC (D.T. Lowery and summer students).
[2]Includes $1,000/yr to Dr. Peryea for collection of aphid samples.

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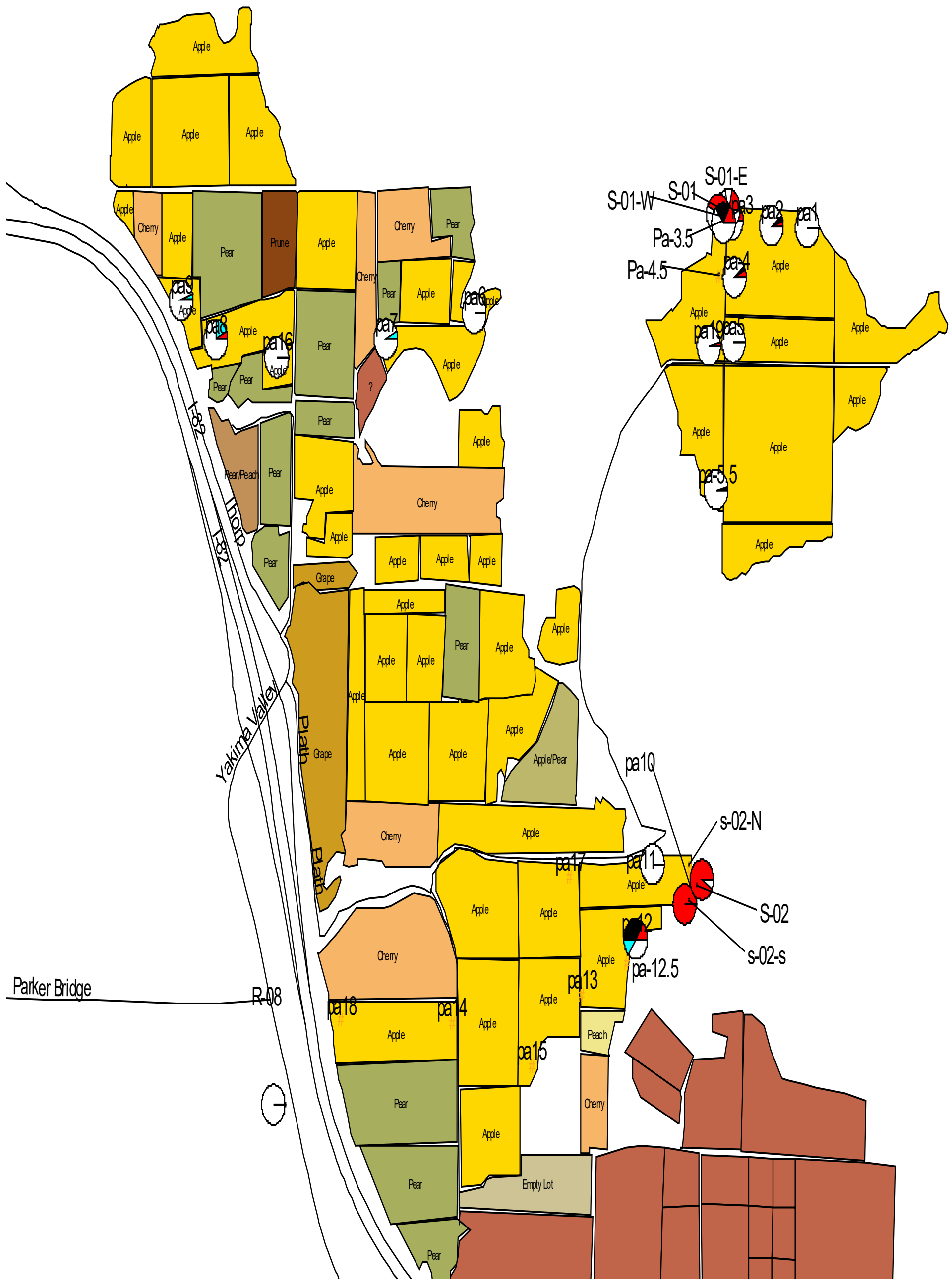

Figure 1. Genetic relationship of green apple aphid and spirea aphid (SA) from B.C., Washington and eastern North America based on distance-neighbour joining analysis of sequenced microsatellite loci (~1600 base pairs) rooted by Nova Scotia samples being used as out groups. Branch length is proportional to genetic change.

Table 1. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to imidacloprid (Admire□, Provado□) for aphids collected in 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	0.15	0.12-0.19	4.40
"	"	"	0.16	0.13-0.20	1.63
"	"	"	0.16	0.13-0.21	1.00
SA	"	"	0.74	0.57-0.94	3.29
"	Wenatchee	Crab Apple	0.74	0.57-0.93	1.36
"	Donald	Conventional	0.76	0.60-0.94	0.50
"	Columbia View	"	0.78	0.62-0.96	2.60
"	Wenatchee	Crab Apple	0.84	0.71-0.98	1.87
"	Grandview	Conventional	0.84	0.69-1.02	1.98
"	Wenatchee	"	0.89	0.71-1.11	1.06
"	Quincy	"	0.90	0.72-1.11	0.60
"	Chelan	"	1.12	0.99-1.39	1.77

Table 2. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to imidacloprid for aphids established in culture from 1997 to 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	0.16 (3)	0.16 (3)
"	SA	"	"	0.88 (7)	0.85 (9)
"	"	"	unsprayed/organic	0.74 (2)	
1999	GAA	B.C.	conventional	0.13 (2)	0.17 (7)
"	"	"	unsprayed/organic	0.19 (5)	
"	SA	"	conventional	0.79 (5)	0.87 (6)
"	"	"	unsprayed/organic	1.31 (1)	
1998	GAA	WA	conventional	0.30 (4)	0.30 (4)
"	SA	"	"	1.25 (1)	1.25 (1)
1997	GAA	B.C.	conventional	0.12 (2)	0.09 (5)
"	"	"	unsprayed/organic	0.07 (3)	
"	SA	"	"	0.55 (1)	0.55 (1)

GAA: overall average LC_{50} = 0.17 ppm AI; range 0.06-0.54 ppm AI; number clones tested = 19SA: overall average LC_{50} = 0.86 ppm AI; range 0.51-1.53 ppm AI; number clones tested = 17

Table 3. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to pirimicarb (Pirimor□) for aphids collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	0.57	0.48-0.66	2.95
"	"	"	1.18	0.99-1.43	4.33
"	"	"	1.66	1.40-1.95	2.11
Spirea	Wenatchee	Crab Apple	1.40	0.85-2.29	5.22
"	"	"	2.17	1.82-2.51	0.40
"	Quincy	Conventional	2.48	2.08-2.93	3.06
"	Columbia View	"	3.58	2.96-4.21	0.44
"	Grandview	"	4.07	3.38-4.87	1.21
"	Chelan	"	4.95	1.31-13.02	7.09
"	Donald	"	5.26	4.33-6.22	4.55
"	Quincy	"	6.20	5.26-7.14	2.06
"	Wenatchee	"	6.30	5.42-7.22	0.01

Table 4. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to pirimicarb (Pirimor™) for aphids established in culture in 1999 and 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	1.14 (3)	1.14 (3)
"	SA	"	"	4.69 (7)	4.05 (9)
"	"	"	unsprayed/organic	1.79 (2)	
1999	GAA	B.C.	conventional	0.56 (2)	0.59 (7)
"	"	"	unsprayed/organic	0.60 (5)	
"	SA	"	conventional	1.92 (5)	2.04 (6)
"	"	"	unsprayed/organic	2.64 (1)	

GAA: overall average LC_{50} = 0.75 ppm AI; range 0.36-1.66 ppm AI; number clones tested = 10SA: overall average LC_{50} = 3.24 ppm AI; range 0.50-6.30 ppm AI; number clones tested = 15

Table 5. Susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to dimethoate (Cygon□) for aphids collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	LC_{50}	95% CI	Chi^2
GAA	Quincy	Conventional	16.2	12.9-20.5	1.10
"	"	"	27.9	23.2-33.7	4.15
"	"	"	55.4	48.3-62.5	0.18
SA	Wenatchee	Crab Apple	8.7	7.4-10.3	1.42
"	Quincy	Conventional	12.3	10.1-14.8	4.07
"	Grandview		15.6	13.9-17.5	2.08
"	Quincy	"	19.9	16.4-24.2	2.75
"	Wenatchee	Crab Apple	20.0	17.1-23.2	1.77

"	Columbia View	Conventional	21.5	18.4-24.8	1.70
"	Chelan	"	25.4	22.2-28.9	2.17
"	Wenatchee	"	25.4	20.9-31.0	1.98
"	Donald	"	30.1	25.5-35.1	3.70

Table 6. Summary of average susceptibilities (LC_{50} ppm AI from probit analyses) of green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, to dimethoate (Cygon™) for aphids established in culture in 1999 and 2000. Numbers of aphid clones indicated in parentheses.

Year	Species	Location	Practice	Avg. LC_{50}	Species Avg. by Year
2000	GAA	WA	conventional	33.2 (3)	33.2 (3)
"	SA	"	"	21.5 (7)	19.9 (9)
"	"	"	unsprayed/organic	14.4 (2)	
1999	GAA	B.C.	conventional	10.0 (2)	10.2 (7)
"	"	"	unsprayed/organic	10.2 (5)	
"	SA	"	conventional	8.8 (5)	8.5 (6)
"	"	"	unsprayed/organic	7.5 (1)	

GAA: overall average LC_{50} = 17.1 ppm AI; range 4.8-55.4 ppm AI; number clones tested = 10SA: overall average LC_{50} = 15.3 ppm AI; range 6.3-30.1 ppm AI; number clones tested = 15

Table 7. Detoxification enzyme activity, esterase activity/minute/mg protein, for green apple aphid (GAA), *Aphis pomi*, and spirea aphid (SA), *A. spiraecola*, collected in fall 2000 from orchards and from ornamental crab apple in Washington State.

Species	Location	Culture	Acetate	Butyrate
GAA	Quincy	Conventional	1.74	0.47
"	"	"	2.19	0.57
"	"	"	2.71	0.67
SA	Wenatchee	Crab Apple	1.37	0.55
"	"	"	2.01	0.69
"	"	Conventional	2.29	0.89
"	Quincy	"	2.77	0.88
"	"	"	3.18	1.02
"	Columbia View	"	2.21	0.66
"	Grandview	"	2.06	0.67
"	Donald	"	2.39	0.83
"	Chelan	"	2.52	0.87

GAA: 2000 acetate average = 2.21; butyrate average = 0.57; number clones tested = 31999 acetate average = 1.23; butyrate average = 0.29; number clones tested = 4SA: 2000 acetate average = 2.31; butyrate average = 0.78; number clones tested = 91999 acetate average = 1.27; butyrate average = 0.49; number clones tested = 6

CONTINUING REPORT **YEAR 2/2**

TITLE: A new pollinator from the homeland of apples: The Tien Shan Mountain Gray Bee

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

TERMINATING YEAR: 2001/2002

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

OBJECTIVES FOR PROJECT PERIOD 2000-2002

- 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 2001: 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.

Year 2002 (continuing funds from 2001): Import 100 queens of *Apis mellifera pomonella* from the Tien Shan Mountains into quarantine on Grande Terre Island. Secure their release to the WSU bee breeding program in summer of 2002 and begin evaluation for pollinating and apicultural characteristics. Develop beekeeper grower collaborators for field testing in 2003.

ACCOMPLISHMENTS:

Characterization and importation plans for honey bee stocks

Data analysis continued from 2000 field and apiary studies of a newly discovered subspecies of honey bee from the Oxul-Jabagly region of the western Tien Shan Mountains. The new subspecies was described scientifically as *Apis mellifera pomonella*. Two articles about the new subspecies and its potential use as an apple pollinator were published in The Goodfruit Grower and Bee Culture.

Initiated importation protocols with USDA-APHIS and LA Department of Agriculture to bring queens into USDA quarantine on Grande Terre Island (LA) in Spring of 2002. Following release from quarantine the bees should be available for evaluation in the WSU breeding program by late summer of 2002. During the evaluation period, the new subspecies will be assessed for overwintering ability, pollinating activity, disease resistance and other apicultural characteristics in Washington State during the winter of 2002 and during the 2003 field season. Evaluation of the stocks will be conducted on crops with early pollinating seasons (cherries) and in sis by side tests with standard honey bee stocks in other tree fruit crops. Anticipated release of stocks for Washington beekeepers could occur as soon as 2004.

Collaborative arrangements
Mr. Urazajev Zufar, a local beekeeper from the village of Jabagly will take part in the queen rearing and stock selection aspects of the project scheduled for 2002. 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. Dr. Roman Jashenko of the Institute of Zoology in Almaty will 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. Dr. Bob Danka of ther same laboratory will take part in pollination research with the new subspecies and is interested in settign up research activities with Washington State growers.

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.

No funds requested for 2002.