2007 Apple Crop Protection Research Review
February 1-2
ATEC at
Big Bend Community College
Moses Lake, Washington

Thursday, February 1, 2006

Time	Page	PI	Proposal Title	Funding period
8:00		McFerson	Introduction and update	
			Final Reports	
8:15	1	Yee	Alternate hosts of apple maggot as a threat to apple	04-06
8:30	13	Yee	Control of apple maggot using bait spray insecticides and traps	04-06
8:45	24	Yee	Temperate fruit fly workshop[1]	05
9:00	35	Beers	Biology and management of secondary pests of apple	06
9:15	46	Horton	Distribution of flower thrips eggs among vegetative, flowering & fruiting structures	05-06
9:30	51	Campbell	Target-specific control of fungal pathogens by natural compounds	04-06
9:45	61	Xiao	Sphaeropsis rot in apple	05-06
10:00			**Break**	
10:15	71	Xiao	Holistic approach to decay management	04-06
10:30	83	Hansen	Auxiliary cold storage component for the Systems Approach (extension)	05
10:45	90	Randall	Acetic acid vapor vs. sterilants to decontaminate storage (extension)	05
	101	Sholberg	Molecular techniques to study apple and pear pathogens in CA storage (written report)	02
11:00		Jones	IPM Decision Aids[2]	05-07
11:15	111	Jones	The importance of dispersal in biological control and IPM[3]	
11:30	118	Jones	Mechanisms underlying mating disruption	04-06
11:45	125	Jones	Evaluation of tachinid parasitoids for OBLR in apples	06
Group			**Poster Session Continuing Reports 1:30pm - 4:00pm**	
1	131	Knight	Developing ULV microencapsulated sex pheromones for CM control (extension)	05-06
1	138	Knight	Direct control of CM with formulations of the pear ester (extension)	05-06
1	145	Beers	Reinstating integrated mite control in apple orchards	06-08
1	152	Unruh	CSI in the orchard: finding the killers of 4 key apple pests (extension)	06
1	156	Brunner	Sustainable management of leafrollers in apple orchards	05-07
2	163	Brunner	Codling moth management with pheromones: key unanswered questions	05-07
2	172	Landolt	Sprayable foam for trap and kill of cocooning codling moth larvae	06-08
3	177	Hanrahan	Collaborative WTFRC research projects	internal
3	183	Mazzola	Employing biological elements of orchard ecosystems	05-07
3	190	Lacey	Improving codling moth granulovirus (CpGV) transmission and activity	05-07
3	195	Lacey	Heterorhabditis nematodes for codling moth and oriental fruit moth control	06-08
3	201	Riga	Synthetic and bio-nematicides for plant parasitic nematodes	05-07

FINAL PROJECT REPORT
WTFRC Project Number: AE-04-426

Project Title: Alternate Hosts of Apple Maggot as a Threat to Apples

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Cooperators: WSU personnel, various growers and homeowners in western and central Washington

Budget History:

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

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

Objectives (2004-2006):
1) Determine apple maggot abundance and prevalence on alternate and normal host trees, including hawthorn, in different regions in Washington, with emphasis in central Washington.
2) Determine effects of fruit maturity and spatial relationships between alternate host trees on apple maggot infestations.
3) Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies in apple or hawthorn.
4) Determine frequency of infestations of hawthorn species and apple varieties.
5) Determine abundance of hawthorns and alternate hosts near representative commercial orchards.
6) Determine acceptance of apple varieties by apple maggots reared from hawthorns and alternate hosts.

Significant Findings:
● Feral apple and black hawthorn fruit were infested by apple maggot larvae in central Washington in Yakima, Nile Valley, Wenas, and Ellensburg, but at low and manageable densities.
● Other than hawthorns, no alternate hosts of apple maggot flies were found in central Washington
● Apple maggot flies infested black hawthorn more frequently than apples in central Washington; whether this suggests a host preference is unclear, but hawthorns should still be considered a threat to apples for now.
● In western Washington, apple maggot flies infested previously unrecorded hosts, including pear, Asian pear, garden plum, Japanese plum, cherry plum, apricot, bitter cherry, mountain ashes, and cotoneasters, indicating flies in Washington have the genetics to utilize a fairly wide range of hosts.
● Flies from black hawthorns are abundant when earlier developing apples are ripening; these apples may be more likely to be attacked by flies from black hawthorn than are later apples.
● Shrubs such as cotoneasters are infested, but are not a threat unless near infested apple/haw trees
● Black and English hawthorns are equally infested, but English hawthorns ripen later; combined, both may contribute flies to early and late developing apples.
● In the Yakima area, black hawthorns are abundant near some apple orchards, but there are few other alternate hosts; removing these hawthorns may further reduce the threat of the flies infesting apples.
● Flies reared from English hawthorn and other alternate hosts accepted apples, suggesting flies from hawthorn trees also can attack apples in the field.

Results and Discussion:

1) Determine apple maggot abundance and prevalence on alternate and normal host trees.
In Yakima/vicinity (Table 1) from 2004-2006, only one pupa (presumably an apple maggot) was found in many apples sampled. None was found in black hawthorn in 2004 and 2005, but 8 were found in this host in 2006. No other plants produced apple maggot pupae, although they produced other species of fruit flies. In the Nile Valley (Table 2) from 2004-2006, none of the apples produced pupae, but black hawthorns from 2003-2006 produced apple maggot pupae and adults in low numbers. None of the other plants produced apple maggot, although again they produced other fly species. In Wenas (Table 3), the relatively few apples found were not infested. However, as in Yakima and the Nile Valley, black hawthorn was infested. Also similar to the Nile Valley, apple maggot populations were low despite the high abundance of native black hawthorn at the collection site. It was estimated that the 49 trees sampled in Wenas represented only about 10% of the available trees, as most were inaccessible along deep ravines. In Ellensburg (Table 4), three pupae were found in apple, but only in the last of the three years of

sampling. None of the other plants was infested by apple maggots. In Goldendale, where sampling was limited to late season in 2006, no apple maggots were detected in apple, crabapple, or other sampled plants (data not shown).

In general, then, in central Washington, breeding apple maggot populations are low, but they are widespread, as they were found in every area sampled (except in Goldendale where sampling was less extensive than in other areas). Results suggest that at present apple maggots can be contained or managed well and kept out of commercial orchards in central Washington because of the low densities. Likely because of the low numbers, apple maggots have not utilized alternative hosts in central Washington. Therefore, in central Washington, alternate hosts do not appear to be a threat to the apple industry, although this could change if fly populations are left unmanaged in the long term in the future, because fly populations in Washington undoubtedly have the ability to exploit alternate hosts (see below).

The low infestations of apple maggots in both apple and black hawthorn in central Washington suggest a recent introduction of the fly or that some environmental factor in this area, such as low moisture, has prevented the population from increasing to higher levels. Alternatively, with respect to black hawthorn, the variety in central Washington may not allow for high larval survival (compared with the variety in western Washington, *C. douglasii suksdorfii*, see below). The high abundance of black hawthorn trees with heavy fruit loads in the Nile Valley and Wenas suggest the population is not limited by breeding sources. An interesting and potentially important result in central Washington was that black hawthorns were more frequently infested than apples. Several possible explanations can account for this. One, the apple maggots here are a race that prefers black hawthorns. Two, the varieties of apples found along roadsides or feral settings are those less preferred by the flies. Three, black hawthorns are more abundant than unmanaged apples, and the numbers of flies finding them correspondingly are higher than those finding the less abundant apples.

In western Washington in Puyallup (Table 5) there were clear differences in patterns from those at the central Washington sites. Nearly all apple and English hawthorns (*Crataegus monogyna*) were infested with apple maggot. Significantly, alternate hosts were found, as Asian pear and European mountain ash and milkflower cotoneaster were infested. In Vancouver, its vicinity, and Portland (Table 6), again there were clear differences from results in central Washington. Similarly high percentages of apples and black and English hawthorns were infested. Smooth and cockspur hawthorns were also infested. Also, there were several alternate hosts in this area - apricot, European pear, Asian pear, garden plum, Japanese plum, cherry plum, western mountain ash, cranberry cotoneaster, milkflower cotoneaster, and possibly Japanese honeysuckle (depending on the outcome of fly rearing in 2007).

The western Washington results are important because they show that Washington apple maggots are able to exploit a fairly wide range of hosts (Table 7) under some conditions. The possibility that flies from western Washington have been (and will be) transported by humans from western to central Washington is real. It can be assumed, unless evidence to the contrary is found, that flies in central Washington potentially can also attack alternate hosts, for example, apricot or pear.

2) Effects of fruit maturity and spatial relationships between alternate host trees and normal trees on maggot infestations. In western Washington, black hawthorn generally matured in July and August and had highest fly populations during this period. Thus flies from this host most likely would attack earlier developing apple varieties (assuming these flies live on average about 30 days). There was about a one-month period between peak ripeness of black hawthorn and English hawthorn, keeping the fly populations on the two hosts somewhat separated. Early apples such as “Early Transparent’ in general were more frequently infested than late, firmer varieties (e.g., ‘Gravenstein’), although exceptions to this were seen in 2006, which made generalizations difficult. Early-developing plums were less frequently attacked than

later-developing plums, but their relationship to apple was not clear. The cotoneasters and ashes seem to mature too late to make them a real threat to most apples (see below).

3) Determine conditions under which ornamental shrubs and alternate hosts are used by apple maggot through manipulative host studies. The distance that ornamental shrubs (between rows of infested apples or 20 ft outside orchards), in this case cotoneasters (*Cotoneaster dammeri*), were from an infested apple orchard had no bearing on infestation by apple maggots. No cotoneaster fruit produced pupae. Earlier developing species or varieties of cotoneaster may have led to different results. However, cotoneasters in the field surveys were found to be infested by apple maggot, although at relatively low levels (Tables 5 and 6). Cotoneasters likely do not represent a threat to apples unless unmanaged, heavily infested apples or hawthorns are nearby.

4) Determine frequency of infestations of hawthorn species and apple varieties. In central Washington, black hawthorn appeared to be more infested than English and other ornamental hawthorns, but numbers were too low to draw strong conclusions. If true, however, black hawthorns are a larger threat to early-mid season apples than English hawthorns in central Washington. In western Washington, black hawthorn and English hawthorn appear to be infested at equal frequencies (Tables 5 and 6) despite the large differences in their fruiting phonologies. English hawthorns may be a threat to later apples.

5) Determine abundance of hawthorns and alternate hosts near representative commercial orchards. Black hawthorns were found in abundance close ($\leq$1/2 mile) to some commercial orchards in western Yakima. These were mostly roadside trees, although some were in residential yards. The fly-infested hawthorns in 2006 were found in a back yard with a small planting of apples about 3-4 miles from a commercial orchard. However, despite extensive searches around 10 orchards here and in the Naches area, few hawthorns or other alternate hosts (as determined in western Washington) were found. The major plants found around orchards were roses, which, up to this point, have not been found to be infested by apple maggot in Washington (although it is/was a host in the eastern U.S.).

6) Determine acceptance of apple varieties by apple maggots reared from hawthorns and alternate hosts. Flies reared from English hawthorn did not accept 'Fuji' apples that had been cold stored. However, of 20 female flies (9-14 days old) reared from this hawthorn, 50% accepted 'Early Transparent' apples within 30 min. Flies probed (a series of short stings) or stung the fruit and dragged their ovipositors over the fruit. This indicated that flies reared from hawthorn do accept apples. Other observations indicated flies reared from pear also accepted apple. More data using larger fly sample sizes are needed to determine the frequencies of acceptance.

Significance to the Industry and Potential Economic Benefits. The research is significant for the apple industry because it helps identify which plants are used by apple maggots and how prevalent infestations are in major apple-growing regions. Apple maggot establishment on apples is the major problem, but its establishment on alternate hosts near commercial orchards would also be a major problem because this would increase the fly trapping and detection efforts, potentially at a high labor cost. Also, if the flies need to be controlled around the orchards, spray applications need to be made at additional costs to growers. Results reported here suggest that black hawthorns may be a threat to apples if they occur close to orchards (unclear how far, but possibly ½ -1 mile away), but that other alternative hosts are not likely to threaten apples in central Washington. This is probably because of the relatively low numbers of flies in this region at present. The fact that apple maggots attack both apples and black hawthorns here indicates we need to continue to monitoring for the fly. It is unknown whether the situation represents the beginning of one similar to that postulated to have occurred in western Washington prior to 1979, when apple maggot populations apparently were too low to be easily detected. Our results from western Washington also indicate apple maggots in Washington have the ability to exploit several abundant plants such as pear in central Washington, assuming the genetics of the flies in the two regions are similar. This is a real possibility because it is likely western Washington was a source

of at least some populations in central Washington. If so, even small populations left unmanaged to reproduce in central Washington may increase and eventually utilize alternate hosts such as pear, plum, and others.

Table 1. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in Yakima and vicinity, Yakima County, WA, June to October 2002-2006

Plant Species	Sample Year	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	2004	12	233	0	0	---
	2005	30	1,446	0	0	---
	2006	127	8,592	1	0.8	__, AM
Crabapple	2005	74	12,499	0	0	---
	2006	122	26,207	0	0	---
Black Hawthorn	2004	41	6,741	0	0	---
	2005	39	4,643	0	0	---
	2006	77	24,237	8	2.6	__, AM
Ornamental Hawthorns[a]	2005	25	9,667	0	0	---
English Hawthorn	2006	11	5,691	0	0	---
Smooth Hawthorn	2006	26	12,606	0	0	---
Washington Hawthorn	2005	3	120	0	0	---
	2006	42	45,457	0	0	---
European Pear	2004	5	70	0	0	---
	2005	2	6	0	0	---
	2006	20	965	0	0	---
Ornamental Pear	2006	22	4,882	0	0	---
Garden Plum	2005	6	381	0	0	---
	2006	18	1,266	0	0	---
Apricot	2006	23	1,184	0	0	---
Peach	2006	1	10	0	---	---
Sweet/Sour Cherry	2002	32	6,026	1,679	71.9	672[a], CFF
	2003	12	3,249	1,136	100.0	454[a], CFF
	2004	9	1,800	1,338	100.0	535[a], CFF
	2005	20	3,025	2,428	95.0	324, CFF
	2006	11	4,059	60	81.8	___, CFF
Choke Cherry	2002	3	596	0	0	---
	2003	44	27,354	0	0	---
	2004	4	520	0	0	---
	2005	16	3,608	0	0	---
	2006	10	3,942	0	0	---
Roses[c]	2004	15	1,316	65	73.3	30, RM
	2005	29	2,900	130	31.0	3, RM
	2006	97	18,178	726	87.6	__, RM
Japanese Quince	2004	1	21	0	---	---
	2005	3	32	0	0	---
	2006	1	31	0	---	---
Firethorn	2004	4	582	0	0	---
	2006	26	26,152	0	0	---
Serviceberry	2005	4	384	0	0	---
European Mountain Ash	2005	13	2,644	0	0	---

	2006	30	20,212	0	0	---
Korean Mountain Ash	2006	1	359	0	---	---
Cotoneaster sp.	2005	1	400	0	0	---
Rockspray Cotoneaster	2006	21	3,814	0	0	---
Common Snowberry	2004	5	619	13	60.0	4, SBM
	2005	10	600	77	100.0	10, SBM
	2006	31	5,667	425	80.6	__, SBM
Blue Elderberry	2004	6	1,070	0	0	---
	2005	9	1,800[b]	0	0	---
	2006	6	17,900[b]	0	0	---
Red Osier Dogwood	2005	6	2,892	0	0	---
	2006	13	4,411	3	15.4	__, DF
Flowering Dogwood	2004	1	140	0	0	---
	2006	43	10,786	0	0	---
Gooseberry	2004	2	244	0	0	---
Russian Olive	2004	1	157	0	0	---
	2006	17	4,554	0	0	---
Golden Currant	2005	14	2,953	2	14.3	0
Tall Oregon-Grape	2005	34	10,520	60	20.6	36, OG
	2006	4	978	0	0	---
English Walnut	2005	36	1,080	7,835	97.2	432, WHF
	2006	3	45	119	100.0	___, WHF
Holly	2004	2	200	0	0	---
	2005	1	145	0	---	---
	2006	4	1,052	0	0	---
Juniper	2005	1	25	0	---	---
	2006	2	363	0	0	---
European Cranberry Bush	2005	1	145	0	---	---
Viburnum species	2006	5	2,301	0	0	---
Common Buckthorn	2006	6	761	0	0	---
English Yew	2006	24	3,280	0	0	---
Virginia Creeper	2006	15	3,997	0	0	---
Bittersweet Nightshade	2006	16	1,866	0	0	---
Garden Asparagus	2006	3	399	0	0	---
Netleaf Hackberry	2006	2	290	0	0	---
Barberry	2006	3	825	0	0	---
Tartarian Honeysuckle	2006	2	442	0	0	---
Euonymus	2006	2	414	0	0	---

[a]Combined English and smooth hawthorns; [b]Estimated; [c]Nootka and Woods' roses combined. AM, *R. pomonella*; CFF, *R. indifferens*; RM, *R. basiola*; SBM, *R. zephyria*; OG, *R. berberis*; WHF, *R. completa* (Note: the same abbreviations for fly species are used in all tables); ___, not emerged yet.

Table 2. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in the Nile Valley, Yakima County, WA, June to September 2004-2006

Plant Species	Sample Year	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	2004	23	1,030	0	0	---
	2005	73	2,905	0	0	---
	2006	27	1,323	0	0	---
Crabapple	2005	4	642	0	0	---
Black Hawthorn	2003	12	926	1	8.3	1, AM
	2004	40	10,402	15	22.5	11, AM
	2005	46	18,425	1	2.2	1, AM
	2006	60	21,907	4	5.0	2, AM
European Pear	2004	6	320	0	0	---
	2005	5	125	0	0	---
	2006	5	209	0	0	---
Garden Plum	2004	4	319	0	0	---
	2005	6	350	0	0	---
	2006	4	387	0	0	---
Apricot	2006	1	47	0	---	---
Sweet/Sour Cherry	2005	6	1,161	0	0	---
Bitter Cherry	2003	30	10,954	373	73.3	88, CFF
	2004	20	3,784	308	65.5	51, CFF
	2005	23	3,947	76	21.7	11, CFF
	2006	41	8,819	560	68.3	__, CFF
Choke Cherry	2003	23	9,751	0	0	---
	2004	20	5,592	0	0	---
	2005	41	8,200	73	12.2	3, CFF
	2006	17	5,378	0	0	---
Roses[a]	2004	45	4,223	585	84.4	129, RM
	2005	52	5,200	548	69.2	96, RM
	2006	21	1,845	489	100.0	__, RM
Serviceberry	2005	22	3,293	0	0	---
Common Snowberry	2004	32	5,343	178	50.0	61, SBM
	2005	23	1,557	522	100.0	30, SBM
	2006	37	4,640	644	97.3	__, SBM
Blue Elderberry	2004	18	3,103	0	0	---
	2005	41	8,200[a]	0	0	---
	2006	14	35,698[a]	0	0	---
Golden Currant	2005	12	2,985	28	50.0	0
Red Osier Dogwood	2005	20	7,959	4	20.0	1, DF
	2006	23	6,221	38	73.9	_, DF
Tall Oregon-Grape	2005	2	254	0	0	---

[a]Estimated; [b]Nootka and Woods' roses combined.

Table 3. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in Wenas, Yakima County, WA, August to September 2006

Plant Species	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	9	450	0	0	---
Black Hawthorn	49	18,195	21	20.4	__, AM
Choke Cherry	12	2,194	0	0	---
Roses[a]	21	1,469	32	71.4	__, RM
Common Snowberry	21	2,349	186	61.9	__, SBM

[a]Nootka and Woods' roses combined.

Table 4. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in Ellensburg, Kittitas County, WA, June to September 2004-2006

Plant Species	Sample Year	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	2004	194	4,850	0	0	---
	2005	139	6,950	0	0	---
	2006	64	2,496	3	1.6	__, AM
Crabapple	2004	151	15,700	0	0	---
	2005	108	13,800	0	0	---
	2006	35	4,916	0	0	---
Black Hawthorn	2004	19	3,800	0	0	---
	2005	15	1,450	0	0	---
	2006	27	4,198	0	0	---
Ornamental Hawthorns[a]	2005	30	5,580	0	0	---
English Hawthorn	2006	4	1,936	0	0	---
Smooth Hawthorn	2006	18	5,610	0	0	---
Washington Hawthorn	2006	9	7,419	0	0	---
European Pear	2004	96	2,400	0	0	---
	2005	48	1,645	0	0	---
	2006	2	27	0	0	---
Garden Plum	2004	55	2,612	0	0	---
	2005	32	960	0	0	---
	2006	14	1,239	0	0	---
Peach	2006	1	33	0	0	---
Sweet Cherry	2005	1	500	103	---	59, CFF
Choke Cherry	2005	14	5,300	0	0	---
	2006	10	2,026	0	0	---
Bitter Cherry	2006	10	2,265	32	70.0	__, CFF
Apricot	2004	11	300	0	0	---
	2005	6	180	0	0	---
Roses[c]	2004	12	2,400	10	25.0	1, RM
	2005	53	8,000	179	43.4	18, RM
	2006	30	3,020	122	53.3	__, RM
Quince	2006	1	189	0	---	---
Firethorn	2006	11	6,662	0	0	---
Serviceberry	2005	3	300	0	0	---
Red Osier Dogwood	2006	2	553	0	0	---
European Mountain Ash	2006	21	13,590	0	0	---

Rockspray Cotoneaster	2006	8	2,069	0	0	---
Peking Cotoneaster	2006	21	3,729	0	0	---
Peach	2005	4	110	0	0	---
Common Snowberry	2004	3	600	0	0	---
	2005	9	1,520	88	55.6	2, SBM
	2006	26	5,098	519	96.2	_, SBM
Blue Elderberry	2004	10	>2,000[b]	0	0	---
	2005	11	>2,200[b]	0	0	---
	2006	11	30,000[b]	0	0	---
Tall Oregon-Grape	2005	10	1,000	93	80.0	8, OG
	2006	4	613	0	0	---
Juniper	2005	3	225	0	0	---
English Yew	2006	5	204	0	0	---
Virginia Creeper	2006	5	1,969	0	0	---
Tartarian Honeysuckle	2006	3	48	0	0	---

[a]Combined English and smooth hawthorns; [b]Estimated; [c]Nootka and Woods' roses combined.

Table 5. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in Puyallup, Pierce County, WA, June to October 2004-2006

Plant Species	Sample Year	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	2004	7	345	208	100.0	>23, AM
	2005	13	820	816	100.0	297, AM
	2006	13	1,150	1,221	100.0	__, AM
English Hawthorn	2004	2	400	125	100.0	>8, AM
	2005	6	1,200	290	100.0	235, AM
	2006	8	3,200	271	87.5	__, AM
Asian Pear	2004	1	8	12	---	7, AM
	2005	2	35	24	100.0	11, AM
	2006	1	20	3	---	__, AM
Garden Plum	2004	2	60	0	0	---
	2005	2	100	0	0	---
	2006	3	150	0	0	---
Roses[a]	2005	3	70	0	0	---
European Mountain Ash[b]	2005	1	100	13	---	1, AM[a]
	2006	10	4,000	20	20.0	_, AM
Black Berry	2004	2	100	0	0	---
Salal	2005	2	200	0	0	---
Blue Elderberry	2006	2	100	0	0	---
Milkflower Cotoneaster	2006	2	800	17	50.0	_, AM
Rockspray Cotoneaster	2006	3	1,200	0	0	---
English Holly	2006	1	400	0	0	---
Oregon Grape	2006	2	800	0	0	---

[a]Unidentified species; [b]New host record.

Table 6. Plants sampled and numbers of *Rhagoletis* pupae and adults from various fruit collected in Vancouver, Clark County, WA, Skamania, Skamania County, WA, and Portland, Multnomah County, OR, June to October 2005-2006

Plant Species	Sample Year	No. Plants	No. Fruit	No. Pupae	% Plants Infested	No. Adults
Apple	2005	47	1,733	1,032	93.6	43, AM
	2006	62	2,876	1,384	72.6	__, AM
Crabapple	2005	1	189	6	---	3, AM
	2006	10	950	169	60.0	__, AM
Flowering Crabapple	2006	10	4,543	9	20.0	__, AM
Black Hawthorn	2005	17	6,558	331	70.6	81, AM
	2006	22	11,170	293	68.2	__, AM
English Hawthorn	2005	54	33,165	2,393	87.0	847, AM
	2006	45	37,221	788	88.9	__, AM
Smooth Hawthorn[a]	2005	1	272	188	---	57, AM[a]
	2006	1	1,339	112	---	__, AM
Washington Hawthorn	2006	5	6,174	0	0	---
Cockspur Hawthorn	2006	6	831	4	≤50.0[b]	__, AM
Chinese Hawthorn	2006	1	123	0	---	---
Apricot[c]	2005	4	146	44	100.0	20, AM[c]
European Pear	2005	7	268	0	0	---
	2006	22	936	3	9.1	__, AM
Asian Pear	2006	8	314	100	37.5	__, AM
Garden Plum	2005	6	367	35	16.7	12, AM
	2006	20	1,095	107	40.0	__, AM
Japanese Plum[a]	2005	1	51	5	---	2, AM[a]
Sweet/Sour Cherry	2005	3	625	203	100.0	52, CFF
	2006	5	1,085	121	60.0	__, CFF
Bird Cherry	2006	10	5,632	451	20.0	__, CFF
Bitter Cherry	2006	12	2,287	264	≥41.7	__, CFF
Cherry Plum[c, a]	2005	3	201	28	66.7	9 AM[c], 1 CFF[a]
Cherry Laurel	2005	6	696	75	50.0	34, CFF
	2006	2	665	0	---	---
Portugal Laurel	2006	1	421	0	---	---
Roses[d]	2005	3	827	279	63.2	218, RM
	2006	5	726	102	80.0	__, RM
Firethorn	2006	5	3,495	0	0	---
Western Mountain Ash[a]	2005	1	552	8	---	8, AM[a]
	2006	41	47,046	2	4.9	__, AM
European Mountain Ash	2005	1	643	0	---	---
Cranberry Cotoneaster	2005	3	132	5	---	4, AM
	2006	1	475	0	---	---
Milkflower Cotoneaster[a]	2005	2	1,078	3	50.0	1, AM[a]
	2006	2	1,790	0	---	---
Rockspray Cotoneaster	2006	33	4,148	0	0	---
Littleleaf Cotoneaster	2006	6	1,816	0	---	---
Aronia Species	2006	5	1,547	0	---	---
Common Snowberry	2005	18	5,151	574	94.4	279, SBM
	2006	23	13,678	596	100.0	__, SBM

Blue Elderberry	2005	5	5,045	0	0	---
	2006	8	13,044	0	0	---
Orange Honeysuckle	2005	1	38	0	---	---
Honeysuckle[e]	2006	10	438	0	0	---
Twinberry	2006	1	368	0	---	---
Red-Flowering Currant	2006	4	4,614	0	0	---
Japanese Honeysuckle	2006	1	74	12	---	__, AM?
Highbush-Cranberry	2005	1	372	0	---	---
	2006	10	6,659	0	0	---
Western Viburnum	2006	4	561	0	0	---
Cornelian Cherry	2005	1	541	0	---	---
Pacific Dogwood	2005	2	2,875	0	0	---
	2006	4	1,319	0	0	---
Red Osier Dogwood	2005	1	694	0	---	---
	2006	18	15,100	25	22.2	__, DF
Japanese Dogwood	2006	7	450	0	0	---
Flowering Dogwood	2006	11	696	0	0	---
Cherry Olive	2005	1	446	0	---	---
	2006	2	665	0	0	---
Tall Oregon-Grape	2005	1	547	0	---	---
	2006	29	4,641	53	17.2	__, OGF
Dull Oregon-Grape	2005	1	252	82	---	56, OGF
	2006	9	899	0	----	---
English Walnut	2005	1	62	454	---	---[f], WHF
	2006	6	422	491	100.0	__, WHF
Black Walnut	2005	2	116	370	100.0	163, WHF[f]
	2006	6	273	110	16.7	__, WHF
Blueberry	2005	3	1,663	0	0	---
Grape	2005	2	1,060	0	0	---
	2006	8	6,497	0	0	---
Porcelainberry	2006	1	113	0	0	---
Cascara	2005	4	1,352	4	50.0	0
	2006	6	3,391	55	33.3	__, CFF
Bittersweet Night Shade	2005	1	571	0	---	---
	2006	13	3,968	0	0	---
English Yew	2005	1	228	0	---	---
	2006	8	419	0	0	---
Strawberry Madrone	2005	1	57	0	---	---
Pacific Madrone	2005	1	1,320	0	---	---
Camellia	2006	4	79	0	---	---
Salal	2006	18	4,649	0	0	---
Burning Bush	2006	1	411	0	---	---
Western Burning Bush	2006	1	63	0	---	---
Purple Beauty	2006	1	475	0	---	---

[a]New host record; [b]Tree samples pooled; [c]New Washington State record; [d]Nootka and Woods' roses combined; [e]Several unidentified species. [f]Combined for both walnut species.

Table 7. Complete updated list of 18 confirmed apple maggot developmental hosts in nature in Washington, 2002-2006

Common Name	Scientific Name	Family	WA Nativity
Apple	*Malus domestica* (Borkh.) Borkh.	Rosaceae	Introduced
Black Hawthorn	*Crataegus douglasii* Lindl.[b]	Rosaceae	Native
Black Hawthorn	*Crataegus suksdorfii* (Sarg.) Kruscke[b]	Rosaceae	Native
English Hawthorn	*Crataegus monogyna* Jacq.	Rosaceae	Introduced
Smooth Hawthorn	*Crataegus laevigata* (Poiret) DC.[c]	Rosaceae	Introduced
Cockspur Hawthorn	*Crataegus crus-galli* L.	Rosaceae	Introduced
Crabapples	*Malus* spp.	Rosaceae	Introduced
European Pear	*Pyrus communis* L.	Rosaceae	Introduced
Asian Pear	*Pyrus serotina* L.	Rosaceae	Introduced
Garden Plum	*Prunus domestica* L.	Rosaceae	Introduced
Japanese Plum	*Prunus salicina* Lindl.	Rosaceae	Introduced
Cherry Plum	*Prunus cerasifera* Ehrh.	Rosaceae	Introduced
Apricot	*Prunus armeniaca* L.	Rosaceae	Introduced
Bitter Cherry	*Prunus emarginata* (Dougl. ex Hook.) D. Dietr.	Rosaceae	Native
Western Mountain Ash	*Sorbus scopulina* Greene	Rosaceae	Native
European Mountain Ash	*Sorbus aucuparia* L.	Rosaceae	Introduced
Cranberry Cotoneaster	*Cotoneaster apiculatus* Rehd. & Wils.	Rosaceae	Introduced
Milkflower Cotoneaster[d]	*Cotoneaster lacteus* W. W. Smith	Rosaceae	Introduced

Aults were reared from all hosts; [a]Termed 'native' if in our Washington collection sites, but not exclusive to Washington; [b]Some authorities consider black hawthorns west of the Cascade Mountains to be *C. suksdorfii* (Sarg.) Kruscke (Love 1999); [c]Also known previously as *Crataegus oxyacantha* L.

[d]Still need confirmation, even though identified as this species.

Note: only apple and black hawthorn were infested in central Washington.

FINAL PROJECT REPORT
WTFRC Project Number: AE-04-427

Project Title: Control of Apple Maggot Using Bait Spray Insecticides and Traps

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Cooperators: WSU personnel, growers, and homeowners in Vancouver and Puyallup

Budget History:

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

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

Objectives (2004-2006):

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

Significant Findings:

● GF-120 bait mixed with ammonium carbonate was more attractive than GF-120 mixed with ammonium acetate when placed with traps, suggesting attraction to the bait can be increased, and that ammonia form is important.
● In the laboratory, the percentages of flies feeding on GF-120 with or without ammonium carbonate or ammonium acetate were low, suggesting low attractiveness of even enhanced bait
● In the field, more flies were attracted to and fed on GF-120 mixed with ammonium carbonate and ammonium acetate than on GF-120 alone, indicating GF-120 attractiveness can be increased, even though overall responses over 30-min periods were low.
.● Inconsistent with attraction and feeding tests, in two tests, no reduction in larval infestations in apples were seen when GF-120 alone or with ammonium carbonate and ammonium acetate was sprayed on apple trees; possibly due to habitat effects and to dispersing flies.
.● In three other tests, GF-120 alone was very effective (>90% control) against apple maggot infestation of apples; however, in these tests, GF-120 with additional ammonia did not increase control, and Entrust (spinosad alone) was equally effective.
● In tests of different baits, larval infestations of apples were reduced equally by spinosad alone, GF-120, and Mazoferm + spinosad, but were not reduced by Nulure + spinosad, suggesting spinosad alone and the less expensive Mazoferm can both be substituted for GF-120.
● Use of GF-120 sprays and use of ammonium carbonate-baited red spheres were equally and very effective in reducing larval infestations in apple at two sites; however, in no case were infestations eliminated.
● When GF-120 and red spheres were used together, there was no further decrease in larval infestations, perhaps because flies were more attracted to the red spheres than the bait.

Results and Discussion:

1) Determine release of ammonia and other volatiles from bait sprays. Only low amounts of ammonia could be detected from 40% GF-120, and only from fresh drops, not those aged for three or seven days in a fume hood. The calculated release of ammonia from fresh 40% GF-120 was 2.84 nanograms per 6-mm drop per hour, which is much lower than the optimal 2 mg/h release from a single ammonium lure. Other volatiles were released, but could not definitively be identified, although they presumably included acetic acid, a component of the ammonium acetate in the bait. Although other volatiles were not identified, these results alone suggest attraction to GF-120 is low in part because of the low ammonia release from them.

2) Determine attraction to bait sprays and feeding behaviors on baits. In a test to determine the effects of GF-120 with ammonia compounds (ammonium carbonate = AC; ammonium acetate = AA) on attraction to traps at Vancouver, WA the 10 g AC lure attracted more flies than the 40% GF-120 + 10% AC lure, which attracted more than the control and the 17% and 40% GF-120 lures, although not more than the 40% GF-120 + 10% AA lure (Table 1). At Saint Cloud Ranch, the 40% GF-120 + 10% AC lure attracted more flies than the control and other GF-120 lures, including the 40% GF-120 + 10% AA lure (Table 1). Numerically, slightly more flies were caught in the 40% GF-120 alone treatment than in the control. Results show that GF-120 can be modified to increase fly responses from moderate distances. This assumes that flies trapped were not initially in the immediate vicinity of traps, but flew to them either from other parts of the tree

or from the closest neighboring trees. GF-120 + 10% AC was more attractive than GF-120 + 10% AA, suggesting AA is repellent to a degree, perhaps due to release of acetic acid. Also, when amounts of the compounds are the same, AC may have released more ammonia than the AA.

In a test to determine the effects of GF-120 with ammonia compounds on feeding responses in the laboratory, there were low responses to the baits, but female flies responded less to water than to 13% sucrose and 17% and 20% GF-120. Responses to 40% GF-120 and 40% GF-120 + AA were lower, and the response to 40% GF-120 + AC was intermediate (Table 2). Unlike females, however, males did not respond less to water than any of the baits. Results show that the presence of bait can bring flies onto apples more than the absence of baits. Female flies are more likely to feed on baits, which is consistent with the attraction results in the trapping test. The lack of differences between AC and AA was surprising given the results using the lures with traps in experiment 1. Possibly tests in the laboratory lacked the cues flies need to home in on odors.

Several tests were conducted to determine the effects of GF-120 with ammonia compounds on attraction and feeding in the field by watching flies as they approached or fed on drops sprayed on leaves, At Saint Cloud Ranch in 2005, fly visits were infrequent given the 3.7 total h of continuous observations per treatment, but there were greater percentages of sightings of flies near or feeding on 40% GF-120 + 10% AC or 10% AA than sucrose, 17% GF-120, and 40% GF-120 (Table 3). No differences were detected using AC and AA treatments with GF-120 sprayed on leaves. At Saint Cloud Ranch in 2006, fly responses were also low given the 5.7 total h of observation/treatment and similar results were obtained, with percentages of sightings of flies near or feeding on GF-120 + 10% AC or 10% AA higher than water, sucrose, 40% GF-120, and spinosad only (Table 3). In Puyallup in 2005, percentages of sightings of flies feeding on or near GF-120 + 2.5% AC or 2.5% AA were higher than near or feeding on water, sucrose, 17% and 40% GF-120, and spinosad only (Table 4). Also, no differences were seen between AC and AA treatments. This was also true in Puyallup in 2006, although the percentage of sightings of flies near or feeding on GF-120 + 2.5% AC was slightly higher than on or near GF-120 + 2.5% AA (Table 4). Overall results show that additional ammonia clearly increased the attractiveness of GF-120 when it was sprayed on apple leaves, although it was equally clear that no differences existed between adding AC or AA in the bait. There was also evidence that GF-120 with or without AC or AA was more attractive than water and spinosad only. However, because numbers of flies responding were low (especially at Saint Cloud Ranch), ammonia release from the enhanced GF-120 drops seemed insufficient to elicit strong or immediate responses from a large percentage of a fly population. Point sources of ammonia that emanate from lures such as those in the trapping test certainly are difficult to duplicate from spray drops on leaves and it may take most flies longer than the 30-min observation periods to find the bait. The slightly greater response to GF-120 alone on leaves (compared with sucrose) suggests the bait is somewhat attractive, whether because of olfactory cues, visual cues, or both. Numbers of feeds were lower than numbers of non-feeding visits near GF-120 in Puyallup, possibly due to arrestment of flies, although more evidence is needed to confirm this.

3) Determine effects of bait sprays under different habitat types. Five tests determined the effects of GF-120 with added ammonia compounds on infestations in apples. In Woodland in 2005 using orchard trees (100 ml spray/tree), there were no effects of any treatment on adult fly numbers, which were low in the orchard (Table 5). Numbers of larvae/fruit did not differ statistically among the control and treatments, although statistically there were fewer larvae/fruit in 40% GF-120 and 40% GF-120 + 10% AA than in other treatments (Table 5). Results from Woodland/Vancouver in 2005 using isolated trees (100 ml spray/tree) were similar in that no statistical differences were detected, but numerically there were fewer larvae/fruit in all treatments than in the control (Table 5). In Vancouver in 2006 using orchard trees (200 ml spray/tree), numbers of flies were low, and although there were significantly more adults in the

40% GF-120 + 10% AC treatment than in the control, there were significantly fewer larvae/fruit in all treatments than in the control (Table 6). However, the GF-120 + 10% AC or 10% AA treatments did not perform better than GF-120 alone, and statistically no better than spinosad only. In Puyallup in 2005 using orchard trees (100 ml spray/tree), statistically fewer adults were caught on traps in all treatments than in control trees. There were high levels of control using all the treatments, and similar to Vancouver in 2006, GF-120 + 2.5% AC or 2.5% AA treatments did not perform better than GF-120 alone, and statistically no better than spinosad only (Table 7). In Puyallup in 2006 using orchard trees (also 100 ml spray/tree), all treatments reduced the numbers of adults caught compared with the control, but spinosad only decreased it the most, whereas 40% GF-120 + 2.5% AC reduced it the least (Table 7). Despite different effects on adult captures, all treatments again resulted in high levels of control of larvae/fruit. Also, again no differences among GF-120 with and without AA or AC and spinosad only were detected (Table 7).

In tests comparing various baits, it was found that in the laboratory, flies fed on the various baits at similar frequencies (Table 8), numbers of times, and durations (Table 9). When baits were sprayed on apple leaves in the field, flies responded most to Mazoferm (Table 10). When baits were sprayed on infested apple trees, GF-120, Mazoferm (with spinosad), and spinosad alone were equally effective and all reduced infestations >80% (Tables 11 and 12). Nulure was least effective. These tests were conducted using 100 ml of spray per tree in an orchard.

Results show that GF-120 treatments resulted in high levels of larval control in three of five tests, but adding AC or AA to GF-120 did not enhance its effectiveness in reducing adult fly numbers and larval infestations compared with using GF-120 alone, which was surprising given the consistently greater attraction to enhanced fresh GF-120 in the behavioral observations. It was unclear why results differed among tests, but differences in habitat type probably was one reason, as this affected numbers of dispersing flies around test trees. Fruiting phenology, susceptibility of apple cultivars, numbers of spray applications, spray coverage, and the way fruit were collected (from ground versus tree) among tests also could have affected results. No significant rainfall occurred that could explain them.

With respect to adult flies, in Vancouver in 2006 and in Puyallup in 2005, more flies were caught on traps hung in trees sprayed with GF-120 + AC than in trees sprayed with GF-120 alone, suggesting the enhanced bait brought more flies in from surrounding trees. This apparent influx of adults to the trees did not increase larval infestations, however, suggesting flies were caught before they oviposited. With respect to larval infestations, one possible explanation for the lack of differences between GF-120 and GF-120 + AC or AA treatments is that ammonia release rates from enhanced drops decreased quickly after sprays, so after a few days the enhanced GF-120 was the same as GF-120 alone in attractiveness. If so, ingredients that prolong the release of ammonia may be beneficial. It is possible that at the spray volumes tested, flies were able to find drops even after they lost their attractiveness through normal foraging movements. Coverage of all single trees in an area or of entire orchards with GF-120 may lead to greater suppression than obtained by spraying randomly selected single trees in this study or may even eliminate fly populations over time.

Despite the high levels of control obtained using GF-120 in three of five tests, the similarly high levels of control using 100 ml of spinosad only/tree suggests bait may not be needed with spinosad for it to be effective. Spinosad seems unattractive compared with 40% GF-120 alone, so its effectiveness even at a low volume probably is unrelated to attraction. This suggests the flies found drops of spinosad and fed on them during the course of normal foraging. Also, it is possible there was some contact activity, as spinosad drops are smaller than bait drops and result in greater coverage. More work is needed to determine if baits are needed with spinosad for controlling flies, especially at volumes < 100 ml/tree. The hypothesis is that GF-120 or other baits have more beneficial effects at these volumes, which result in very low coverage of leaves.

The effectiveness of GF-120 and Mazoferm with spinosad suggests that any number of baits will work. However, Nulure with spinosad was not as effective, suggesting not all baits are

equally effective and that the flies can detect differences among them in the field, although apparently not in the confined conditions laboratory, and although the taste of Nulure apparently is not repellent.

4) Determine effects of bait sprays and trapping methods on apple maggot control. In 2004 in Puyallup, there was a significant reduction in numbers of larvae in apples when trees were trapped used 6 baited red spheres alone, sprayed with GF-120 alone (540 ml/tree), or when spheres and GF-120 were combined, as compared with the control (Table 13). Presumably, the baited red spheres alone removed enough gravid flies to reduce larval infestations, while GF-120 sprays killed enough flies to reduce infestations. The combination of the two surprisingly did not further reduce infestations, perhaps because flies were more attracted to the red spheres than to the bait spray, and were caught before they fed on the bait. When ammonia-baited spheres were absent, the flies apparently fed on the bait and suffered high mortality, resulting in reduced infestations. Red spheres did not eliminate infestations in one season, but they may increase the effectiveness of sprays by reducing populations, making it easier to eliminate populations the following. Trees were relatively small at 5-13.5 ft tall and 4.5-11.5 ft wide, and the 540 ml sprays resulted in very thorough coverage of the trees.

In 2004 in Woodland, there were significant reductions in larval infestations in apple when 6 red spheres were used, when 6 red spheres were combined with weekly GF-120 sprays (540 ml spray/tree), and when 1 yellow panel was combined with weekly GF-120 sprays, as compared with when single yellow traps alone were used (Table 13). Possibly results may have even been more dramatic had no yellow panels been placed in the control, for some female flies that would have oviposited were removed from the population in these trees. Because the test was conducted in isolated trees, larval infestations in the single yellow trap treatment may have reduced due to lack of many immigrating flies. Trees were relatively large at 12.5-20 ft tall and 12-20 ft wide, so coverage was not as high as in Puyallup. However, as in Puyallup, the results indicate that either baited red sphere traps or GF-120 can reduce larval infestations.

Significance to the Industry and Potential Economic Benefits: The results are significant to the apple industry in that they show GF-120, spinosad alone, and other protein baits (in particular Mazoferm) appear to be highly capable of managing apple maggot flies in feral or backyard apple trees, and likely in hawthorn trees as well, although this has yet to be tested. Use of sprays represent a second line of defense against flies moving into commercial apples orchards, after flies are detected using traps. By suppressing fly populations using spinosad-based bait sprays or even spinosad alone, the risks of flies spreading into previously un-infested commercial apple-growing areas and of these areas being placed under quarantine are greatly reduced. GF-120 and other baits with spinosad are safer alternatives to the main organophosphate currently used (Imidan) to control the flies and thus are more desirable to use near residential areas and near creeks and rivers. Results also show the potential of mass trapping to reduce larval infestations, suggesting baited red spheres may be useful in feral and backyard trees. Results of this study show that GF-120 and other baits can be improved with respect to attractiveness, but that this increased attractiveness does not necessarily result in increased control. It is possible that GF-120 is so effective that it did not need added ammonia at the spray volumes used, and that addition of ammonia in GF-120 for control or the use of baits in general may be more critical at spray volumes much lower than those used in this study. Even though spinosad baits are highly effective, infestations were not eliminated using them, so there should be further benefits of optimizing the bait, e.g., by making it effective at very low amounts or making it attractive over longer periods of time. The best possible bait spray should eliminate fly infestations after one season and virtually ensure that there is no chance flies will invade orchards.

Table 1. Mean total numbers of apple maggot flies ± SE caught over the season per sticky yellow panel trap with different GF-120 bait lures at two sites, WA, 2005

	Vancouver	Saint Cloud Ranch
Treatment	19 July-22 August	28 July-22 September
Control	1.2 ± 0.9c	9.0 ± 2.0b
10 g AC	15.5 ± 2.7a	----
17% GF-120	1.8 ± 1.1c	17.3 ± 1.8b
40% GF-120	1.8 ± 1.0c	66.0 ± 47.9b
40% GF-120 + 10% AC	7.0 ± 2.3b	220.3 ± 39.1a
40% GF-120 + 10% AA	2.8 ± 1.8bc	41.8 ± 4.7b
Randomized Block	$F = 8.4$; df = 5, 15	$F = 13.0$; df = 4, 12
ANOVA	$P = 0.0006$	$P = 0.0003$

Blank GF-120 used; AC, ammonium carbonate; AA, ammonium acetate.
Four replicates of the control and each treatment in both tests.
10 ml of each GF-120 or GF-120 + ammonia compound mixture in 15 ml polypropylene bottles. Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 2. Percent of single apple maggot flies that drank or fed on water or GF-120 baits on apples and that were on apples over 1-h observations in the laboratory

	Females			Males		
Treatment	*N*	% Drank or Fed	% all Flies on Apple[a]	*N*	% Drank or Fed	% all Flies on Apple[a]
Water	22	0.0	9.1	18	0.0	16.7
13% Sucrose	27	33.3	81.4	20	15.0	40.0
17% GF-120	25	20.0	64.0	21	0.0	28.6
20% GF-120	22	27.3	86.4	18	11.1	33.3
40% GF-120	27	7.4	25.9	24	16.7	37.5
40% GF-120 + 10% AC	21	9.5	52.4	20	5.0	35.0
40% GF-120 + 10% AA	12	0.0	25.0	10	10.0	70.0
Fisher's Exact Test[b]		$P = 0.0054$[b]	$P < 0.0001$[c]		$P = 0.2826$[b]	$P = 0.2072$[c]

Blank GF-120 used; AC, ammonium carbonate; AA, ammonium acetate.
[a]Feeders and non-feeders combined; [b]Comparing % that fed and not fed; [c]Comparing total fly numbers on apple and not on apple.

Table 3. Total numbers of apple maggot fly sightings every 2 min feeding on or near GF-120 baits sprayed on apple leaves at Saint Cloud Ranch, WA, 2005-2006

2005[a] Treatment	No. <15 cm From Bait[b]	No. Feeding[c]	Total Fly Sightings	% of Total
13% Sucrose	0	1	1	2.9
17% GF-120	1	1	2	5.7
40% GF-120	1	1	2	5.7
40% GF-120 + 10% AC	6	8	14	40.0
40% GF-120 + 10% AA	12	4	16	45.7
Total fly sightings: Chi square = 30.9; df = 4; $P < 0.0001$.				
2006[d] Treatment	No. <15 cm From Bait[b]	No. Feeding[c]	Total Fly Sightings	% of Total
13% Sucrose	0	4	4	15.4
40% GF-120	0	2	2	7.7
40% GF-120 + 10% AC	1	9	10	38.5
40% GF-120 + 10% AA	2	7	9	34.6
Spinosad Only	1	0	1	3.8
Total fly sightings: Chi square = 12.8; df = 4; $P = 0.0121$.				

Blank GF-120 used in 2005; GF-120 and spinosad only in 2006 had 0.0096% spinosad (wt/vol).
AC, ammonium carbonate; AA, ammonium acetate.
[a]Total sightings from three to six replicate trees from each of eight d.
[b]Not drinking or feeding.
[c]Expected cells <5, data not analyzed.
[d]Total sightings from four or five replicate from each 11 d.

Table 4. Total numbers of apple maggot fly sightings every 2 min feeding on or near GF-120 baits sprayed on apple leaves in Puyallup, WA, 2005-2006

2005[a] Treatment	No. <15 cm From Bait[b]	No. Drinking or Feeding[c]	Total Fly Sightings	% of Total
Water	1	0	1	0.4
13% Sucrose	1	0	1	0.4
17% GF-120	13	0	13	5.5
40% GF-120	38	3	41	17.4
40% GF-120 + 2.5% AC	82	6	88	37.4
40% GF-120 + 2.5% AA	83	6	89	37.9
Spinosad Only	2	0	2	0.8
Total fly sightings: Chi square = 286.7; df = 6; $P < 0.0001$.				
2006[d] Treatment	No. <15 cm From Bait[b]	No. Drinking or Feeding[c]	Total Fly Sightings	% of Total
Water	1	0	1	1.2
13% Sucrose	3	2	5	6.1
40% GF-120	13	1	14	17.1
40% GF-120 + 2.5% AC	30	6	36	43.9
40% GF-120 + 2.5% AA	23	3	26	31.7
Spinosad Only	0	0	0	0.0
Total fly sightings: Chi square = 51.8; df = 4; $P < 0.0001$; spinosad only not included.				

GF-120 and spinosad only in 2005 and 2006 had 0.0096% spinosad (wt/vol); AC, ammonium carbonate; AA, ammonium acetate. [a]Total sightings from four replicate trees from four d.
[b]Not feeding; [c]Expected cells <5, data not analyzed; [d]Total sightings from 10 replicate trees from five d.

Table 5. Mean numbers of adult apple maggot flies and larvae per apple fruit ± SE in GF-120 bait spray tests in Woodland and Woodland/Vancouver, WA, 2005

Woodland				
Treatment	No. Flies/Trap	% Lower	No. Larvae/Fruit	% Lower
Control	0.2 ± 0.2	----	0.22 ± 0.08	----
17% GF-120	0.8 ± 0.8	----	0.21 ± 0.12	4.5
40% GF-120	0.5 ± 0.3	----	0.09 ± 0.05	59.1
40% GF-120 + 10% AC	0.2 ± 0.2	----	0.31 ± 0.22	----
40% GF-120 + 10% AA	0.2 ± 0.2	----	0.08 ± 0.05	63.6
Randomized Block	$F = 0.2$		$F = 0.7$	
ANOVA, df = 4, 12	$P = 0.9175$		$P = 0.5916$	
Woodland/Vancouver				
Treatment	No. Flies/Trap	% Lower	No. Larvae/Fruit	% Lower
Control	2.0 ± 0.7	---	1.71 ± 0.89	---
17% GF-120	4.0 ± 2.2	---	0.42 ± 0.25	75.4
40% GF-120	3.3 ± 2.6	---	0.30 ± 0.07	82.5
40% GF-120 + 10% AC	3.8 ± 2.4	---	0.30 ± 0.05	82.5
40% GF-120 + 10% AA	2.8 ± 1.6	---	0.84 ± 0.46	50.8
Randomized Block	$F = 0.1$		$F = 1.4$	
ANOVA, df = 4, 12	$P = 0.9705$		$P = 0.3092$	

GF-120 and spinosad only had 0.0096% spinosad (wt/vol).
AC, ammonium carbonate; AA, ammonium acetate; 100 ml spray/tree.
Four replicates of the control and treatments.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 6. Mean numbers of adult apple maggot flies and larvae per apple fruit ± SE in GF-120 bait spray test in Vancouver, WA, 2006

Treatment	No. Flies/Trap	% Lower	No. Larvae/Fruit	% Lower
Control	0.0 ± 0.0b	----	0.030 ± 0.009a	---
40% GF-120	0.17 ± 0.17b	----	0.000 ± 0.000b	100.0
40% GF-120 + 10% AC	1.00 ± 0.36a	----	0.000 ± 0.000b	100.0
40% GF-120 + 10% AA	0.17 ± 0.17b	----	0.007 ± 0.004b	76.7
Spinosad Only	0.0 ± 0.0b	----	0.013 ± 0.013b	56.7
Randomized Block	$F = 4.2$		$F = 2.9$	
ANOVA, df = 4, 20	$P = 0.0129$		$P = 0.0480$	

GF-120 and spinosad only had 0.0096% spinosad (wt/vol).
AC, ammonium carbonate; AA, ammonium acetate; 200 ml spray/tree.
Six replicates of the control and treatments.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 7. Mean numbers of adult apple maggot flies and larvae per apple fruit ± SE in GF-120 bait spray tests in Puyallup, WA, 2005-2006

2005				
Treatment	No. Flies/Trap	% Lower	No. Larvae/Fruit	% Lower
Control	35.2 ± 1.9a	---	1.22 ± 0.59a	---
40% GF-120	11.6 ± 3.1b	67.0	0.07 ± 0.02b	94.3
40% GF-120 + 2.5% AC	28.2 ± 2.2b	19.9	0.08 ± 0.04b	93.1
40% GF-120 + 2.5% AA	17.8 ± 2.6b	49.4	0.16 ± 0.03b	86.7
Spinosad Only	14.0 ± 2.8b	60.2	0.05 ± 0.004b	96.0
One-Way ANOVA	$F = 12.5$		$F = 4.4$	
df = 4, 20	$P < 0.0001$		$P = 0.0098$	
2006				
Treatment	No. Flies/Trap	% Lower	No. Larvae/Fruit	% Lower
Control	119.4 ± 10.1a	---	0.99 ± 0.23a	---
40% GF-120	15.0 ± 1.4c	87.4	0.08 ± 0.02b	91.9
40% GF-120 + 2.5% AC	24.4 ± 1.6b	92.0	0.05 ± 0.02b	86.9
40% GF-120 + 2.5% AA	9.6 ± 1.5c	79.6	0.13 ± 0.05b	94.9
Spinosad Only	1.8 ± 0.8d	98.5	0.01 ± 0.01b	99.0
One-Way ANOVA	$F = 158.7$		$F = 19.4$	
df = 4, 20	$P < 0.0001$		$P < 0.0001$	

GF-120 and spinosad only had 0.0096% spinosad (wt/vol).
AC, ammonium carbonate; AA, ammonium acetate; 100 ml spray/tree.
Five replicates of the control and treatments.
Means within columns followed by the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

Table 8. Percentages of apple maggot flies responding to protein baits inside vials in the laboratory

	Females		Males	
Treatment	*N*	% Response	*N*	% Response
Control	36	8.3	30	3.3
GF-120	38	34.2	34	17.6
Nulure	30	46.7	30	23.3
Mazoferm	36	33.3	38	21.1
Spinosad Only	37	5.4	35	14.3
Fisher's Exact Test		$P = 0.000039$		$P = 0.1785$

All baits and spinosad only had 0.0096% spinosad (wt/vol).
When Fisher's exact test was conducted within treatments and between sexes to determine if responses to a bait were dependent on sex, there were no significant differences ($P = 0.1033$ to 0.6198).

Table 9. Numbers of feeds and feeding duration ± SE of apple maggot flies on protein baits inside vials in the laboratory

	No. Drinks/Feeds		Durations (seconds)	
Treatment	Females	Males	Females	Males
Water	0.08 ± 0.05	0.03 ± 0.03	0.58 ± 0.45	0.13 ± 0.13
GF-120	0.45 ± 0.11	0.21 ± 0.08	8.13 ± 2.84	4.35 ± 2.08
Nulure	0.70 ± 0.16	0.27 ± 0.10	10.30 ± 3.33	3.27 ± 2.26
Mazoferm	0.61 ± 0.17	0.21 ± 0.07	5.89 ± 2.02	1.13 ± 0.53
Spinosad Only	0.05 ± 0.04	0.14 ± 0.06	0.05 ± 0.04	0.20 ± 0.10
Two-way ANOVA	No. Drinks/Feeds		Durations (seconds)	
Treatment	$F = 7.5$, df = 4, 334, $P < 0.0001$		$F = 6.1$, df = 4, 334, $P < 0.0001$[b]	
Sex	$F = 11.4$, df = 1, 334, $P = 0.0008$		$F = 8.0$, df = 1, 334, $P = 0.0051$	
Treatment × Sex	$F = 2.8$, df = 1, 334, $P = 0.0276$[a]		$F = 1.4$, df = 1, 334, $P = 0.2410$	

All baits and spinosad only had 0.0096% spinosad (wt/vol); N, same as in Table 1; [a]Because of the interaction, one-way ANOVA was performed within sexes; for females, $F = 6.9$; df = 4, 172, $P < 0.0001$; water = spinosad <GF-120 = Nulure = Mazoferm (Fisher's LSD test); for males, not significant ($P = 0.2508$).
[b]Water = spinosad >GF-120 = Nulure, Mazoferm not different than any treatment (Fisher's LSD test).

Table 10. Total numbers of apple maggot flies seen feeding on or near (and not feeding on) protein baits sprayed on apple leaves in Puyallup, WA, 2006

Treatment	No. Feeding	No. Not Feeding[a]	Total Sightings of Flies	% of Total Sightings
Water	0	0	0	0.0
13% Sucrose	2	2	4	6.0
GF-120	7	4	11	16.4
Nulure	10	0	10	14.9
Mazoferm	34	5	39	58.2
Spinosad Only	1	2	3	4.5
Total Sightings: Chi-Square Goodness of Fit Test = 64.9; df = 4; $P < 0.0001$				

All baits and spinosad only had 0.0096% spinosad (wt/vol).
Totals from 10 trees (two trees per day over five days of observations)
[a]≤15 cm from water or baits.

Table 11. Mean numbers of adult apple maggot flies and larvae per apple fruit ± SE in bait spray test in Puyallup, WA, 2005

Treatment	No. Adults/trap	% Reduction	No. Larvae/fruit	% Reduction
Control	43.8 ± 7.7a	---	0.846 ± 0.025a	---
GF-120	12.3 ± 2.8b	71.9	0.095 ± 0.032b	88.8
Nulure	13.5 ± 3.1b	69.2	0.746 ± 0.160a	11.8
Mazoferm	18.0 ± 7.6b	58.9	0.104 ± 0.21b	87.8
Spinosad Only	14.8 ± 5.5b	66.2	0.108 ± 0.026b	87.2
1-way ANOVA	$F = 4.2$		$F = 31.3$	
df = 4, 15	$P = 0.0184$		$P < 0.0001$	

All baits and spinosad only had 0.0096% spinosad (wt/vol).
Four replicates of the control and treatments.
Means within columns followed by the same letter are not significantly different ($P > 0.05$).

Table 12. Mean numbers of adult apple maggot flies and larvae per apple fruit ± SE in bait spray test in Puyallup, WA, 2006

Treatment	No. Adults/trap	% Reduction	No. Larvae/fruit	% Reduction
Control	145.4 ± 18.8a	---	0.92 ± 0.21a	---
GF-120	7.2 ± 2.2bc	95.0	0.19 ± 0.04bc	79.3
Nulure	4.2 ± 0.4cd	97.1	0.38 ± 0.08b	58.7
Mazoferm	14.4 ± 1.8b	90.1	0.18 ± 0.03bc	80.4
Spinosad Only	0.6 ± 0.2d	99.6	0.03 ± 0.01c	96.7
1-way ANOVA	$F = 117.8$		$F = 13.0$	
df = 4, 20	$P < 0.0001$		$P < 0.0001$	

All baits and spinosad only had 0.0096% spinosad (wt/vol).
Five replicates of the control and treatments.
Means within columns followed by the same letter are not significantly different ($P > 0.05$).

Table 13. Effects of GF-120 sprays and red sphere traps on larval infestations, 2004

Test 1: Puyallup			Test 2: Woodland		
Treatment	Adults	Lv/Apple	Treatment	Adults	Lv/Apple
Control	---	0.580b	Control, Panel	41.7	1.795b
GF-120	---	0.011a	GF-120, Panel	12.2	0.027a
6 Red Spheres	220.4	0.059a	6 Red Spheres	113.5	0.167a
GF-120 + 6 Red Spheres	180.2	0.096a	GF-120 + 6 Red Spheres	44.5	0.023a
RBD ANOVA *F*	23.1	---	RBD ANOVA *F*	2.8	6.4
df	3, 12	---	df	3, 8	3, 8
P	< 0.0001	---	*P*	0.1071	0.0163

Means followed by the same letter within columns with the same letter are not significantly different (Fisher's LSD test, $P > 0.05$).

FINAL REPORT

Project Title: Temperate Fruit Fly Workshop

PI: Wee Yee
Organization: USDA-ARS

Telephone/email: (509) 454-6558/wlyee@yarl.ars.usda.gov
Address: 5230 Konnowac Pass Rd
City: Wapato
State/Province/Zip WA/98951

Cooperators: Jim McFerson, Tom Unruh, Pete Landolt, Vince Jones and other university and industry participants (see below)

Budget History:

Item	**Year 1:** 2006
Salaries	0
Benefits	0
Wages	0
Benefits	0
Equipment	0
Supplies	0
Travel	5,000[1]
Miscellaneous	0
Total	5,000

[1]The $5,000 (and another $5,000 from Cherry Research) was used to reimburse 7 scientists for their travel to and stay in Yakima for the workshop.

Objectives 2006
1) Have a focused update on the nature of our problem with fly pests.
2) Provide updates on what research is ongoing and relevant – both in the Pacific NW and nationally.
3) Discuss how we ought to revise our research strategy to develop more collaborative, productive research and implementation.

Significant Outcomes:
At the end of the workshop, the following were identified as items of high research priority for apple maggot fly:

- **IDENTIFICATION (WHAT IS IT?)**
  - A. Differentiate apple maggot fly (*Rhagoletis pomonella*) from snowberry maggot fly (*Rhagoletis zephyria*) using molecular markers.
  - B. Identify possible host races of apple maggot on apple and hawthorn using morphometric, genetic, and behavioral approaches.
  - C. Hybridization of and gene flow between apple maggot and snowberry maggot.
- **DETECTION (FINDING WHAT WE HAVE)**
  - A. Develop better attractants and traps used for detecting and monitoring flies on apples and on hawthorns.
  - B. Determine spatial distributions of flies within WA; genetic, ecological, host, and behavioral factors affecting apple maggot abundance in western and eastern WA.
- **APPLE MAGGOT CONTROL AND MANAGEMENT**
  - A. Improve attractants, bait formulations (efficacy, retention), and packaging for apple maggot kill.
  - B. Improve and test toxicants (neonicotinoids such as Assail) and repellents (such as kaolin, Rainguard) and better sprays to use in riparian areas for fly containment.
  - C. Sampling and trapping to determine where flies come from.
  - D. Determine meaning of fly catches; reproductive status of flies caught on traps.
  - E. Combined tactics, including parasitoid release, for fly containment in feral areas.
  - F. Determine dispersal of flies; where flies are coming from.
  - G. Validation of phenology model in Washington.
  - H. Host plant resistance; resistant apple varieties and chemical, other factors.
  - I. Systems approach for management to satisfy domestic and foreign markets.
- As a result of the workshop, collaborations were planned: Wee Yee, Peter Landolt, and Charlie Linn to on work on host attractants for apple maggot; Wee Yee, Tom Unruh, Jeff Feder, and Stewart Berlocher to work on morphometric and molecular diagnostics of apple maggot, apple maggot host races, and snowberry maggot flies.

History and Methods

The Washington Tree Fruit Research Commission initiated a discussion to address research that could expedite the management of cherry fruit flies on January 7, 2005 at the Cherry Institute Meeting in Yakima. Discussion initially centered on the cherry fruit fly, but at a second discussion that took place at the Apple Entomology Research Review in Yakima on January 28, 2006, it was expanded to include apple maggot. At the Review, discussion was centered on what fly problems occur in the Northwest and how the Commission can help in funding projects that potentially can solve these problems. A third discussion took place at the USDA's Yakima Agricultural Research Laboratory (YARL) on March 1, 2006, and it was here at YARL that the idea arose to hold a fly workshop at the Cherry Research Review in The Dalles in November

2005. Between this time and the Cherry Priority Setting session in Ellensburg on August 11, 2005, various fruit fly researchers were contacted for their possible participation for a November meeting. However, the November meeting conflicted with the Entomological Society of America's Annual Meeting, which is attended by almost all professional entomologists, and it was decided to postpone the fruit fly workshop to a later time. At the Cherry Research Review in The Dalles on November 4, 2005, a question and answer session about the proposed fruit fly workshop was conducted in which Commission support for the workshop was gauged (research participants were Wee Yee, Pete Landolt, Vince Jones, and Mike Willett, moderated by Jim McFerson).

After more discussion, it was decided a meeting to plan the workshop should be held at the Western Orchard Pest and Disease Management Conference in Portland from January 11-13, 2006 to come up with a tentative agenda. The eight attendees in Portland were: Diane Alston, Utah State University, Rufus Isaacs, Michigan State University, Vince Jones, Washington State University, Gary Judd, Ag Canada, Pete Landolt, USDA-ARS, Howard Thistlewood, Ag Canada, Tom Unruh, USDA-ARS, and Dave Biddinger, Penn State University.

One of the main conclusions of the group was that several areas of research are relevant and need to be emphasized; a few additional ones (f and g) were added after the meeting in Portland:

a. Identification problems (esp. apple maggot and snowberry maggot) using molecular techniques
b. Behavioral studies, particularly migration of mated females, population biology, and phenology
c. Detection/security
d. What happens in the soil? Including biological control, possible use of nematodes
e. Management, including bait sprays, area-wide approaches, pesticide efficacy
f. Host range – likelihood of a fruit fly species infesting a certain host. Any objective measure of a commercially significant host range that could be explored
g. Survival of flies in different habitats in Washington

It was further agreed that the idea was not to ask Washington people to present their research, but to let experts from other parts of the country do this. Because there were seven identified areas, potentially seven outside researchers would participate, although there may be two participants under some of the areas. The plan was to have a maximum of 10 researchers invited to the workshop.

On April 10, 2006 in Ellensburg, a meeting was held to draft a workshop agenda and to decide which fruit fly researchers to invite to the workshop. The meeting was attended by Wee Yee, Vince Jones, Tom Unruh, and Jim McFerson, with Mike Willett calling in. From this meeting a draft was generated.

After much more correspondence, the list of invited researchers and the researchers' general areas of expertise was finalized:

1- Dr. Sue Opp, California State University – Dispersal of walnut husk fly
2- Dr. Charles Linn, Cornell University – Attraction of apple maggot races to fruit volatiles
3- Dr. Jeff Feder, University of Notre Dame – Genetic differences among apple maggot fly host races
4- Dr. Stewart Berlocher, University of Illinois – Genetics of and taxonomic relationships among fruit flies
5- Dr. Russ Messing, University of Hawaii – Biological control of fruit flies
6- Dr. Diane Alston, Utah State University – Insecticide control of western cherry fruit fly
7- Dr. Larry Gut, Michigan State University – Management of eastern cherry fruit fly and apple maggot fly using insecticides and baits

After several revisions, the final agenda was as follows:
Date and Location: August 28-29, 2006, USDA-ARS Lab in Wapato, WA
SUN, AUGUST 27
– Researchers fly in; evening get together of researchers, at Tom Unruh's house.

MONDAY, AUGUST 28

1. WELCOME & INTRODUCTION – WEE YEE – 8:00-8:10

2. OVERVIEW 8:10-8:30
MIKE WILLETT -- NORTHWEST HORTICULTURAL COUNCIL
Magnitude of Problems of Apple Maggot and Cherry Fruit Fly,
Quarantine issues; Distribution of apple maggot, etc.

3. OVERVIEW OF WASHINGTON RESEARCH LAST FIVE YEARS
Wee Yee and Tom Unruh USDA-ARS Wapato (8:35-8:55)

SECTION I. GENETICS AND LIFE HISTORY*

4. GENETIC VARIATIONS: (9:00-9:20)
Jeff Feder and Stewart Berlocher
- Host Use
- Identification
- Implications for Management

5. LIFE HISTORY: (9:25-9:45)
Charlie Linn
- Behavior
- Odor and Visual Cues
- Learning
- Detection- Trapping

BREAK (9:50-10:05)

6. FACILITATED DISCUSSION AND SYNTHESIS – SECTION I (10:05-12:00)
- Focus on areas ripe for collaboration, areas where info is missing or inadequate

LUNCH (12:-1:30)

***each person summarizing should give us at the end of their presentation, 3 areas that are researchable and key to understanding the life history and management of the flies**

SECTION II. POPULATION BIOLOGY AND MANAGEMENT*

7. POPULATION BIOLOGY: (1:30-1:50)
Sue Opp
- Dispersal
- Phenology
-Survival, abiotic and dietary factors

8. BIOLOGICAL CONTROL: (1:55-2:15)
Russ Messing
–Parasitoids & Predators
–Potential to reduce problems

9. MANAGEMENT: (2:20-3:05)
Larry Gut **(2:20-2:40)**
Diane Alston **(2:45-3:05)**
-Area Wide Suppression
- Bait Sprays
- Attract-and-Kill
- Pesticide Efficacy
- Thresholds

BREAK (3:10-3:25)

10. FACILITATED DISCUSSION AND SYNTHESIS – SECTION II (3:25-4:30)

4:30-5:15 – BREAKOUT GROUPS FOR FUTURE COLLABORATION

5:15-5:45 - CONTINUE BREAKOUT GROUPS AND/OR LAB TOUR

6:00 – 9:00 SILVERLAKE WINERY TOUR & SOCIAL WITH INDUSTRY REPS

***each person summarizing should give us at the end of their presentation, 3 areas that are researchable and key to understanding the life history and management of the flies**

TUESDAY, AUGUST 29

1. INTRODUCTIONS INDUSTRY AND SCIENTISTS (8:20-8:30)

SCIENTISTS' AND ORGANIZER'S MEETING (Synthesis of Monday's presentations and discussions) 8:30-10:30

(**Scientists and** Willett, Brunner, Landolt, McFerson, Yee, Unruh, Jones)

1. Give synthesis of where we are in PNW as of now
2. Emphasize the areas of needed research, areas ripe for collaboration, areas where info is missing or inadequate
3. Address in the presentation issues that we can't control
    a. Zero tolerance effects on IPM

10:30-10:45 BREAK

2. DISCUSSION WITH INDUSTRY (10:45-11:45)

Willett & McFerson

– Go through each area again (summary points only up on screen)
    - Ask for questions, comments, and suggestions in each area
    - Was anything missing?
    - Throw open for interactions

END BY 12:00; after lunch, researchers can leave.

1:30-3:00; ORGANIZER'S COMMITTEE (Willett, Brunner, Landolt, McFerson, Wee, Unruh, Jones)

- **Meets and modify presentation dependent on interactions in morning**
- **Set research priorities for industry. Or should this be done over a week's time?**

PARTICIPANTS IN ADDITION TO THE 7 SCIENTISTS:

Industry	Researchers and Others
McFerson	Klaus
Willett	Brunner
Craver	Landolt
Doornink	Yee
Hayden	Jones
Tim Smith	Unruh
Milne	Barcenas
Dan Griffith	

On August 28 and 29, 2006, the workshop was held at the YARL as planned. All invited scientists were present: Sue Opp, Charles Linn, Jeff Feder, Russ Messing, Stewart Berlocher, Diane Alston, and Larry Gut. The Washington entomologists and industry people were: Mike Willett, Vince Jones, Jim Doornink, Jim McFerson, Timothy Smith, Michael Klaus, Jay Brunner, Brent Milne, Dain Craver, Tom Unruh, Pete Landolt, and Wee Yee.

Not all the invited people could attend. The August 29 attendees were: Brent Milne, Jay Brunner, Dan Griffith, Charlie Linn, Russ Messing, Mike Willett, Vince Jones, Diane Alston, Larry Gut, Jim Doornink, Michael Klaus, Sue Opp, Tom Unruh, Pete Landolt, and Wee Yee.

The workshop in general followed closely the agenda outline for the first day.

The invited scientists were asked prior to the workshop to come up with three key research areas that will help understand fly biology and fly management:

The following were ones pertinent to apple maggot fly:

Jeff Feder: Genome Project using AM

Stewart Berlocher:

1-Surveys to determine basic ecological and biogeographical data on western *Rhagoletis*

2-Find molecular markers to distinguish *R. pomonella* (AM) from *R. zephyria* (SBM).

3 - Measure gene flow between AM & SBM

Russ Messing:

1 -Selectivity of parasitoids (long-term, high risk)

2-Mass-rearing technology and field testing of augmentation (medium-term, high risk)

3-Comparative economics of weekly GF-120 sprays area-wide, systems approach to population management strategies with integrated techniques (chemicals, parasitoids, nematodes) (medium-term, low risk)

Larry Gut:

1-Mode of insecticides; relationship to application timing

2-Improve bait formulations; potential use in non-commercial setting

3-Test, develop attractants/use in baits/monitoring

There was much discussion throughout the first day. Notes were taken during the workshop by Wee Yee. A condensed summary of the discussion pertinent to apple maggot flies follows:

The meeting began with an introduction by Wee Yee, who reiterated the purposes of the meeting:

1) Have a focused update on the nature of our problem with fly pests.

2) Provide updates on what research is ongoing and relevant – both in the Pacific NW and nationally

3) Discuss how we ought to revise our research strategy to develop more collaborative, productive research and implementation

This was followed by an overview of the fruit industry in the NW and issues with the World Trade Organization. Three areas important to export countries and the way these countries perceive our fruit fly problems were mentioned: 1) control of cherry fruit fly/apple maggot; 2) monitoring and ID; need more accurate traps, because we do not have much confidence in ones we have currently; 3) the difference between the biology of apple maggot vs. tropical fruit flies; host range (example, apricots use by apple maggot) and climate/adaptability. Indonesian examples: 1) flies from China are tropical species; 2) if we provide scientific, biological information to the export countries, this will stand up over the political issues.

SECTION I. GENETICS AND LIFE HISTORY

Jeff Feder and Stewart Berlocher presented on genetic variations in apple maggots. In addition to their technical points, they presented their researchable areas (see above):

Charlie Linn then presented on work done on developing assays to determine responses of flies to host odors and host odor discrimination.

Following the presentations, there were questions and comments.

A question arose as to the presence of host races in the NW. It was brought up that Stewart Berlocher and Bruce McPheron had done some work in the NW in the past - but Bruce did not sample enough to evaluate whether there are host races in NW. Now we can do that.

The situation in NW is different than in the East, because of different host plants – there are native haws, ornamental haws, and apples. We should also look at red haw. Can it be assumed that if there is no suitable host, flies will attack apples? There have been multiple catches in Yakima on hawthorn, but prior to just a few years ago, all larvae have been from hawthorns; now we know they infest apples too (2005, AM larvae in apples in the Selah).

Question: How much does not knowing where flies came in screw things up, as opposed to behavioral assays? Need both. 1) There are problems with host plants. There are 2 types of snowberries and several hawthorns. We have distribution records of hawthorns, but hybrids make ID difficult. Do fruit loads of trees here versus in the east differ? Do flies survive differently because of wet versus dry environments? However, in the SW (Texas), the environment is harsh, but AM can still survive there. 2) East coast eclosion times, are the emergence times here similar? We need flight tunnel tests, genetic tests of flies, and surveys of flies in eastern and western WA; maybe some flies not responding to apple? 3) Ovipositor lengths: problems; molecular tools needed to separate apple maggot from snowberry maggot; add more markers; none shows 100% separation, 7 or 8 markers; SNIPS may be valuable. 4) Morphometrics, may be more practical to use now than molecular tools?
WSDA keeps specimens in hard medium; can extract DNA out of them.

LUNCH was between 12 pm and 1 pm.

After lunch, there was further discussion and synthesis of the talks in SECTION I, GENETICS AND LIFE HISTORY.
Notes, summaries of discussion and researchable areas and possible collaborations follow:

1) It was suggested that Charlie Linn get involved in studies on responses to fruit odors of flies in the East vs. West, flight tunnel tests, develop better detection methods; **2)** Charlie Linn can ID volatiles of snowberries; have candidate blends already; **3)** Charlie Linn can ID profiles of volatiles from black hawthorn, *Crataegus douglasii*; **4)** Question of genome project; Jeff Feder, Stewart Berlocher:
Find how fly's brain organized; how genes expressed. Extension of USDA project; additional funds helpful. Jim McFerson-Set priorities – need robust set of markers. Not start with genome? The studies of Feder, Berlcoher with allozymes, microsatellites tie in with eclosion and wind tunnel studies. Then expand, additional monies data for all loci; put markers on linkage map; tissue samples. This is both applied and basic science – understanding physiological mechanisms. The question arose: Is there competition for funding with other insects? Mosquitoes, *Glossina* (Tsetse flies) genome project; honeybee genome project wrapping up. Apple maggot work supportable from 2 camps (USDA and basic research); **5)** Survey work of *Rhagoletis*: Basic survey work can be tied in with allozymes; CAPS money; Characterize the insects – this work supportable by NRI.

This was followed by a discussion on systems approaches: There are NAPPO standards for phytosanitary measures; systems approaches – partial quarantine areas- show areas are pest free; surveys, traps, research to back up use of traps; risk assessments. This includes odor perception, host races; molecular approaches.

SECTION II. POPULATION BIOLOGY AND MANAGEMENT
Talks were presented by Sue Opp, Russ Messing, Larry Gut, and Diane Alston.

Following their presentations were discussions.

The first topic was GF-120 and how it may be improved, and other areas; discussion was somewhat a free exchange of ideas sometimes not entirely following one train of thought. The idea was put forth that GF-120 simply protects the spinosad, keeping it toxic longer. Poor spray coverage resulted in poor control. It was stated that for control studies, it is good to know your fly population, whether you are catching mostly mature or immature flies. Is a greater proportion of flies that is caught immature? GF-120 is not attractive to apple maggot. Black spots are attractive in flight tunnel, so perhaps flies attracted to baits visually? Adding fruit volatiles to baits may improve attractiveness? When trapping in hawthorns, are haw volatiles useful? Does a dot, clumped bait made to resemble a fly increase attractiveness? A deterrent material such as Surround (kaolin) causes flies to disperse, which would not be good? Do flies feed on GF-120 on non-hosts? Yes, flies are found on non-host plants, and presumably would feed on the bait. Is MSG is a feeding stimulant? Stimulates feeding in Lepidoptera (moths), but for flies?

Other areas not related to GF-120 were then discussed: It was stated that apple maggot populations in arid areas have not gone very far; are there ecological relationship to survival of apple maggot; in neglected trees under which soil moisture is low? The tree still fruits, so climate is suitable for trees and flies? Hot in central WA.

This was followed by a discussion on fly survival in arid environments and central Washington:

Marshall Johnson (Univ. Calif.) has done work with olive fly and temperatures – why not a problem: hot weather in CA and no food and water. It is hard to imagine how flies find water in arid environments; what effects on adult mortality, longevity? For olive fly, there was still 60% survival even after 5 days with no water; work by Jorge Hendrichs on apple maggot behavior of regurgitating droplets to concentrate the bubble. Way of conserving water? Actually the bubbling was to concentrate dilute sugar foods. Is penetration by females of fruit a factor in why populations are low in Nile Valley? Is the black hawthorn, *C. douglasii,* not a perfect host? Sheri Smith showed that apple maggot emergence was good even when soil was dry; so in some non-irrigated areas, soil moisture not a significant mortality factor? It was stated that there is variability in haws. Question was raised: do the xeric conditions affect quality of host and not fly itself? Suggestion that xeric conditions affect quality of host and not directly on the pupae.

A question was asked of two regulators – Mike Willett, Mike Klaus: what remediation measures have been taken against apple maggot? Willett: We need to keep insecticides out of the water; pest boards using up Imidan this year; took all the fruit from haws over water; the plan is to use traps to detect; then Pest Board sprays; Counties go in and treat where flies are caught so next year no flies; a few orchards are threatened this year. Willett stated that we want to create a low prevalence area, but Pest Boards are not likely to spray consistency.

Another participant stated that we have no idea of the effectiveness of remediation measures; we do not know the extent of the fly distribution; should we do something to make an area fly free?

Klaus was asked if there are researchable areas he sees for apple maggot: He stated that it would be valuable to determine high and low apple maggot population years as determined by trapping in the Nile Valley, a remote area, using sentinel-type sites over 10 years: and then relate to local climate data and infestation rates in fruit

Are there any trap crops? Haws for apple maggot? Maybe, but it is probably more of a risk if we use trap crops.

SETTING THE RESEARCH AGENDA: AUGUST 29, 2006

8/29 Attendees: Brent Milne, Jay Brunner, Dan Griffith, Charlie Linn, Russ Messing, Vince Jones, Diane Alston, Larry Gut, Jim Doornink, Michael Klaus, Sue Opp, Tom Unruh, Pete Landolt, Wee Yee

McFerson stated we need to maintain dialog with other scientists

Pete Landolt stated that what he gathered from the first day's open discussion fit into 3 research areas:

1) A problem area is finding out what we have, taxonomy, etc
2) Finding it, using traps
3) Management

The question was asked: Do we all agree: Are all these problems, anything overlooked? No one publicly disagreed. It was stated that we still need to know basic biology; if different, will affect control and all three areas. Characterization of biology, host species range, control options, and biology are threaded through all these three areas. Is it possible any of the "apple maggots" are actually dogwood flies – do we need continued work on rearing flies from fruit – dogwood, serviceberry, cascara, pyracantha, etc? Why are we (WSDA) picking up single flies, are we missing some? Why are we not successfully finding them in higher numbers? Is the dogwood fly is the same fly as apple maggot? The dogwood fly is similar to apple maggot, but separate and may be a different species.

1. APPLE MAGGOT, WHAT WE HAVE:

Question: why apple maggot is not established in orchards, if the fly is all around us?

There is a disconnect - why do we detect, but do not manage the fly; hesitant to feel comfortable; are we detecting apple maggot in commercial areas? Yes, we have some threatened orchards in west Valley (of Yakima); if I were grower, I would file complaints against owners with infested backyard trees; there is a lot of development, some trees are cut, some trees are unmanaged; if we start to find flies, it may be because of these unmanaged trees. There is an educational component: for example, there is a giant hedgerow of black hawthorn near an orchard, which shouldn't be there. Can you detect apple maggot? Yes. Apple maggot is in both apple and black hawthorn. Where is apple maggot is coming from, and when will it infest the cities? It will be a mess when that happens.

The third cover spray is when apple maggot is emerging. There is a push into organics, but we don't hear any growers complain about apple maggot. Do flies from haws attack apples? How ID? The best we have now for species ID is morphometrics. The preference for ID is morphometrics. Further, under the basic umbrella info, we also need to make methods available to people doing surveys, to separate apple maggot from snowberry maggot flies from dogwood flies. Hawthorn vs. apple fly phenologies are different. Is there any interest in volatiles - yes.

McFerson asked if everyone agrees that a major barrier is sibling species and races?

An important researchable area is morphometrics. We also need to know if flies act/behave differently. There are at least two researchable areas: 1) Separation of snowberry maggot and apple maggot; 2) Ecology of host races. It depends on underlying questions, what interests are. Methods to use odors to ID the flies can be done. Flies can look alike, but act differently. If no "black hawthorn flies" go to volatiles, it will still mean something. We don't know if we have an apple maggot or black hawthorn fly. Emergence time is the critical thing; are some flies later emergers? Temperatures will shift the emergence period of flies. But this work has been done; flies do emerge at different times. Unlike in east, in Washington, when fruit are removed from each host, placed under a tree, all flies emerged at same time. However, on a calendar day basis, emergence may differ, but on a degree-day basis, emergence is the same.

A researchable issue then is: Determine eclosion/ecological data to characterize host races? Another researchable area then: snowberry maggot vs. apple maggot; within apple maggot, are there apple and haw races? There will be different methods for each analysis. Morphometrics is important, but boring. But it is possible to take from each specimen a leg for molecular work, and the rest of body for morphometrics. Morphometrics are for the short term, but maybe not.

2. APPLE MAGGOT, WHERE IT IS, DISTRIBUTION:

How to detect them: Detection and distribution are different. McFerson asked: what do we need to know about it? We need historical data. We are talking about different scales: in one orchard, the other along a specific creek, for example. One problem is that we are not sure what the flies are. What about wild vs. commercial settings? We don't trap commercial orchards; do we need to know?
WSDA has been mapping apple maggot distribution in WA for 20 yrs; we need to slightly re-direct it; give WSDA tools to do better job. In the future, we need to know how apple maggot expands in commercial areas. McFerson stated that if we had more tools, we can analyze the situation better, right? We need detection in feral vs. commercial settings. We need new tools; traps become critical.
Do we have a trap that works as well as we would like – the answer is no. What volatiles, traps, etc., will help do research? McFerson asked: We don't have tools yet? No, not the optimal tools. Also, we can do it, but we don't always know which fly is apple or snowberry maggot. It comes down to the trap, what's now; contrary to experience in the East. There, traps are giving information you need; people in NY don't think of races. Traps in WA do not work same way as in East. Put traps around orchards – But trapping doesn't occur around orchards here.
Volatiles work in the East. Ladd traps (yellow panel with red sphere in middle) work, but cumbersome to use. McPhail traps work better in hot dry areas, but McPhail-type traps (yellow jacket traps) don't work well for apple maggot in WA. New volatile research needed; behavioral research also needed. McFerson asked if it is possible to pursue aggressive field sampling without new tools?
Yes, we can start with current tools, but better tools should be pursued. Regulators can do distribution studies using what we have; we are not talking about that; we need new IPM tools; need something more attractive. We need to do IPM instead of calendar sprays – need better traps.
How flies are moving, how far, understanding what's happening; are there techniques to release-recapture, understand fundamental info? We don't have good info yet.

The problem with looking at dispersal is that with low populations, we will not recover any flies. There are areas in WA State with high apple maggot populations. How far can populations move from small orchards; is movement by apple maggot natural or caused by humans? One wonders if flies are moved by humans, because flies are found in recreation areas. Flies can go into cars also.
It is coincidental if apple maggot is moving north. Some rate of movement has been plotted in the 1980's – first nothing in Seattle, now all over Skagit and Whatcom; natural movement? Was this a function of trapping intensity, as in Spokane? But these are not researchable areas. What happens when apple maggot moves from western to eastern WA? Suggest marking a source area and seeing where flies go. Some may fly, disperse a long way. The Manson (in Chelan County) catch in 2006 may be a result of long range dispersal. We may be seeing only the tip of the iceberg; a mated female may fly far. We detect larvae in tree, then come back to it, and find no larvae. What does this mean about spread?

Has anyone taken apple maggot from haw and see how well it develops in apples?
This is a researchable area: How apple maggot flies do in different hosts. Messina's work: very different- haw flies did not do well in apples.

3. APPLE MAGGOT, MANAGEMENT AND CONTROL:
Here in control, apple maggot and cherry fruit fly diverge; in one, management is keeping it out of the orchards, in the other, management is outside the orchards. Are the balls with the toxicants (curveballs) effective? These developed for eastern areas; needs moisture; not looked at in dry areas. It is not easy to make a ball, but maybe easier without rain. Do curveballs have relevance

here in the NW (central WA)? Cannot put the curveballs on trees all over the roadsides, etc. It is important to make GF-120 better. Possible usefulness of Kaolin (Surround), and the use of a combination of tactics; whether they will ever be implemented is another question. Yes, a combination of tactics important, including the spread of parasites, everything. Agreed a combination of different strategies needed. There will be other benefits also.

This was followed by discussion on whether apple maggot can be prevented from spreading:
There is a bifurcation in control in feral vs. commercial settings. Researchable area: what are the chances of controlling apple maggot? It is better to use the terms containment or suppression, not control. McFerson asked if it was possible to contain it? Yes, in Kittitas County, the Pest Board is keeping it in feral trees. The Board is using Imidan now; we don't know what will happen if Assail (acetamiprid) is used. But Ellensburg (in Kittitas County) is a small area; apple maggot will spread in Yakima, since much bigger: 100,000 vs. 12,000 people, area much bigger. If apple maggot is not there currently, we can contain it; problem is if it infests towns; restricted areas right now.
How do we do it effectively? With apple maggot, growers never had to deal with it in central Washington; hope we don't get to that point; some trees have them, why don't we have it?
In the past, the consensus was that apple maggot would never get over to eastern WA; but it was only a matter of time, it will get here; making it here; it is here now. Yes, agree that apple maggot is new here (central Washington), a big unknown. We need to keep it where it's at; look at history, it's only a matter of time; we need to keep it out of orchards. We need treatments for organic apples.
Same question as with cherry fruit fly, if orchard is 100 m from a riparian area; can a fly disperse to the orchard? We don't know. With apple maggot, we are dealing with a completely different thing; need bait attractive to apple maggot and one that works. Which chemicals are effective for apple maggot? Neonicotinoids work for apple maggot. We have overstated the negatives – there is so much basic biology on apple maggot that needs to be done; is mating only visual? What are parasitoid effects on flies moving onto apples? For flies in riparian area, spray trees and follow dispersal. What does it mean when one fly is caught, are there many flies out there? When we catch flies, we need to pay attention to which type of flies, males or females, whether they are reproductively mature or immature.
END OF PERTINENT DISCUSSION
On the second day, the workshop ended at 12:00 pm, earlier than scheduled.

In summary: the research plan generated for the next three years was to work on host attractants and diagnostics: collaboration between Wee Yee, Peter Landolt, and Charlie Linn on host attractants for apple maggot; collaboration between Wee Yee, Tom Unruh, Jeff Feder, and Stewart Berlocher to work on morphometric and molecular diagnostics of apple maggot, host races, and snowberry maggots.

FINAL PROJECT REPORT
WTFRC Project Number: AE-06-600 (WSU Project No. 13C-3643-7386)

Project Title: Biology and Management of Secondary Pests of Apple

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Budget History:

Item	Year 1: 2006
Salaries	43,409
Benefits	8,864
Wages	7,000
Benefits	770
Supplies	2,000
Travel	1,500
Total	63,543

Objectives:

1. Woolly apple aphid.

1a. Test chemical control tactics for aerial colonies with field trials.
1b. Test chemical control tactics for root colonies with both greenhouse assays and field studies.
1c. Determine the life history of woolly apple aphid in different growing regions of Washington.
1d. Evaluate widely planted as well as newly developed rootstocks for resistance to local woolly apple aphid biotypes.

2. Rosy apple aphid.

2a. Determine the herbaceous hosts of rosy apple aphid in Washington.
2b. Determine if rosy apple aphid is capable of continuous reproduction on apple.
2c. Determine species and relative abundance of natural enemies of the rosy apple aphid on both woody and summer hosts.
2d. Determine the time of susceptibility of fruit to injury by rosy apple aphid colonies.

3. Western flower thrips.

3a. Determine source of thrips attacking fruit using protein markers.
3b. Determine phenology of thrips oviposition on apple around bloom.
3c. Determine the optimum timing of thrips control with insecticides.

Significant findings:

1. Woolly apple aphid.

1a. Oil+Lorsban at delayed dormant reduced infestation immediately, delayed population increase until late summer, and resulted in a lower population by late summer. Oil alone caused a temporary reduction. NNI-0101 and BeLeaf 50SG looked promising for summer control. The 1.5% v/v rate of Safe-T-Side oil was slightly more effective than the 1% rate. Tank-mixing oil with Assail improved the performance but increasing the rate did not. Diazinon 50W was consistently the most effective material for woolly apple aphid control.

1b. Venom and Admire Pro (soil drench) and spirotetramat (foliar spray) controlled root and shoot colonies on potted seedling apple trees. Results of field trials were more variable and generally less effective. Admire Pro provided the most consistent suppression of root and shoot colonies. Spirotetramat showed a (non-significant) trend to suppress woolly apple aphids in a foliar application in May.

1c. The peak period of upward woolly apple aphid crawler movement occurred nearly 7 wk later in 2006 (late July) than in 2005 (early June). Shoot colonies had a small peak in mid- to late June and a second, usually larger, peak in mid- to late August. Alate adults were found at only one site (mid-September to early October).

1d. Geneva 202, Geneva 41, and 4210 rootstocks are much more resistant than MM.111 to a Washington strain of woolly apple aphid. M.9, M.26, Bud 9, and Bud 118, and seedling rootstocks are highly susceptible.

2. Rosy apple aphid.

2a. Both broadleaf and narrowleaf plantain serve as summer hosts of rosy apple aphid in Washington.

2b. No populations of rosy apple aphid were found to survive on apple under ambient conditions past midsummer. A lab colony on apple was maintained under spring conditions for 15 months.

2c. *Lysiphlebus testaceipes* (Cresson) was the most common parasitoid on apple colonies, while *Aphidius spp.* were the most common on plantain colonies. Syrphids were the most common predators on both plants.

2d. Results of one year show dwarfed apples were caused by aphid feeding before, but not after, 30 May. 'Golden Delicious' and 'Fuji' appear to be particularly susceptible.

3. Western flower thrips.

There appears to be a window for control of thrips damage (pansy spot). Insecticide applications were effective from full bloom to 5.7-mm diameter fruit. When applied at the same timing, Success provided control equivalent to Carzol.

Methods:

1. Woolly apple aphid.

1a. Chemical control tactics of aerial colonies.

Delayed-dormant trial. This test was in apple orchards (replications) in Bridgeport, Brewster, and Quincy. At delayed dormant (6-11 April), growers applied 2 treatments: 1) Lorsban 4E + oil (2 qt+4-5 gal/acre); 2) oil alone (4-5 gal/acre). Treatments were applied with commercial airblast equipment to ca. 2-acre plots, and an additional 1-2 acres were left untreated at this timing as a check. Woolly apple aphid populations were evaluated within 4 wk after treatment and every 4-8 wk thereafter. Woolly apple aphid densities were assessed by conducting a 15-min search in the center row of each plot and recording the number of colonies.

Summer trials. Two randomized complete block trials were conducted in a commercial apple orchard near Royal City. Small plots (1-3 trees) were sprayed with a handgun to the point of run-off on 21 July. Larger plots (10 trees) were sprayed with an airblast sprayer at 200 gpa on 20 July. Live aphids, dead aphids, aphid mummies, and predators were counted before and at 3-7 d intervals after treatment.

Postharvest/dormant trials. A randomized complete block test and three single-block comparisons were started in the fall of 2006 in apple orchards near Quincy, WA. Three to four days before treatment, woolly apple aphid colonies were counted in a 3-min search per plot. Five colonies were collected per plot, and immature and adult aphids, aphid mummies, and predators were counted. In the randomized complete block trial, each block (5 rows × 20 trees) was sprayed with an airblast sprayer at 200 gpa on 10 October. In the single block comparisons, treatments were applied in the same manner to 3-5 rows of trees on 26 October 2006. Treatments at delayed dormant (2007) are planned for next spring.

1b. Chemical control of root colonies.

Bioassays. Two bioassays, randomized complete block design, were conducted in a greenhouse. Seedling apple trees (7/16 inch) were planted in 8-inch pots in a mixture of equal parts peat, perlite and vermiculite. After trees had grown shoots, twigs from infested trees were placed on exposed roots, and crawlers were allowed to settle on both roots and shoots. Solutions (250 ml) of Admire and Venom were poured onto moist potting mix on 10 July. Shoot colonies were assessed before treatment, then every 7 d. At 4 wk, all trees were lifted and root colonies assessed. A second bioassay was conducted using the same methods. Admire was applied as a soil drench. Movento (spirotetramat) with various adjuvants was applied to foliage to run-off with a 1-gal sprayer (ca. 50 ml per tree).

Orchard trials. Four experiments were conducted in three commercial orchards to evaluate systemic insecticides for control of root and shoot colonies. Two were in Mattawa, one in East Wenatchee, and one was near Royal City. Plot size ranged from 3-6 rows and 10-31 trees. Admire Pro 4.6F, Vydate 2L, Venom 20SG, NNP-515, and NNP-516 were applied to the herbicide strip. A swath 2.4 m wide centered on the tree trunk was sprayed 15-18 May with a boom sprayer calibrated to deliver 100 gpa. The trees were irrigated the day before the application to completely wet the soil profile, and then again for 45 min after the application to carry the pesticide into the root zone. Foliar applications of spirotetramat were made with a handgun to the point of drip on 19 May or 21 July.

Population density of root colonies was assessed with sticky bands to trap first instar woolly apple aphid crawlers moving on the trunk. Bands were made of 3 cm wide strips of aluminum foil sized to encircle the trunk, and attached with 3M Spray Adhesive. Aphid crawlers were trapped in a bead of Tree Tanglefoot adhesive placed around the band in a circle. Three trees per replicate plot were banded and bands replaced every month. The number of aerial colonies was evaluated in a 3-min search/plot.

Data from all pesticide evaluation trials were analyzed using the Statistical Analysis System (SAS 1988). Data were tested prior to analysis for homogeneity of variance using Levene's (1960) test. Variances found to be non-homogeneous were transformed [ln(y+0.5)] before analysis. PROC GLM was used to conduct an analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test.

1c. Life history of woolly apple aphid.

Overwintered colonies. One to five woolly apple aphid colonies were collected 30 March to 7 April from the trunks and surface roots of apple trees in Malott, Brewster, Bridgeport, Quincy, and Vantage. The percent parasitized, living aphids, dead aphids, and developmental stages of the aphids were determined.

Crawler movement. Crawlers were monitored in Bridgeport, Vantage and Quincy. Two sticky bands, described in Section 1b, were placed on three trees at each site. One band was placed 15 cm above the soil to trap nymphs moving up the trunk from the roots. A second band was placed 1 cm above the first to trap nymphs moving down from aerial portions of the tree. Bands were set out in April and replaced weekly until frost.

Aerial colonies. The average size and number of aerial colonies were recorded weekly on the three banded and three adjacent trees. On additional trees, up to five colonies were collected weekly. The number of aphid mummies, predators, and the developmental stage of aphids was determined. Predators were also collected at a second site in Quincy.

Fruit infestation. Fruit was sampled in two rows. Five hundred fruit were examined weekly *in situ* and the stem and calyx ends examined for woolly apple aphid. Any fruit with external infestation was cut open and examined for core infestation. Aerial colonies were counted in the same rows in a 3-min search. Samples were also taken weekly in an additional block in Vantage. Two other sites in Quincy and Royal City were sampled at harvest.

1d. Rootstock resistance.

Apple rootstock liners, from ¼- to ⅜-inch diameter, were planted in pots on 21 April. Ten replicates of 10 rootstock types were used: the Geneva line 4210, Geneva 41, Geneva 202, Bud 9, Bud 118, M.9, M.26, MM. 111, seedlings from Washington (Willow Drive Nursery), and seedlings from New York. Trees grew shoots approximately 6 cm long before infestation. Sections of infested shoots 4-6 cm long from an orchard in East Wenatchee, each with 50-200 aphids, were placed at the base of each tree on 19 May and again on 22 May. Trees were arranged on a greenhouse bench in a randomized complete block design.

Aphid densities were evaluated on 16 June. Two types of evaluations were performed, a rating system based on visual inspection and a photographic method. Trees were each digitally

photographed against a black backdrop. The area photographed remained a constant 73.5 cm × 49 cm. The resulting images were imported into a digital imaging program (Photoshop ver. 6.0, Adobe, Inc.) which was used to obtain a count of the number of white pixels in the image (representing woolly apple aphid colony mass). Because the total number of pixels in the image remained constant, the number of selected pixels was used as a direct measurement rather than as a proportion of the total number.

2. Rosy apple aphid.

2a. Determine herbaceous hosts.

No-choice experiments. Seedling domestic apple trees were planted 21 April and infested 10 May with rosy apple aphid from a WSU-TFREC orchard. Three herbaceous species, narrowleaf plantain (*Plantago lanceolata* L.), broadleaf plantain (*P. major* L.), and woolly plantain (*P. patagonica* Jacq.), were grown from seedlings in the greenhouse. Broadleaf dock (*Rumex obtusifolius*) was collected 5 May from an orchard in Brewster. Three replications of each apple-herbaceous plant combination were placed in insect cages on 15 May and grown under natural photoperiod. Trees were removed 4 July; then on 7 September new, uninfested seedling apple trees were placed in the cages. Aphid samples were collected 4 July, 21 July and 12 October. The entire broadleaf dock plants were placed in Berlese funnels to extract aphids. All aphids were identified at WSU-IAREC, Prosser.

*Alternate host surveys***.** Plantain and other weed species in apple orchards were sampled in the following counties (no. sites): Okanogan (2), Douglas (3), Chelan (2), Benton (6), Yakima (16), and Skamania (1) in Washington, and Umatilla (1) in Oregon. Plantain in areas at least 100 m from apple trees were also sampled in the following counties (no. sites) once during the season: Okanogan (3), Grant (2), Chelan (2), Douglas (1), Benton (3), Yakima (13), Klickitat (6), Skamania (4), Clallam (1), Jefferson (1), and Walla Walla counties (1) in Washington. Similar areas were also sampled in Umatilla (3) and Deschutes counties (1) in Oregon, and on Maui, Hawaii (1). At most sites, samples were collected every other week from July through the end of August, but some were sampled once. Plants were cut at ground level and sufficient plant material was collected to fill two 1-gallon plastic bags. The contents of one bag were placed in a Berlese funnel to collect aphids and natural enemies. Parasitoids were reared from both apple and plantain at sites in Okanogan, Douglas and Chelan counties. The contents of the second 1-gallon bag were transferred to 6-liter plastic boxes with ventilated lids and kept at room temperature for one month until adult parasitoids emerged. Parasitoids and aphids were identified as described previously.

Phenology of rosy apple aphid on summer hosts. Three orchards in Douglas and Chelan counties were selected for intensive sampling. Two orchards had narrowleaf plantain and the third had broadleaf plantain. Ten 1-gallon bags of the dominant plantain species were collected every two weeks from late May through October and aphids extracted as described previously.

2b. Continuous reproduction on apple. Three seedling apple trees were grown in a greenhouse as described in section 2a. Trees were infested in May with rosy apple aphid from Orondo and TFREC. Aphids were provided with fresh seedlings every 2-4 wk. The aphids in the indoor colony started in June 2005 were maintained on apple throughout the year under conditions typical of early April (15-20°C, 14:10 light:dark photoperiod).

2c. Natural enemies on apple and summer host. Natural enemies were collected from apple and plantain from sites in Okanogan, Chelan and Douglas counties. Apple trees were examined weekly for rosy apple aphid colonies at three sites beginning in late April until colonies could no longer be found, by early August. Predators were collected directly from colonies; immatures were stored in 70% ethanol and adults pinned. Parasitoids from apple colonies were reared as

described in Section 1. Natural enemies were collected from plantain from late May through October as described in Section 2a.

2d. Timing of injury to fruit. This study was conducted on 'Delicious,' 'Golden Delicious,' 'Fuji,' and 'Gala' apple trees in an experimental orchard at WSU-TFREC. Eight trees of each cultivar were infested at either pink (20 April), petal fall (9 May), 30 May, or 20 June. An additional eight trees were not infested and served as a check. A single fruit spur with a growing shoot was selected on each tree and inoculated with 10-20 rosy apple aphids. Attempts on 20 June failed to establish colonies. Colonies were assessed weekly for three weeks, then eliminated with Assail 70WP at 1.7 oz./100 gal applied with a 16-fl oz. hand-held spray bottle. No chemical thinning agents were applied to the block, and fruit was not hand-thinned.

The condition and circumference of fruit were assessed at harvest. Fruit within 10 cm of the infested shoot (1-10 fruit) were examined on each replicate tree. About 10 fruit were selected randomly from each uninfested tree. The influence of time of infestation on the occurrence of dwarfed/non-dwarfed fruit was examined for each cultivar with a goodness-of-fit test. Size of non-dwarfed (not misshapen) fruit was analyzed using the Statistical Analysis System (SAS 1988). PROC GLM was used to conduct an analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t*-test.

3. Western flower thrips.

3a. Migration of thrips. This experiment was located in an orchard near Bridgeport, Washington. Plant tissues (and presumably thrips) were coated with protein markers to determine where the thrips originated. Two areas were treated with separate markers. With a hand-held fertilizer spreader, an area (50 × 342 ft) of native vegetation adjacent to the orchard was dusted with powdered skim cow's milk at a rate of 1 lb/1,000 ft^2. Four bands on the orchard floor, each 75 × 324 ft, were sprayed with a solution of 10% egg whites v/v at 134 gpa. Each band started at the edge next to native vegetation and spanned the width of the block. Treatments were applied at pink (24 April) and king bloom (1-3 May).

Blossoms from four arrowleaf balsamroot plants in treated native vegetation and 500-2,000 dandelion flowers from the orchard floor were collected before and after each treatment and at full bloom of apple. With the final sample, 150 apple flower clusters were collected in all four bands at the edge, then every five rows up to 24 rows into the block. All flower samples were immediately frozen.

Flowers were examined under a microscope and thrips were collected on a toothpick with a tiny drop of Stickum Special™ adhesive. Each insect was placed in the bottom of a 1.5 ml microcentrifuge vial. The marker was released from the insects with a buffer solution. This solution was tested with an ELISA procedure for the presence of both egg and milk proteins.

3b. Timing of insecticides. This experiment was a randomized complete block design conducted in a 'Cameo' apple block in Bridgeport. Four replicate plots (a single row of 15 trees) were sprayed with either Carzol 92SP or Success 2SC using an airblast sprayer at 200 gpa. Insecticides were applied at king bloom (28 April), full bloom (4 May), and at fruit diameters averaging 5.7 mm (12 May), 12.8 mm (17 May), 17.3 mm (25 May) and 26.7 mm (1 June).

Fruit (110-150/plot) were examined on 6 June for pansy spot. The proportion (p) of damaged fruit was transformed with arcsine [square root (p)]. Data were analyzed with ANOVA. Results from the two insecticides were analyzed at each spray date with a Least Significant Difference test. Data for insecticides were combined, and means for spray dates were separated with a Least Significant Difference test.

3c. Timing of oviposition. Reproductive tissue samples were taken at pink (24 April), then on approximately the same dates as the insecticide sprays in 3b, with an additional sample at

31.7 mm diameter fruit (9 June). One hundred blossom clusters or king fruit were collected from untreated trees next to the spray trial. Thrips were separated from plant material by filling the sample bag with water, adding a few drops of liquid detergent, and agitating for several seconds. Thrips and plant material were separated from the soapy water by pouring through two sieves. Both adult and immature thrips were counted. Average fruit diameter for each sample date after bloom was determined by measuring a subsample of 10 king fruit.

Eggs laid in blossom or fruit tissue were counted by direct observation. The king bloom or fruit was stained with McBride's stain for 12 h to color eggs. Small fruit (<15 mm) were covered with dense trichomes which made eggs difficult to see. These were treated with a clearing solution and skin was removed. Skin was studied under a dissecting scope illuminated from behind to reveal the dark eggs. Larger fruit (≥15 mm) were studied under a microscope without clearing the tissue. Oviposition sites were excised and studied more closely if necessary.

Results and discussion:

1. Woolly apple aphid.

1a. Chemical control tactics of aerial colonies.

Delayed-dormant trial. An application of oil+Lorsban at delayed dormant practically eliminated aerial woolly apple aphid in infested orchards, and populations did not begin to recover until late in the season. Even in mid-September, the density of woolly apple aphid colonies in the oil+Lorsban plots was significantly lower than the check or oil alone. Oil alone significantly reduced woolly apple aphid populations shortly after application, but the effect was transitory; these plots were not different than at any time after June.

Summer trial (handgun). NNI-0101+oil looked promising for woolly apple aphid control at the higher rates (1.59 and 3.19 fl oz /100 gal). Another new nicotinoid insecticide (flonicamid; BeLeaf 50SG at 1.75 lb/100 gal) also significantly reduced populations by 7 DAT.

Summer trial (airblast). Assail 30SG+oil provided better aphid suppression than oil alone; however, the 8 oz rate of Assail+oil was not better than the 4 oz rate. The inclusion of oil with Assail, plus the higher spray gallonages used in this test (200 gpa) may be responsible for the improved performance of this material. Safe-T-Side at 1.5% v/v was slightly more effective than the 1.0% rate. The standard, Diazinon 50W (4 lb/acre), provided the best control, reducing woolly apple aphid populations by 4 DAT which remained low for the rest of the test.

1b. Chemical control of root and aerial colonies.

Bioassays. Both Venom 20SG (21 oz/100gal) and Admire Pro 4.6F (14 fl oz/100 gal) significantly reduced aphids on shoots by 7 DAT, and within 4 wk eliminated woolly apple aphid on both roots and shoots of the seedling trees. The suppression of shoot colonies from soil drench applications is a clear indication that these products were translocated upward in the trees. In a second bioassay, Admire Pro provided the fastest and most complete control of shoot populations. Regardless of formulation or adjuvant used, Spirotetramat worked more slowly and left a small residual population. All treatments, however, gave virtually complete control of the root colonies at the time they were evaluated (5 wk after application).

Orchard trials. Success of treatments in field trials was poorer and more variable than in bioassays. Admire Pro 4.6F (7-10.5 fl. oz./acre) provided suppression of either root or shoot colonies in two trials. Spirotetramat 150OD (12 fl oz/100 gal) + Silwet L-77 applied to foliage in May showed a (nonsignificant) trend to suppress crawlers and aerial colonies. An application in July showed no effect. Soil applications of NNP-515 (1.5 qt./acre) provided good control in one orchard but had no measurable effect in another. Vydate 2L (1-3 gal/acre) was not significantly different from the check in all field trials, although it showed good activity in 2005 potted tree bioassays. Venom 20SG (21 oz./100 gal) and NNP-516 (1 pt./acre), both applied to soil, were not significantly different from the check.

1c. Life history of woolly apple aphid.

Peak upward movement of woolly apple aphid crawlers as evidenced by sticky bands occurred from late June to late July 2006 depending on the site. Peak movement at the site which was sampled for 2 years was nearly 7 wk later in 2006 (late July) than in 2005 (early June). These data indicate substantial year-to-year variation in crawler movement. Captures in the lower band (presumably upward movement) were generally much higher than captures in the upper band (presumably downward movement). Shoot colony development appeared to be bimodal in the study orchards in 2006, with a small peak in mid- to late June and a second, usually larger, peak in mid- to late August (Fig. 1). Immature aphids were the most prevalent form in the shoot colonies throughout the season, indicating potentially growing populations (Fig. 2). Alate adults were found at only one site (Vantage) and only in mid-September through early October. Mummies (aphids parasitized by *Aphelinus mali*) were present starting in mid-June for as long as active colonies were present in the orchard (late October in one study site).

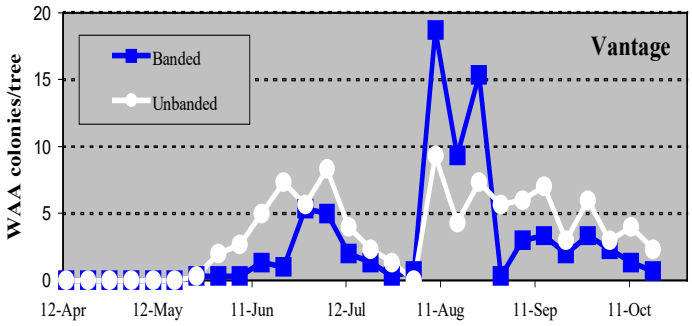

Fig. 1. Woolly apple aphid colonies on three banded and three unbanded trees at one of three sampled sites, 2006.

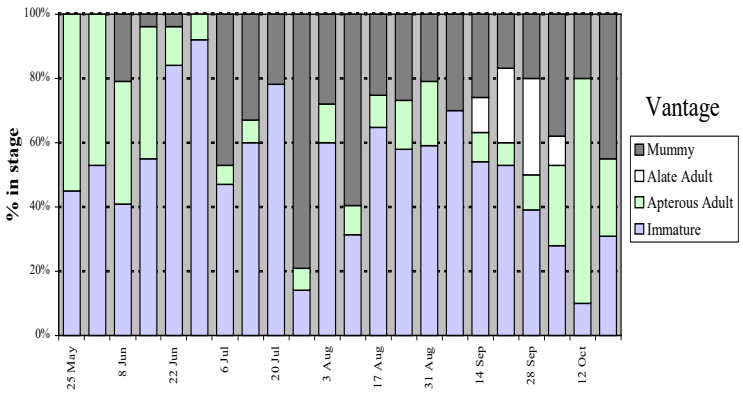

Fig. 2. Percentages of aphids in different stages in one of three sites sampled in central Washington, 2006.

Fruit sampling. At one site, fruit became infested by woolly apple aphids settling in the stem and calyx ends after aerial colonies increased in late August, three weeks before harvest. A few fruit had woolly apple aphids inside the cores, where they had entered through a natural opening in the calyx end (Plate 1). The incidence of infested fruit appeared to depend on the proximity of aerial colonies; however, this relationship needs further investigation.

Plate 1. Woolly apple aphid nymphs inside the core of a 'Fuji' apple at harvest.

1d. Rootstock resistance. Striking differences among the various rootstocks were apparent within a few weeks of artificial infestation. After 4 wk, the susceptible rootstocks (including M.9, M.26, Bud 9, Bud 118, and seedlings from New York and Washington) were heavily infested (Plate 2). On MM.111 (whose resistance is derived from 'Northern Spy'), colonies established successfully but were small and poorly developed. The majority of the replicates of the Geneva 'Robusta 5'-derived resistant rootstocks (G.202, G41, and 4210) were free from infestation (Plate 3), although some replicates had a few very small colonies.

Plate 2. Seedling rootstock heavily infested with woolly apple aphid.

Plate 3. Geneva series rootstock free of woolly apple aphid.

2. Rosy apple aphid.

2a. Determine herbaceous hosts.

No-choice experiments. Rosy apple aphid successfully migrated from apple to all three species of plantain. Alate aphids were observed on potted plantain in the cages from late May through early July, with colony formation of the pale yellow virginoparae starting in June. All woolly plantain plants produced seed and died by early August. Rosy apple aphid was not found on any replicate of dock (*R. obtusifolius)*. These results confirm those of 2005.

*Alternate host surveys***.** Rosy apple aphid was identified from broadleaf plantain at 4 of the 18 sites (22%) where this weed species occurred and on narrowleaf plantain at 5 of the 23 sites (22%) sampled in or directly adjacent to apple orchards. Plantain from 5 out of 42 extra-orchard locations sampled in Yakima and Klickitat counties contained rosy apple aphid. Two of the positive sites were the Bingen City Park and the grounds of the Maryhill Museum (both in Klickitat County). Analysis of the sites and proximity to woody hosts is in progress. The results from 2006 confirm the results from 2005, that is, that both narrowleaf and broadleaf plantain serve as a summer host for rosy apple aphid. The highest numbers of aphids in plantain samples occurred in June and July, and higher aphid populations were found on narrowleaf plantain than on broadleaf plantain.

Phenology of rosy apple aphid on summer hosts. Rosy apple aphid was detected on plantain as soon as sampling began in late May. An early peak occurred in July at TFREC, and a later peak occurred in

August and September at TFREC and Stormy Mountain Orchard. The last sample positive for this species was 20 September, although sampling continued through late October. The absence on the summer host likely marks the migration back to the overwintering host, apple. Although these orchards received minimal pesticides, mowing and other cultural operations may have periodically disrupted aphid development.

2b. Continuous reproduction on apple. Access to new apple shoots did not prevent the typical summer decline of rosy apple aphid colonies from Orondo and Wenatchee when subjected to natural photoperiod and high temperatures in the greenhouse. A colony started from a field population in June 2005 was maintained on apple in a growth room under typical early April conditions throughout 2006.

2c. Natural enemies on apple and summer host. The natural enemy complex on plantain is different, both qualitatively and quantitatively, than that on apple. Several groups of generalist predators were collected feeding on apple colonies. These included earwig nymphs, cecidomyiid larvae, lady beetles, and *Campylomma verbasci* (Meyer-Dür). Syrphid larvae were the most commonly encountered predator. Three primary parasitoid species, *Lysiphlebus testaceipes* (Cresson), *Praon unicum* Smith, and an encyrtid were collected on colonies from apple, with *L. testaceipes* being by far the most common. All others collected were hyperparasitoids. On plantain, syrphid larvae were also the most common predator and *Aphidius* sp. (including *A. matricariae*) the most frequently encountered parasitoid. *L. testaceipes* was also found on plantain but much less commonly than on apple.

2d. Timing of injury to fruit. The four cultivars supported different levels of aphid infestations on the spur shoots from pink through petal fall. 'Golden Delicious' shoots had significantly higher cumulative aphid days than 'Fuji' and 'Delicious' shoots. 'Delicious' had the lowest levels of aphids, which may have been caused by a mild form of antixenosis in this tissue to rosy apple aphid. Aphid populations were not significantly different between cultivars during the other time periods.

Dwarfed fruit developed on 'Fuji' trees infested from pink through petal fall and on 'Golden Delicious' trees infested from petal fall through late May. No dwarfed fruit resulted from aphids feeding 30 May or later, although cumulative aphid days were higher during the late treatment. Among the remaining fruit of normal appearance, a small but detectable reduction in size sometimes occurred. 'Gala' apples were slightly smaller when shoots were infested from petal fall through late May. 'Golden Delicious' apples were slightly smaller on infested branches regardless of the time of infestation. 'Delicious' apples did not sustain any damage from rosy apple aphid treatments.

3. Western flower thrips.

3a. Migration of thrips. At the time of writing, thrips samples were being analyzed. Thrips positive for casein (cow's milk) and chicken albumen (egg white) have been detected in some apple flowers, indicating a successful marking procedure. A complete analysis of data will be done by 31 March 2007.

3c. Timing of insecticides. The effect of spray timing was highly significant, while the effect of insecticide used was not. No significant difference between Carzol 92SP (1 lb./acre) and Success 2SC (8 fl. oz./acre) treatments was found at any timing date. Fruit injury on trees treated at full bloom and 5.7 mm diameter king fruit was significantly lower than injury on trees treated at all other times (Fig. 3). Fruit injury was not significantly different among treatments applied at king bloom or at any timing after 5.7 mm diameter king fruit.

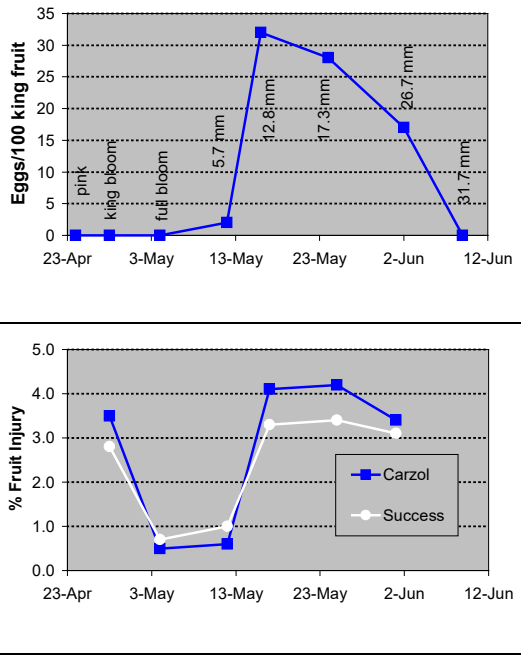

Fig. 3. (Top) Eggs laid in 100 king fruit. (Bottom) Percentage of fruit with pansy spot from trees sprayed with Carzol 92SP or Success 2SC on different dates. The x-axis indicates the spray date; all fruit were evaluated on 6 June 2006.

3b. Timing of oviposition. Adult thrips increased in flower clusters as blossoms opened, peaking at full bloom and declining as fruit grew. Some adults were still present on king fruit at 5- to 12-mm diameter. Immatures did not occur in samples until 12.8-mm diameter king fruit and did not follow the trend of oviposition in king fruit. It appears that immatures on fruit came from eggs laid elsewhere. Eggs were found in fruit beginning at 5.7-mm diameter king fruit and increased sharply by 12.5 mm. Stained eggs were detected in oviposition scars after 25 mm but were no longer found in scars by 31.7 mm in early June.

Two timings provided the best control of pansy spot damage from thrips, full bloom and 5.7-mm king fruit. Applications before or after these times were less successful. The later timing coincided with the period just before peak oviposition as determined by egg sampling. These data confirm previous experiments on the timing of oviposition and its relation to optimum control. This later timing would allow growers to tank- mix the insecticides with some types of early thinning sprays and would permit a greater range of choices in insecticides if applications are made after bees are removed from the orchard.

References cited:

Levene, H. 1960. Robust tests for equality of variances. Chap. 25. *In* Olkin, I., S. G. Ghurye, W. Hoeffding, W. G. Madow and H. B. Mann (Eds.), Contributions to probability and statistics. Stanford University Press, Stanford, CA.

Statistical Analysis Institute. 1988. SAS/Stat User's Guide, Release 6.03 Edition. SAS Institute, Inc., Cary, NC.

FINAL PROJECT REPORT
WTFRC Project Number: #AE-05-505

Project Title: Distribution of flower thrips eggs in apple blossoms

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Budget History:

Item	Year 1: 15000	Year 2: 15000	Year 3:
Salaries	15,000	15000	
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	**15000**	**15000**	

OBJECTIVES:

Describe egglaying preferences of western flower thrips among non-damaging and potentially damaging areas within the flower/fruitlet cluster in apples. Assess whether preference changes with apple phenology.

SIGNIFICANT FINDINGS:

- Developed method to survey tissues for presence of thrips eggs in apple tissues that avoid difficulties in earlier published methods
- Showed that adult thrips numbers in blossoms peaked at full bloom. Egg and nymphal counts lagged adult phenology
- Showed that adults were absent or at low densities at pink, petal fall, and post-petal fall
- Showed that almost no eggs were deposited in damaging areas before petal fall. The calyx was highly preferred until well after petal fall
- As the calyx dried following petal fall, there was a shift of egglaying to the stem and developing fruitlet, but because adults had mostly disappeared from the orchard at this time, only very few damaging eggs were actually found
- To confirm this shift in egglaying preferences, we introduced egglaying adults onto apple clippings in the laboratory. The developing fruitlet became highly preferred as the fruitlet reached the 10-25 mm stage

- **CONCLUSION:** Results suggest that optimal timing for insecticide applications is between full bloom and petal fall.

METHODS

Sampling methods for adults and nymphs followed techniques developed earlier by Miliczky (swishing of clusters in soapy water). Assessment of egg densities was done using methods developed in Horton's laboratory in 2005. Briefly, the tissues are immersed in a warmed solution of white distilled vinegar and blue food coloring. The vinegar and warming allows us to easily tease away the top layer of the plant tissues, exposing the (bluish-colored) oviposition scar and associated egg. Samples were taken at pink, king bloom, full bloom, petal fall, and 10-25 mm fruitlet. Clippings having cut ends placed in water are infested with adult thrips in the laboratory to obtain egglaying at those stages in which adult thrips were absent from clusters in the field.

RESULTS AND DISCUSSION

Figure 1 shows densities of adults, nymphs, and eggs of flower thrips at three orchards. Egg and nymphal population curves trail adult curves, as is expected. Peak egg numbers occurred at full bloom and petal fall. Adults disappeared from clusters at petal fall.

Egg distribution among tissues (combined across orchards) is summarized in Tables 1-2. No or few eggs were found at pink, king, and 10-25 mm fruitlet (as shown also in Figure 1), due to low densities of adult thrips at these times. The calyx was heavily preferred until after petal fall. Eggs deposited in potentially damaging areas were first obtained at full bloom (2005) or petal fall (2006), peaking at petal fall (2005) or 10 mm fruitlet stage (2006).

Because few eggs were present in field material at pink, king, and the 10-25 mm stage, preference could not be reliably assessed. We artificially infested clippings in the laboratory to assess egglaying preferences during these phenological stages (Tables 3-4). At pink and king stages, we obtained large numbers of eggs. None were deposited in potentially damaging areas, suggesting that

even if adults happened to be present in the orchard at those times, eggs would not to be deposited in damaging areas. However, at the 10-25 mm stages, a very high percentage of eggs had been deposited directly on the developing fruitlet. Thus, if egglaying adults happen to be present in clusters at this phenological stage, there appears to be considerable potential for damage. Table 5 summarizes important conclusions from these studies, including timing of infestation and appearance of damaging eggs in the field.

Figure 1. Number of thrips per blossom cluster at each of three orchards, 2005-2006.

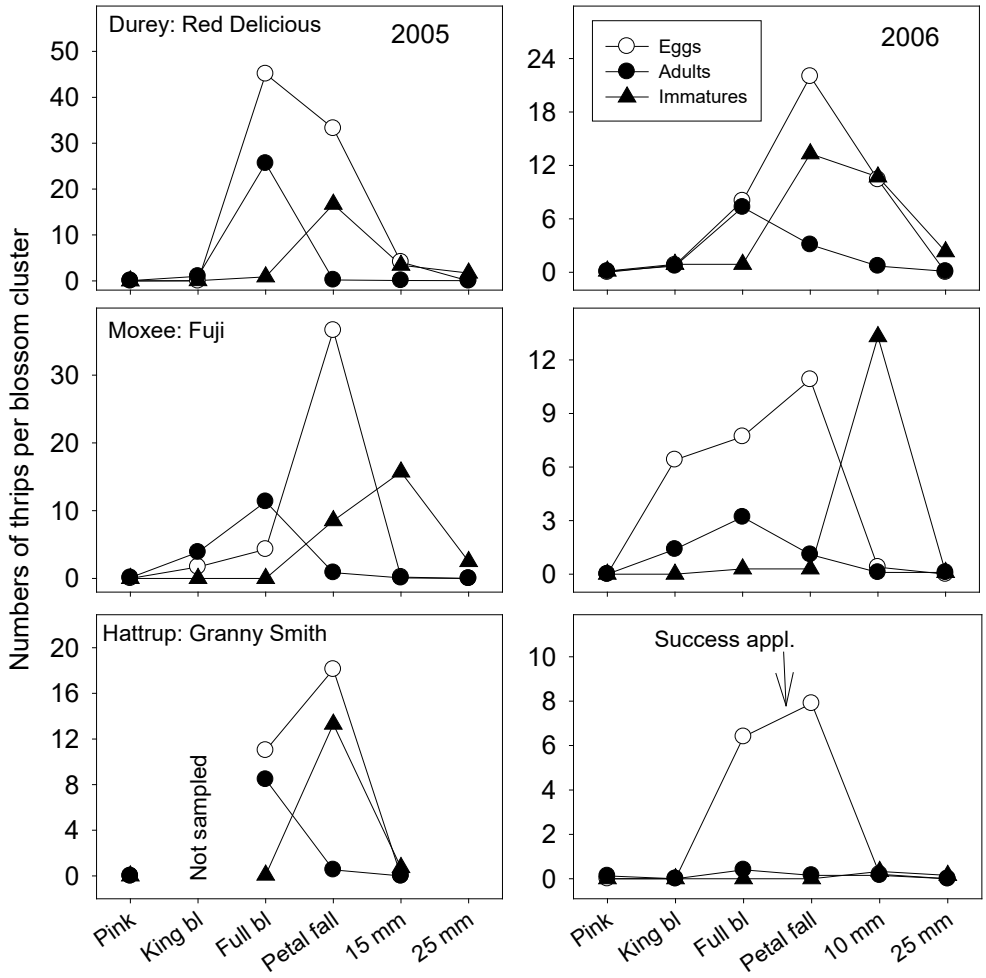

Table 1. Distribution of thrips eggs on field-collected material (2005). Orchards combined.

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	0	--	--	--	--	--
King bloom	16	0	74.2	19.4	6.4	0
Full bloom	286	0.8	84.0	13.0	1.8	0.4
Petal fall	513	4.4	67.2	20.4	4.6	3.4
15-25 mm	31	0	8.5	90.0	0	1.5

*** Potentially damaging eggs

Table 2. Distribution of thrips eggs on field-collected material (2006). Orchards combined

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	0	--	--	--	--	--
King bloom	72	0	50.4	21.7	27.9	0
Full bloom	293	1.0	57.4	27.4	14.2	0
Petal fall	408	2.7	68.6	19.2	8.3	1.2
10 mm	110	4.3	37.6	42.6	3.5	12.1
25 mm	0	--	--	--	--	--

*** Potentially damaging eggs

Table 3. Distribution of thrips eggs on artificially infested material (2005). Includes mix of Red Delicious, Golden Delicious, Fuji, and Granny Smith.

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	769	0	63.7	25.6	10.7	0
King bloom	405	0.4	59.3	29.1	11.2	0
15-25 mm	71	0	31.0	1.4	0	67.6

*** Potentially damaging eggs

Table 4. Distribution of thrips eggs on artificially infested material (2006). Granny Smith clippings.

		Percentage of eggs deposited in:				
Apple stage	**Total # eggs**	**Stamen**	**Calyx**	**Stem**	**Leaves**	***** "Fruitlet"**
Pink	159	0	42.4	29.4	28.3	0
10 mm	50	1.8	45.6	8.8	0	43.9
25 mm	20	0	0	4.8	77.4	17.7

*** Potentially damaging eggs

Table 5. Text summary of results.

	Field-infestation				Artificial infestation: damaging eggs?
	Adults	Eggs	Damaging eggs		
Pink	No	No	No		No
King	Low #'s	Low #'s	No		No
Full	Peak #'s	Peak #'s	No		(not tested)
Petal fall	Low #'s	Peak #'s	Low #'s		(not tested)
10-15 mm	No	Low #'s	Peak #'s		Yes
25 mm	No	No	No		Yes

PRESENTATIONS

Miliczky, E., D. Horton, S. Cockfield and E. Beers. 2005. Spatial and temporal distribution of western flower thrips eggs in apple tissues. 101st Annual Meeting, Washington State Horticultural Assoc., Wenatchee, WA. (Poster)

Miliczky, E., D. Horton, S. Cockfield and E. Beers. 2006. Spatial and temporal distribution of western flower thrips eggs in apple tissues. 80th Annual Conference, Western Orchard Pest and Disease Management, Portland, OR. (Poster)

FINAL PROJECT REPORT
WTFRC Project Number: AH-04-420

Project Title: Target-specific control of fungal pathogens of tree fruit by natural compounds

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State/Province/Zip	CA 94710	**State/Province/Zip:**

Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	14,192	14,618	15,056
Benefits			
Wages			
Benefits			
Equipment	5,000	3,000	2,800
Supplies	2,700	4,000	4,000
Travel	800	800	
Miscellaneous			
Total	22,692	22,418	21,856

[1]Salary for one half-time GS5 Biological Lab. Tech. with 3% projected salary increase for FY05 and 06. [2]2004-Upgrade DNA sequencer; 2005- Plant growth chamber; 2006- PCR thermo-cycler [3]2004- Yeast & fungal strains, kits (nucleic acids work), oligos, plasmids; 2005 & 2006- kits (nucleic acids & protein works), oligos, plasmids, fungal growth media, chemicals. [4]2004 & 2005- Washington State, field trip to orchards to isolate pathogens. [5]Technical support, equipment and supplies are being requested in this proposal for added ability to research on functional genomics and control of phytopathogenic fungi of orchard crops.

Objectives:

Identify new natural compounds effective as antifungals. In apple orchards, controlling a phytopathogenic disease is problematic as chemical controls are currently very limited for several fungal diseases. In the past three years of study, we identified a set of natural compounds with great promise for controlling fungal pathogens. We proved antioxidative stress response/defense systems are essential for fungal tolerance to the natural compounds identified and are potentially useful molecular targets for control of fungal pathogens such as *Penicillium*. We also focused on developing/determining an effective method for delivery of newly discovered natural compounds, leading to a target-specific strategy for an easy, safe and economic approach to pathogen control.

Significant Findings (Last 3 years):

- Molecular targets of identified natural compounds were determined using yeast *Saccharomyces cerevisiae* as a model fungal system.
- Selected phenolic agents were used in target-gene based bioassays in combination with conventional fungicides:

  Mitochondrial superoxide dismutase (Mn-SOD) as a target:

  (1) Targeting Mn-SOD with phenolics such as **vanillylacetone** resulted in a 100 to 1000 fold greater sensitivity to **strobilurin** (inhibitors of complex III of the mitochondrial respiratory chain) or **carboxin** (inhibitors of complex II of the mitochondrial respiratory chain) fungicides. This synergism is significantly greater with strobilurin than with carboxin, suggesting that complex III is a better target than complex II for fungal control, using phenolics.

  (2) Enhancement of antifungal activity of strobilurins was tested using both **berberine** (an alkaloid) and phenolic compounds. With berberine, the most effective phenolic was **veratraldehyde**. The *sod2*Δ mutant (Mn-SOD mutant) of *S. cerevisiae* was highly sensitive to berberine and veratraldehyde. Functional complementation analysis verified these compounds target Mn-SOD.

  (3) Activity of **strobilurin** (25 to 50 μM) was significantly elevated on *Aspergillus fumigatus* and *A. nidulans* by co-application with berberine (2 mM) and/or by veratraldehyde (2-4 mM) on *Penicillium expansum* and most aspergilli.

  Antioxidative signal transduction (MAPK) or vacuolar transport system as targets:

  (1) These compounds also prevented *A. fumigatus* mitogen-activated protein kinase (MAPK) mutants from escaping toxicity of **fludioxonil**, a phenylpyrrole fungicide potentiated by the MAPK pathway that regulates osmotic/oxidative stress-responses in fungi.

  (2) Activity of fludioxonil is elevated by co-application of the aspirin/salicylic acid metabolite, **2,5-dihydroxybenzoic acid** (2,5-DHBA). 2,5-DHBA disrupts cellular GSH (reduced glutathione)/GSSG (oxidized glutathione) homeostasis, further stressing the oxidative stress-response system, enhancing fludioxonil activity. The 2,5-DHBA treatment also prevents tolerance of MAPK mutants resistant to fludioxonil.

  (3) Positive interaction between phenolics and **concanamycin A**, an inhibitor of V-ATPase, or berberine was observed where combined application of test phenolics with either of these compounds greatly enhanced the inhibition of fungal growth.

- These results show certain natural compounds are effective antifungals or synergists to conventional fungicides and can be used for improving control of food-contaminating pathogens. Use of such compounds for fungal control reduces environmental and health risks associated with commercial fungicides, and lowers cost for control and the probability for development of resistance to these fungicides.

Methods:

***In vitro* susceptibility bioassays.** Phenolics (*i.e.,* cinnamic, *o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic, ferulic, benzoic, vanillic, 4-hydroxybenzoic, 3,4,5-trimethoxybenzoic, 3-chlorobenzoic and salicylic acids, benzaldehyde, veratraldehyde, vanillin, vanillylacetone, thymol), carboxin, strobilurins and berberine hemisulfate were examined in fungi. For yeast assays, ~10^6 cells were cultured in YPD (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) and serially diluted from 10-fold to 10^5-fold in SG (0.67% Yeast nitrogen base w/o amino acids, 2% glucose with appropriate supplements: 0.02 mg/ml uracil, 0.03 mg/ml amino acids). Cells from each serial dilution were spotted adjacently on SG agar medium incorporated with each phenolic to be tested. Cells were grown at 30 ºC for 7 days. Numerical scoring is: 6= colonies were visible in all dilutions, 0= no colonies were visible in any dilution, 1= only the undiluted colony was visible, 2= the undiluted and 10-fold diluted colonies were visible, *etc.* Sensitivity of *S. cerevisiae* was also tested with the modifications of the guidelines of NCCLS document M27-A (National Committee for Clinical Laboratory Standards, 1997). An inoculum size of ~ 5 X 10^3 colony forming units (CFU)/ml yeast cells were incubated in flat-bottomed microtiter plates in SG liquid medium (200 µl/well; 30 ºC) containing phenolics, and the spectrophotometric reading (OD at 600 nm) was performed after 48 h of incubation. All treatments were performed in triplicate. For fungal assays ~200 spores were diluted in Phosphate-Buffered Saline (PBS) and spotted in the center of Potato Dextrose Agar (PDA) plates containing phenolic reagents and/or inhibitors of mitochondrial respiration and cell growth was monitored after 7 days at 28 ºC (37 ºC for *A. fumigatus*). Colony growth was measured based on percent radial growth compared to control colonies.

***S. cerevisiae sod2*Δ complementation bioassay.** We examined if complementation of the yeast *sod2*Δ mutant lacking Mn-SOD gene with the orthologous fungal gene (*i.e*., *sodA* from *A. flavus*; encodes Mn-SOD; GenBank accession# AY585205) reversed any effects observed in the *sod2*Δ mutant to berberine hemisulfate and phenolics. This complementation bioassay was done as described previously to examine effects of compounds on Mn-SOD (Kim *et al.* 2005). *S. cerevisiae sod2*Δ with pYES2 empty vector (*sod2*Δ + pYES2; negative control; Invitrogen), wild type with pYES2 empty vector (WT + pYES2; positive control), wild type with pYES2 vector containing PCR-amplified *sodA*, the *A. flavus* Mn-SOD gene (WT + *sodA*; Mn-SOD overexpression), and *sod2*Δ with pYES2 vector containing PCR-amplified *sodA* (*sod2*Δ + *sodA*; functional complementation) were cultured in raffinose medium (0.67% Yeast nitrogen base without amino acids, 110 µM raffinose, 200 µM amino acids) at 30 ºC. Yeast cells were serially diluted as described above with raffinose liquid medium and spotted adjacently on SGAL (0.67% Yeast nitrogen base w/o amino acids, 110 µM galactose, 200 µM amino acids). Functional expression of *sodA* was achieved under the yeast *GAL1* promoter (30 ºC, 10 days). Functionality of *sodA* was assessed based on yeast cell dilution having visible growth in the presence of berberine hemisulfate and phenolics. If the dilution showing yeast growth was similar to the positive control or better than the negative control, *sodA* was considered to have been functionally complemented *sod2*Δ. Reduced sensitivity after complementation would signify test compounds targeted the yeast oxidative stress response system, *i.e*., Mn-SOD.

Enhanced growth inhibition of fungi by co-application of test compounds: synergistic inhibition. Phenolic compounds were added to the medium with strobilurin, carboxin, concanamycin A (vacuolar ATPase inhibitor) or berberine hemisulfate. The cell growth was monitored for 5 to 7 days at 30 ºC or 28 ºC for yeast or fungal pathogens, respectively. The types of medium, culture condition and measurement of the cell growth were as described above. To test the positive interaction, *i.e*., synergistic inhibition, with concanamycin A, *o*-coumaric acid was chosen and combined in the medium for target fungi (*Penicillium,* aspergilli, and yeast). Positive interaction between berberine hemisulfate (0.5 mM) and phenolics (vanillylacetone 10 mM, veratraldehyde 5 mM, vanillic acid 3 mM, vanillin 1 mM, cinnamic acid 0.1 mM, *m*-coumaric acid 5mM) were tested in the yeast as described above. For other fungi, *i.e*., *Penicillium* and aspergilli, berberine hemisulfate (0.5 or 1 mM) and vanillylacetone (5 or 10 mM) were given together in the PDA medium, and the cell growth was monitored for 5 to 7 days.

Results and discussion:

Identification of molecular targets of phenolic agents in the model yeast *S. cerevisiae.* As an approach to identify vulnerable gene targets in pathogenic fungi, we first tested the levels of sensitivity in our model fungal yeast system. We examined forty-six *S. cerevisiae* deletion mutants defective in the antioxidative stress response system against seventeen phenolic agents (Kim *et al.* 2005). We chose minimum effective concentration (MEC) for each compound where the growth of the wild type yeast was almost not affected but reduced colony size. Molecular target(s) most crucial for cellular tolerance/detoxification against the antifungal phenolics could be identified under this condition. Four classes of mutants showing hypersensitivity to the test compounds were identified, as follows: i) regulators of pH responsive transcription (*rim101*Δ) or glutathione transferase transporter (*ure2*Δ), ii) V-ATPase system (*tfp1/vma1*Δ, *vph2*Δ), iii) MAPK kinase (*hog4*Δ; MAPKK), and iv) antioxidative enzymes for glutathione biosynthesis (*gsh1*Δ, *gsh2*Δ)/superoxide dismutation (*sod1*Δ, *sod2*Δ). Genes identified are suggested to be important molecular targets for control of phytopathogenic fungi. The levels of antifungal activity of phenolics were not correlated to their acidic nature, but were due to certain structural characteristic(s).

Identification of an effective antifungal target on the mitochondrial respiratory chain. Phenolics such as vanillylacetone affect the normal function of mitochondrial respiration of yeast (Kim *et al.* 2006). To target mitochondrial respiration and the antioxidative stress response system more efficiently for fungal control, we applied vanillylacetone to synergize the effects of the fungicides carboxin or strobilurin. Application of vanillylacetone enhanced the level of growth inhibition by these fungicides (**Table 1**). There was greater synergism in activity of strobilurin than that of carboxin by vanillylacetone, suggesting complex III of the respiratory chain is a more efficient target for fungal control. Thus, using vanillylacetone as a synergist may significantly reduce potential for development of resistance to these types of fungicides that inhibit mitochondrial respiration; a frequent problem with conventional fungicides.

Table 1. Effects of different concentrations of fungicides (μM) carboxin and strobilurin combined with vanillylacetone (mM) on growth of fungi *Penicillium expansum* (*Pe*), *Aspergillus niger* (*An*) and *A. flavus* (*Af*)[a].

Vanillylacetone (mM)	0	10	15	20
Fungal species	*Pe An Af*	*Pe An Af*	*Pe An Af*	*Pe An Af*
Fungicide (μM)				
None 0	100,100,100	64, 60, 91	40, 19, 58	24, 0, 29
Carboxin 50	75, 100, 98	29, 32, 64	~0, 17, 29	0, 0, 0
100	71, 100, 96	19, 24, 47	0, 0, 17	0, 0, 0
Strobilurin 50	71, 100,100	0, 0, 18	0, 0, 0	0, 0, 0
100	69, 100, 98	0, 0, 0	0, 0, 0	0, 0, 0

[a] Growth of fungi is represented as a percentage of radial growth of the fungal mat of treated compared to control (no inhibitors of mitochondrial respiratory chain). Values are means of three replicates, standard deviations of all measurements were <2%. ~0 means barely germinated.

Identification of berberine analog as a natural synergist for targeting mitochondrial superoxide dismutase (Mn-SOD). Berberine targets the activity of Mn-SOD. Berberine also acts as a synergist for fungal control with phenolics. The antifungal interaction between berberine (0.5, 1 mM) and veratraldehyde (2.5, 5 mM) on several different aspergilli and *P. expansum* showed that germination was completely inhibited in *A. fumigatus*, *A. parasiticus*, *A. oryzae*, *A. niger* and *A. nidulans* at 0.5 mM berberine (1 mM for *A. niger*) and 5 mM veratraldehyde (**Table 2**). However, the growth of *A. flavus*, *A. ochraceous* and *P. expansum* was reduced but not completely inhibited at the highest doses tested of both compounds (**Table 2**).

Table 2. Relative growth of *P. expansum* and aspergilli treated with varying amounts of veratraldehyde or berberine hemisulfate, individually or in combination*

Veratraldehyde (mmol l^{-1})	0			2.5			5		
Berberine (mmol l^{-1})	0	0.5	1.0	0	0.5	1.0	0	0.5	1.0
A. fumigatus AF293	100	60±17	56	100	0	0	94	0	0
A. flavus NRRL 3557	100	98	94	98	94	83	90	73	0
A. parasiticus NRRL 5862	100	96	92	90	76	60	72	0	67
A. ochraceous NRRL 5175	100	85	81	88	73	63	69	50	0
A. oryzae FGSC A815	100	89	83	87	62	51	70	0	48
A. niger NRRL326	100	80	78	106	84	78	80±27	64±13	0
A. nidulans FGSC A4	100	76	64	84	0	0	0	0	0
P. expansum NRRL 974	100	85	81	100	81	74	89	59	52

* Fungal growth is presented as a percentage of radial growth compared to control colonies grown on PDA plates receiving only DMSO. Values are means of three replicates. Standard deviations of all measurements are <5%, except where noted.

The *sodA*/*sod2*Δ complementation assay (See Kim *et al.* 2005 for the method) showed that Mn-SOD plays a role in tolerance to berberine and phenolics. The *sod2*Δ mutant overcame berberine/phenolic-mediated growth inhibition by functional expression of *Aspergillus sodA*. Co-application of berberine and veratraledehyde or vanillylacetone greatly reduced yeast survivability in all strains, including the wild type, compared to individual treatment of these compounds (**Figure 1**). The *sod2*Δ complemented with the functional *sodA* gene (*sod2*Δ-*sodA*) had the same level of growth as the wild type when exposed to berberine and the phenolics. The *sod2*Δ with the empty vector, however, was 10 to 100 times more sensitive to these compounds. The *sod2*Δ-*sodA* complementation also resulted in greater tolerance to individual treatments of veratraldehyde or vanillylacetone. Over-expression of Mn-SOD (wild type + *sodA*) had no effect on hypertolerance. This result further demonstrates Mn-SOD plays an important role for fungal tolerance to our test compounds.

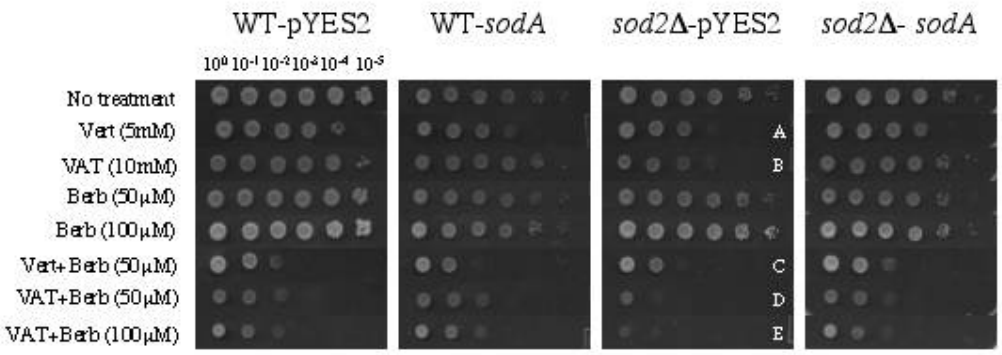

Figure 1 Functional complementation assay of the Mn-SOD gene from *Aspergillus flavus* (*sodA*), using the vector pYES2, in yeast wild type (WT) or *sod2*Δ, lacking the orthologous gene. Assay shows *sod2*Δ-pYES2 (no Mn-SOD + empty vector) is 10 to 100 times more sensitive to 5 mM veratraldehyde (Vert) (A) and 10 mM vanillylacetone (VAT) (B) and to combined treatments with berberine (Berb) (C, D and E) than complemented *sod2*Δ-*sodA* and wild type (WT) strains.

2,5-DHBA inhibits fungal growth by disrupting cellular glutathione homeostasis. 2,5-Dihydroxybenzoic acid (2,5-DHBA) is a cellular metabolite of salicylic acid (2-hydroxybenzoic acid), in turn a metabolite of aspirin (acetylsalicylic acid) (Forth *et al*., 1987). Lower doses of 2,5-DHBA relieved oxidative stress in *S. cerevisiae* in accordance with prior evidence showing that 2,5-DHBA had antioxidant activity (Ashidate *et al*., 2005; See **Figure 2**). We tested the antifungal activity of 2,5-DHBA against 46 additional yeast deletion mutants, using the dilution bioassay. These mutants were previously identified as lacking various genes in the oxidative stress response pathway (see Materials and methods; Kim *et al*., 2005). Of these 46 strains, *ure2*Δ (putative glutathione transferase mutant), *vph2*Δ (vacuolar H(+)-ATPase assembly mutant), *ste20*Δ (protein ser/thr kinase mutant), *glr1*Δ (glutathione reductase mutant), *gsh1*Δ (γ-glutamylcysteine synthetase mutant), *gsh*2Δ (glutathione synthetase mutant), *sod1*Δ (Cu/Zn superoxide dismutase mutant), *sod2*Δ (Mn superoxide dismutase mutant), *hog1*Δ (MAPK mutant) and *hog4*Δ (MAPK kinase mutant) were 10 to 1,000 times more sensitive than the wild type, when exposed to 6 to 18 mM 2,5-DHBA. Based on this observation using *S. cerevisiae* as a model system, we concluded that these 10 genes, or their orthologs, played relatively more significant roles than other fungal genes, in responding to or tolerating toxic levels of 2,5-DHBA.

Supplementation of GSH at 0.1 mM resulted in almost complete recovery of the *glr1*Δ strain to 2,5-DHBA-induced toxicity, whereas supplementation of GSSG had no effect (**Figure 2**). This response in recovery of the *glr1*Δ strain to GSH and not GSSG suggested that 2,5-DHBA may play a role in disrupting regulation of reduced *vs.* oxidized levels of glutathione in the cell.

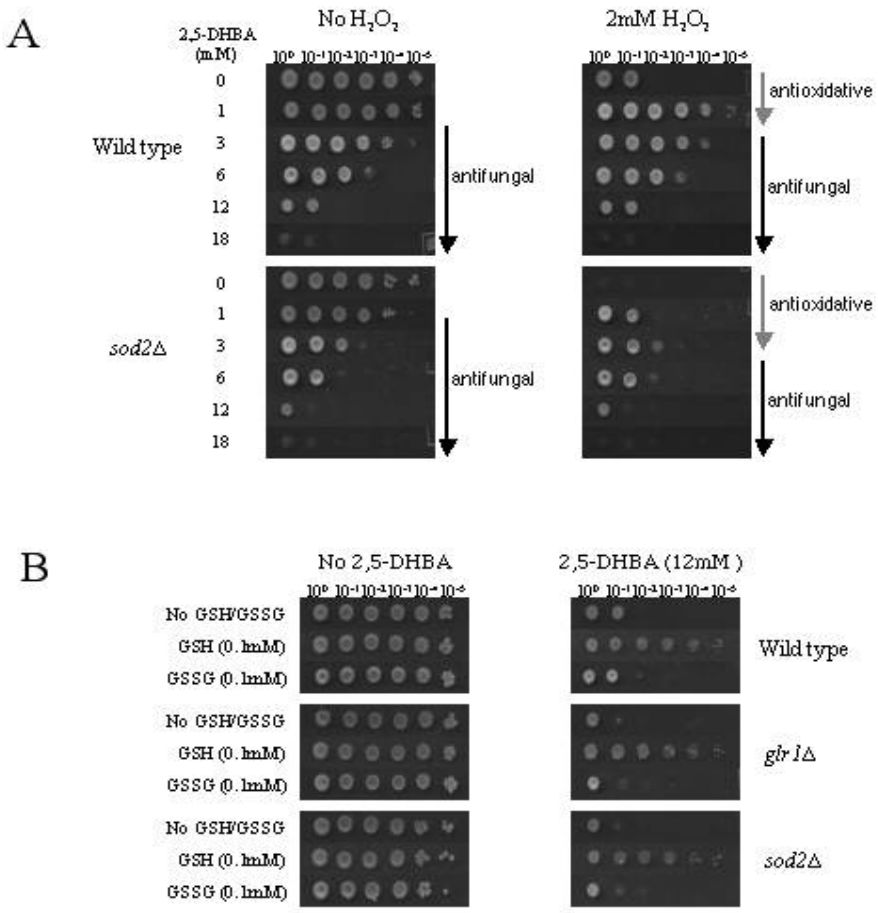

Figure 2. (**A**) Antioxidant/antifungal activities of 2,5-DHBA using a yeast bioassay based on 10-fold serial dilutions of yeast cultures placed sequentially on the growth medium. For testing antioxidant activity of the compounds, different concentrations of hydrogen peroxide (H_2O_2; 1, 2, 3 mM) were applied to the culture medium and the antioxidant/antifungal effects of 2,5-DHBA were monitored as described in the text. The representative bioassays shown here are of the 2 mM H_2O_2 treatment. (**B**) Yeast bioassay showing treatment with GSH (0.1, 0.5 mM) induced recovery of cell growth (wild type, *glr1*Δ, *sod2*Δ) from 2,5-DHBA toxicity, whereas GSSG (0.1, 0.5 mM) did not. This shows 2,5-DHBA prevents reduction of GSSG to GSH. The representative bioassays shown here are of the 0.1 mM GSH/GSSG treatment.

2,5-DHBA enhances fludioxonil activity in wild type and MAPK mutants of fungi. Certain fungi having MAPK mutations are tolerant to phenylpyrrole fungicides (Kojima *et al*., 2004). Both *sakA*Δ/*mpkC*Δ MAPK mutants of *A. fumigatus* escaped fludioxonil toxicity (**Figure 3A**). However, we found co-applying 2,5-DHBA with fludioxonil prevented these mutants from developing tolerance to this fungicide (**Figure 3A**). Presumably, this tolerance is prevented because 2,5-DHBA targets genes downstream in these MAPK pathways targeted by fludioxonil and destabilizes GSH/GSSG homeostasis (**Figure 3B**). Considering MAPK signaling pathway is highly conserved in different organisms, we are currently focusing on this pathway in *Penicillium* or other apple pathogens as a target for effective fungal control.

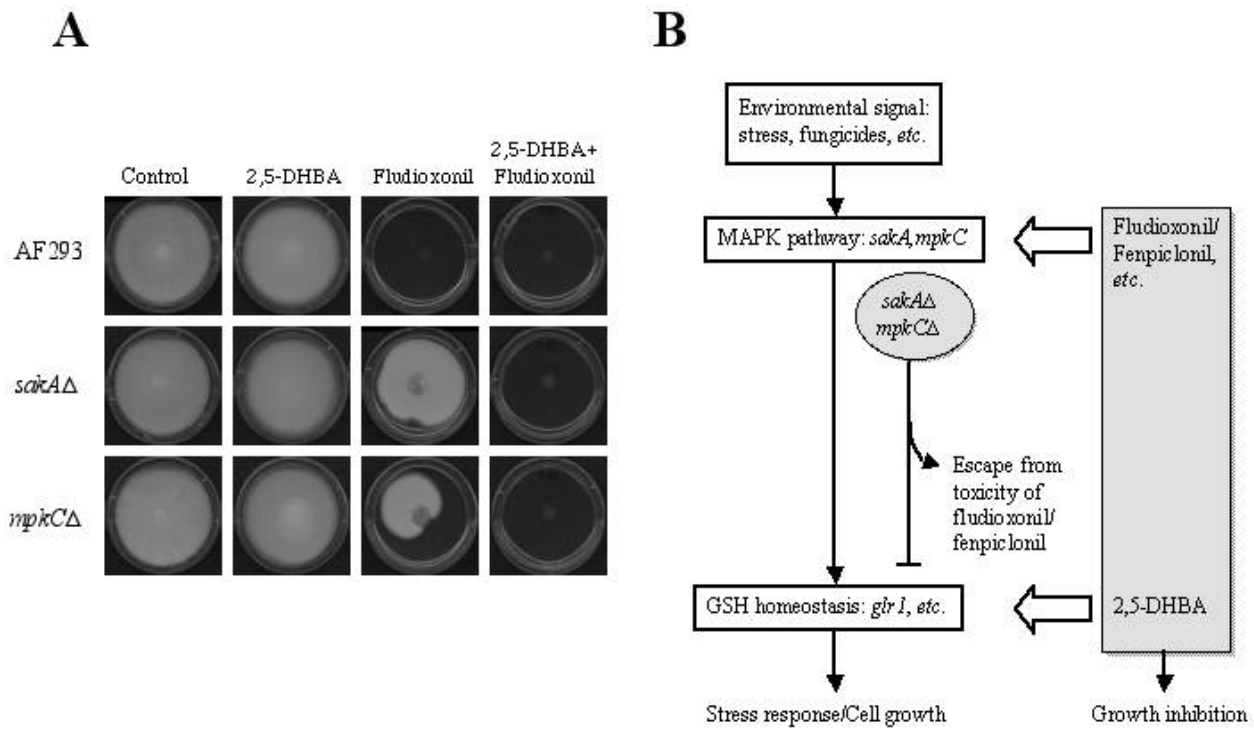

Figure 3. Inhibiting tolerance (escape) of *sakA*Δ and *mpkC*Δ MAPK mutants of *A. fumigatus* to phenylpyrrole fungicides by co-application of 2,5-DHBA. (**A**) Representative bioassays of wild type (AF293) and MAPK mutants with no treatment (Control), 2,5-DHBA (12 mM), fludioxonil (50 µM) and fludioxonil (50 µM) + 2,5-DHBA (12 mM). Note that co-treatment of 2,5-DHBA prevents *sakA*Δ and *mpkC*Δ MAPK mutants from escaping fludioxonil toxicity. (**B**) Scheme showing where phenylpyrrole fungicides (*e.g.*, fludioxonil) target MAPK signaling pathway genes. MAPK mutants escape toxicity by missing the signal stimulated by phenylpyrrole fungicides and, thus, avoiding the induced osmotic/oxidative stress response. Application of 2,5-DHBA disrupts cellular GSH homeostasis, which enhances the toxicity in the wild type cells or helps prevent escape of MAPK mutants from antifungal effects.

We also compared the activity of structural derivatives of 2,5-DHBA on *Penicillium expansum* and more species of aspergilli. Acetylsalicylic acid had the highest antifungal activity in all fungi tested. 2,3-DHBA and 2,4-DHBA/2,5-DHBA showed decreased toxicity, respectively (**Table 3**). 3,4-DHBA had little antifungal activity, even at the highest concentration (21 mM). In a separate assay, benzoic acid (no –OH group on the aromatic ring) had the highest antifungal activity, followed by 2-hydroxybenzoic (salicylic) acid and acetylsalicylic acid. These responses, based on chemical structure, indicate additional hydroxyl groups, and further away from the ortho position in the aromatic ring, gradually decrease antifungal activity.

Table 3. Structure-activity relationship of effect of dihydroxybenzoic acid (DHBA) derivatives on growth of *P. expansum*, *A. fumigatus* and *A. flavus*[1].

Conc. (mM)	Control	Acetyl-salicylic acid	2,3-DHBA	2,4-DHBA	2,5-DHBA	3,4-DHBA
A. fumigatus						
0	100	-	-	-	-	-
9	-	0	22	72	76	92
12	-	0	0	44	58	92
15	-	0	0	46	44	92
18	-	0	0	32	28	92
21	-	0	0	0	12	90
A. flavus						
0	100	-	-	-	-	-
9	-	60	88	92	80	96
12	-	0	84	80	74	96
15	-	0	0	72	60	96
18	-	0	0	42[2]	42	98
21	-	0	0	0	0	98
P. expansum						
0	100	-	-	-	-	-
9	-	52	74	77	77	90
12	-	0	58	68	58	87
15	-	0	0	52	48	81
18	-	0	0	23	32	81
21	-	0	0	0	23	77

[1] Responses are percentage of radial growth compared to control colonies grown on PDA plates receiving only DMSO. Values are means of three replicates. Standard deviations of all measurements are <3% except where noted. For fungal assays, ~200 spores were diluted in phosphate-buffered saline (PBS) and spotted onto the center of potato dextrose agar (PDA) plates containing test compounds and incubated at 37 ºC and 28 ºC for *A. fumigatus* and *A. flavus*/*P. expansum*, respectively, for 5 days. *A. flavus* NRRL3357 and *P. expansum* NRRL974 were obtained from National Center for Agricultural Utilization and Research, USDA, Peoria, IL (http://nrrl.ncaur.usda.gov/index.html) and A. fumigatus strains were kindly provided by Dr. Greg May (University of Texas M.D. Anderson Cancer Center).

[2]Standard deviation: 4%

Summary. During last three years we identified a potentially effective approach to fungal control using newly discovered natural compounds that have a target-specific basis of activity. Antioxidative stress response systems of fungi can be an efficient molecular target of phenolics for pathogen control. We proved positive interaction between phenolics and conventional fungicides or berberine significantly augment the fungicidal effects of commercial fungicides. Certain phenolics disrupt cellular redox homeostasis by targeting the fungal antioxidative stress systems. We also showed how 2,5-DHBA greatly improved effectiveness of fludioxonil, a phenylpyrrole fungicide. Our results indicate this improvement is from the ability of 2,5-DHBA to disrupt glutathione homeostasis, resulting in cellular GSH/GSSG imbalances. Such supplementation, using safe, natural compounds to augment effectiveness of commercial fungicides or antifungal drugs, lowers dosages of commercial fungicides required for effective control. Consequently, this lower dosage reduces environmental impact and risks to human health by lowering exposure to fungicides. Additionally, there is decreased potential for development of fungal resistance. We conclude natural compounds such as phenolic agents that do not have any significant medical or environmental shortcomings could be useful in control programs involving conventional antifungal agents.

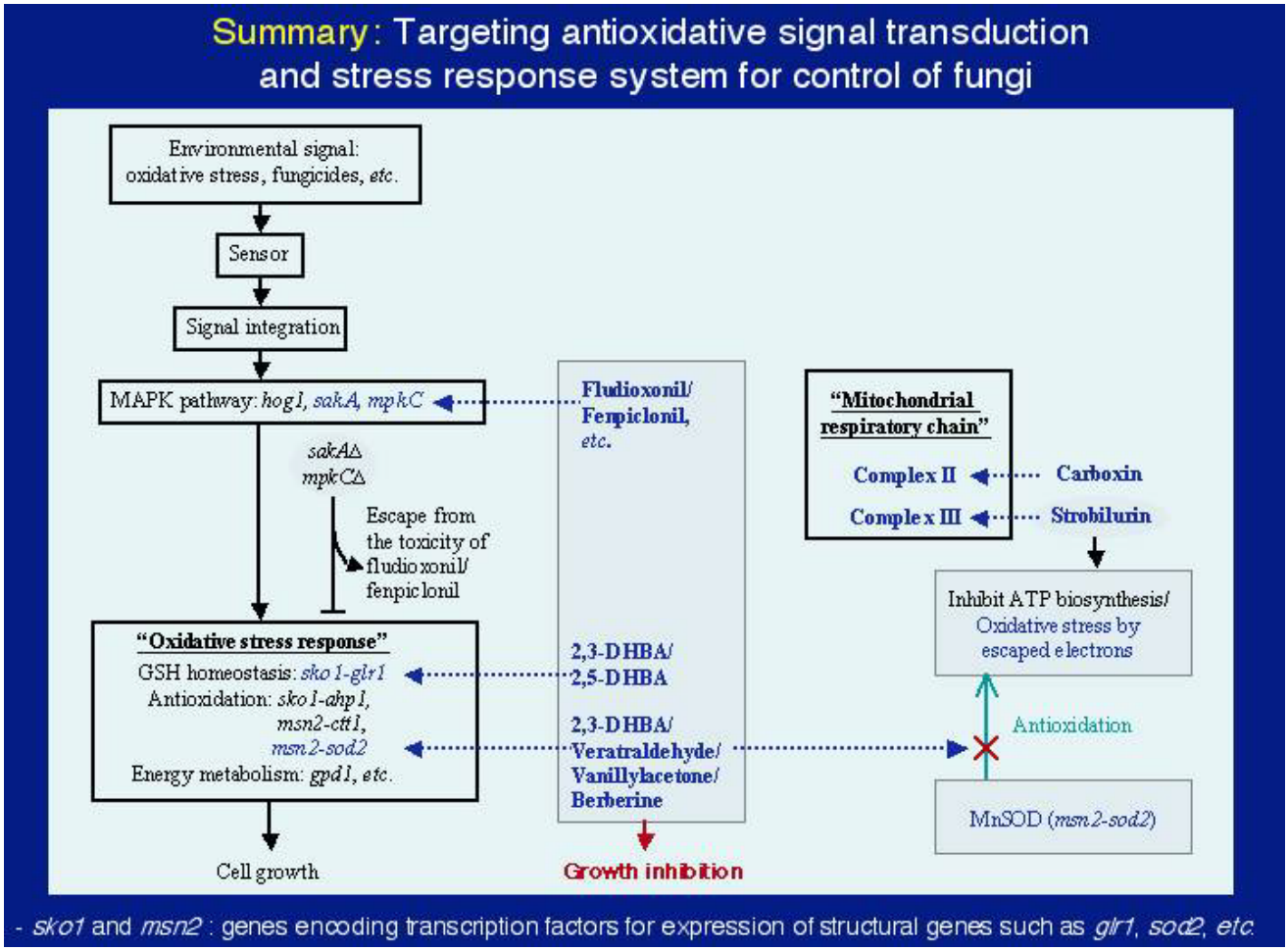

Figure (Summary). Elucidation of targeting antioxidative signal transduction and stress response system for control of fungi. DHBA, dihydroxybenzoic acid. See text for the description of other genes.

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FINAL PROJECT REPORT
WTFRC Project Number: AH-05-510 (WSU Project No. 13C-3661-5366)

Project Title: Sphaeropsis Rot in Apple

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Cooperators: Fruit packinghouses

Budget History:

Item	Year 1: 2005	Year 2: 2006
Salaries	27,037	35,020
Benefits	10,815	14,709
Wages		3,000
Benefits		300
Supplies	6,000	3,000
Travel	3,000	1,000
Total	46,852	57,029

Objectives:

1. Determine the sources and availability of inoculum of *Sphaeropsis pyriputrescens* in the orchard during apple-growing season.
2. Determine seasonal susceptibility of apple trees to infection by *S. pyriputrescens* in the orchard.
3. Determine susceptibility of apple fruit at different growth stages to infection by *S. pyriputrescens* in the orchard in relation to Sphaeropsis rot during storage.
4. Test sensitivity of the fungus to various fungicides including new postharvest fungicides.
5. Determine the prevalence and incidence of Sphaeropsis rot as well as other postharvest diseases on major apple varieties under various handling systems.
6. Evaluate effectiveness of preharvest fungicides and postharvest drench with fungicides in controlling Sphaeropsis rot.

Significant findings:

- Over the three-year statewide survey of postharvest diseases on Red Delicious, Fuji and Golden Delicious, blue mold caused by *Penicillium* spp., gray mold caused by *Botrytis cinerea* and Sphaeropsis rot caused by *Sphaeropsis pyriputrescens* accounted for 32.4%, 29.4% and 18.4% of the total decay, respectively. Sphaeropsis rot was observed in all seven counties that we sampled in central Washington State. Sphaeropsis rot was observed in 73% of the grower lots we sampled. Sphaeropsis rot varied from grower lot to grower lot. Instances of severe losses of fruit due to Sphaeropsis rot in storage have been observed in both Red Delicious and Fuji apples.
- Our three-year survey results indicate that gray mold, blue mold and Sphaeropsis rot should be considered major targets for decay control. Bull's eye rot should also be considered a target disease on Golden Delicious.
- In 2006, 60-100% of sampled apple trees in a commercial Fuji orchard were infected by the Sphaeropsis fungus; 27-57% of the sampled dead fruit spurs or twigs were infected by the fungus. Over 90% of sampled crabapple trees were infected by the Sphaeropsis fungus.
- In the Red Delicious orchard, crabapple trees were not commonly planted. The focus was on dead fruit spurs or twigs and dead bark. Approximately 20-40% and 0-50% of the sampled trees were infected by the fungus in 2005 and 2006, respectively; 1-16% and 0-14% of the sampled fruit spurs had pycnidia of the fungus in 2005 and 2006, respectively.
- The results indicate that dead tissues on fruit spurs, dieback twigs or cankers, and crabapple trees were important sources of inoculum responsible for infection of apple fruit in the orchard leading to Sphaeropsis rot during storage. The results also indicate that viable inoculum of the fungus was available throughout the fruit-growing season.
- Cankers and dieback twigs of crabapple trees were not the only sources of inoculum. Crabapple trees, if present, likely facilitated spread of the fungus from crabapple trees to apple trees in the orchard. Fruiting bodies of the fungus on apple trees were the immediate inoculum responsible for infection of fruit in the orchard leading to Sphaeropsis rot during storage.
- Significant cankers developed on twigs inoculated in November and early spring. No significant cankers developed on twigs inoculated in June. It appeared that trees were more susceptible to infection during the dormant period and that Fuji trees seemed to be more susceptible to infection than Red and Golden Delicious.
- Sphaeropsis rot developed on early-inoculated fruit as well as late-inoculated fruit, indicating that when conditions were met the fungus was able to colonize the fruit even in the early season and to remain latent throughout the fruit-growing season. Overall, incidence of Sphaeropsis rot on inoculated fruit increased as the inoculation date approached harvest except for Fuji inoculated in October.
- On Red Delicious, stem and calyx infections both were common; on Golden Delicious, stem infection was more common than calyx infection; on Fuji, calyx infection was more common than stem infection.

- Scholar, Mertect and Pristine were highly effective in inhibiting mycelial growth of the fungus, and Penbotec was effective only at higher rates. Pristine was also effective in inhibiting spore germination. The information has been used for developing pre- and postharvest fungicide programs for control of Sphaeropsis rot.
- Ziram applied at two weeks before harvest provided inconsistent results in trials conducted in a commercial orchard (in the first year it was effective but not effective the following year). In a trial conducted in a research block of Red Delicious, ziram was effective to control Sphaeropsis rot. Timing of infection may affect its efficacy.
- In the experiment conducted in 2005-06 season, Pristine and Topsin applied at seven days before harvest significantly reduced Sphaeropsis rot on apple fruit that were inoculated with the Sphaeropsis fungus at either five or two weeks before harvest. Pristine and Topsin reduced Sphaeropsis rot by 53-71% and 39-59%, respectively.
- Timing of infection of fruit by the Sphaeropsis fungus may affect the effectiveness of preharvest fungicides for control of Sphaeropsis rot. This needs to be further evaluated.
- Mertect applied as a pre-storage drench treatment was consistently effective to control Sphaeropsis rot.
- We are currently evaluating new pre- and postharvest fungicides (Pristine, Topsin M, Scholar and Penbotec) for control of Sphaeropsis rot.

Methods:

Sources and availability of inoculum of *Sphaeropsis pyriputrescens* were monitored in two commercial apple orchards during the apple-growing season.

Experiments were conducted four times a year to determine susceptibility of trees of Fuji, Red Delicious and Golden Delicious to *S. pyriputrescens*. To determine susceptibility of apple fruit to infection by *S. pyriputrescens*, fruit of Fuji, Golden Delicious and Red Delicious were inoculated with the pathogen four to five times during the growing season. Fruit were harvested and stored at 32ºF for decay development. Decay incidence and infection sites on the fruit were recorded.

Effectiveness was tested of new fungicides (Scholar, Penbotec, Pristine) and other fungicides in inhibiting mycelial growth and spore germination. Ten representative isolates were included. An experiment was conducted in a commercial Red Delicious orchard with a history of severe Sphaeropsis rot to evaluate effectiveness of preharvest fungicides and postharvest drench with fungicides in controlling Sphaeropsis rot.

Results and discussion:

Sources and availability of inoculum in the orchard

In 2005 and 2006, we monitored inoculum availability of the Sphaeropsis fungus in two commercial orchards (Table 1 for 2006 data). The results from the two-year study were similar. The 2005 data were reported in the 2005 report. In summary, in the Fuji orchard sources of inoculum likely responsible for infection of fruit included: 1) Twig dieback and dead fruit spurs. In 2006, 60-100% of sampled apple trees were infected by the Sphaeropsis fungus; 27-57% of the sampled dead fruit spurs or twigs were infected by the fungus. 2) Fuji fruit mummies on the trees. Fifty-five percent of the mummies sampled in mid-May were infected by the fungus. 3) Cankers, twig dieback and infected fruit of crabapple. Over 90% of sampled crabapple trees were infected by the Sphaeropsis fungus.

In the Red Delicious orchard, crabapple trees were not commonly planted. The focus was on dead fruit spurs or twigs and dead bark. Approximately 20-40% and 0-50% of the sampled trees were infected by the fungus in 2005 and 2006, respectively; 1-16% and 0-14% of the sampled fruit spurs had pycnidia of the fungus in 2005 and 2006, respectively.

The results indicate that dead tissues on fruit spurs, dieback twigs or cankers, and crabapple trees were important sources of inoculum responsible for infection of apple fruit in the orchard leading to

Sphaeropsis rot during storage. The results also indicate that viable inoculum of the fungus was available throughout the fruit-growing season.

Table 1. Sources and availability of inoculum of the Sphaeropsis fungus in two apple orchards in 2006.

Date	Orchard	Variety	Sample Type	% Trees with Pycnidia	% Samples with Pycnidia
16-May-06	1	Crabapple	Twigs	100	96.7
		Fuji	Spurs/Twigs	80	43.3
	2	Red Delicious	Bark	0	0.0
			Spurs	0	0.0
21-Jun-06	1	Crabapple	Twigs	90	90.0
		Fuji	Spurs/Twigs	80	46.7
	2	Red Delicious	Bark	20	2.0
			Spurs	20	4.0
10-Aug-06	1	Crabapple	Twigs	100	100.0
		Fuji	Spurs/Twigs	90	56.7
	2	Red Delicious	Bark	0	0.0
			Spurs	50	14.0
27-Sep-06	1	Crabapple	Twigs	100	93.3
		Fuji	Spurs/Twigs	100	76.7
	2	Red Delicious	Bark	0	0.0
			Spurs	50	14.0

[1] In the Fuji orchard, at each sampling time, 3 dieback twigs from each of 10 crabapple trees and 3 dead fruit spurs or twigs from each of 10 Fuji trees were sampled. In the Red Delicious orchard, 5 pieces of dead fruit-spur tissues and 10 pieces of dead bark tissues from each of 10 trees were sampled.

Seasonal susceptibility of apple trees to cankers caused by the Sphaeropsis fungus

We inoculated trees of Fuji, Golden Delicious and Red Delicious at four different times each year. Some results were presented in the previous report and the experiments are still in progress. Some available results (canker sizes at six months after inoculation) are presented in Table 2.

Significant cankers developed on twigs inoculated in November and early spring. No significant cankers developed on twigs inoculated in June. It appeared that trees during the dormant period were more susceptible to infection and that Fuji trees seemed to be more susceptible to infection than the other two varieties.

Susceptibility of apple fruit to infection by the Sphaeropsis fungus in the orchard in relation to Sphaeropsis rot during storage

In 2004-2006, we inoculated fruit of Fuji, Golden Delicious and Red Delicious at different growth stages. The 2005-06 data are presented in this report. The fruit from the 2006 inoculation study are currently in storage for decay development. We observed that Sphaeropsis rot developed on early-inoculated fruit as well as late-inoculated fruit, indicating that when conditions were met the fungus was able to colonize the fruit even in the early season and to remain latent throughout the fruit-growing season. Overall, incidence of Sphaeropsis rot on inoculated fruit increased as the inoculation date approached harvest except for Fuji inoculated in October (Fig. 1). The Fuji fruit inoculated in October had a lower incidence than those inoculated in September. This is likely due to low temperature in October when inoculation of Fuji was conducted.

On Red Delicious, both stem and calyx infections were common; on Golden Delicious, stem infection was more common than calyx infection; on Fuji, calyx infection was more common than stem infection (Fig. 2).

Table 2. Susceptibility of apple trees to infection by the Sphaeropsis fungus.

Inoculation Date	Variety	Isolate	Canker Size in mm[a]	
			Average	Range
19-Nov-04	Fuji	check	6.6	6.0 - 8.0
		Sphaeropsis	30.8	8.0- 110.0
	Golden	check	7.1	6.0 - 9.0
		Sphaeropsis	9.7	7.0 - 18.0
	Red	check	6.9	6.0 - 10.0
		Sphaeropsis	18.2	7.0 - 61.0
22-Mar-05	Fuji	check	8.8	6.0 - 12.0
		Sphaeropsis	12.6	6.0 - 25.0
	Golden	check	6.9	6.0 - 10.0
		Sphaeropsis	10.8	6.0 - 18.0
	Red	check	8.6	6.0 - 13.0
		Sphaeropsis	12.1	7.0 - 24.0
21-Jun-05	Fuji	check	10.4	6.0 - 17.0
		Sphaeropsis	8.5	5.0 - 13.0
	Golden	check	6.3	6.0 -8.0
		Sphaeropsis	6.5	5.0 - 8.0
	Red	check	7.0	5.0 - 10.0
		Sphaeropsis	7.0	5.0 - 10.0
20-Sep-05	Fuji	check	5.6	5.0 - 7.0
		Sphaeropsis	8.9	5.0 - 25.0
	Golden	check	6.1	5.0 - 7.0
		Sphaeropsis	7.9	7 - 11.0
	Red	check	5.5	5.0 - 6.0
		Sphaeropsis	7.6	5.0 - 23.0
15-Nov-05	Fuji	check	7.5	7.0 - 8.0
		Sphaeropsis	25.6	7.0 - 170.0
	Golden	check	7.4	7.0 - 8.0
		Sphaeropsis	10.4	7.0 - 20.0
	Red	check	7.6	6.0 - 9.0
		Sphaeropsis	9.9	8.0 - 15.0
28-Mar-06	Fuji	check	8.7	6.0 - 14.0
		Sphaeropsis	11.8	7.0 - 17.0
	Golden	check	8.0	7.0 - 10.0
		Sphaeropsis	9.1	7.0 - 15.0
	Red	check	8.7	6.0 - 12.0
		Sphaeropsis	10.5	7.0 - 17.0

[a] Twigs were inoculated and canker sizes were measured at six months after inoculation.

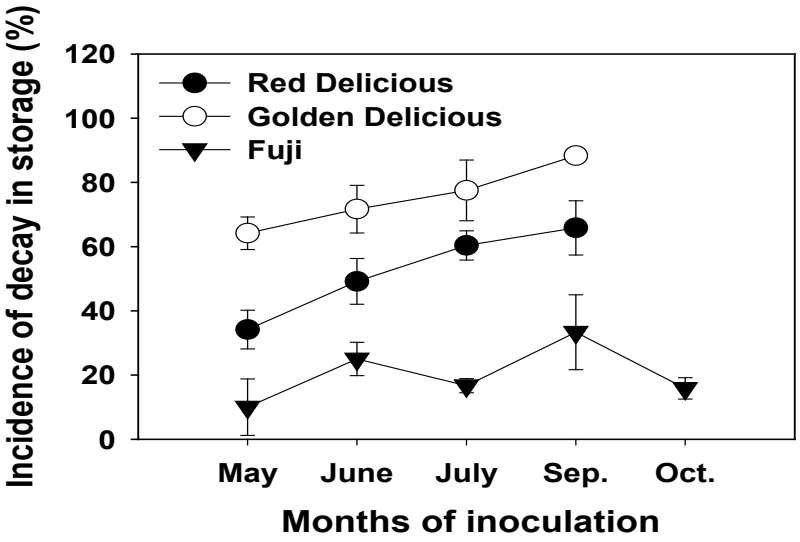

Fig. 1. Development of Sphaeropsis rot on apple fruit after 9 months of storage at 32ºF. Fruit were inoculated with *Sphaeropsis pyriputrescens* in the orchard during the fruit-growing season.

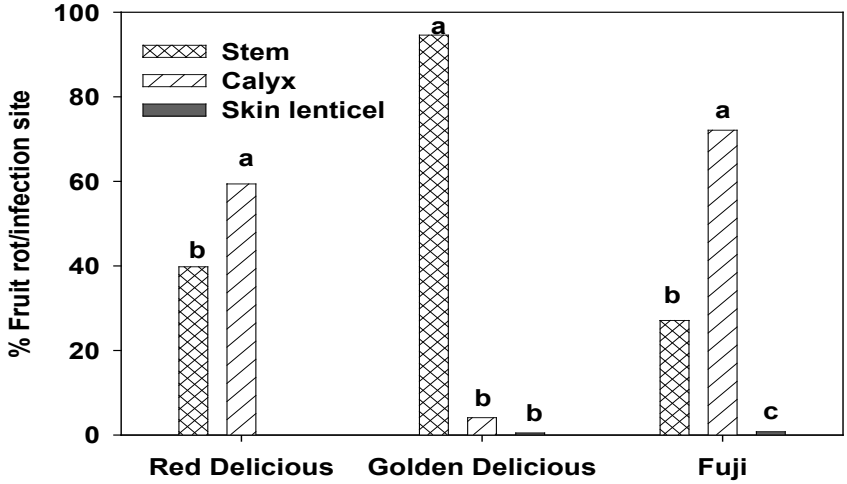

Fig. 2. Infection sites of the Sphaeropsis fungus on the fruit of three apple varieties.

Sensitivity of the Sphaeropsis fungus to newly registered pre- and postharvest fungicides

In 2004 we tested sensitivity of the fungus to various preharvest fungicides. In 2005, we tested sensitivity of mycelial growth and conidial germination to new fungicides fludioxonil (Scholar), pyrimethanil (Penbotec) and Pyraclostrobin+boscalid (Pristine). Scholar, Mertect and Pristine were highly effective in inhibiting mycelial growth of the fungus, and Penbotec was effective only at higher rates. Pristine was also effective in inhibiting spore germination. The information has been used for developing pre- and postharvest fungicide programs for control of Sphaeropsis rot.

Table 3. Effectiveness of three postharvest fungicides and Pristine against the Sphaeropsis fungus

Fungicide	Class	Concentration	Inhibition (%)	
			Range	Average
Mertect	Benzimidazole	X/4 (label rate)	100	100
		10^{-1}X/4	95.8-100	99.6
		10^{-2}X/4	99.5-100	99.9
		10^{-3}X/4	89.3-100	94.5
		10^{-4}X/4	92.2-100	98.3
Penbotec	Pyrimethanil	X/4 (label rate)	95.8-100	99.1
		10^{-1}X/4	26.7-58.2	49.4
		10^{-2}X/4	0-1.0	0
		10^{-3}X/4	0-9.4	0
		10^{-4}X/4	0	0
Scholar	Fludioxinil	X/4 (label rate)	100	100
		10^{-1}X/4	100	100
		10^{-2}X/4	100	100
		10^{-3}X/4	100	100
		10^{-4}X/4	73.8-87.4	82.6
Pristine		X (label rate)	100	100
		10^{-1}X	100	100
		10^{-2}X	96.2-98.7	97.6
		10^{-3}X	82.0-84.3	82.4
		10^{-4}X	23.9-39.0	33.7

Pristine was tested with 3 isolates starting from the label rate. Others were tested with 11 isolates starting from ¼ label rate.

Prevalence and incidence of Sphaeropsis rot and other postharvest diseases

Surveys of Sphaeropsis rot and other postharvest diseases were conducted in 2003, 2004 and 2005. Decayed apple fruit were sampled from six commercial packinghouses, representing orchards in various apple producing areas including north-central Washington, the Columbia Basin, and the Yakima area. Approximately 50 decayed fruit from each grower lot were randomly sampled from cull bins, dump tanks, or sorting tables depending on packing or pre-sizing operations in the packinghouses. In this study, each grower lot represents one orchard. Twenty-six grower lots of Red Delicious were sampled during June to August in 2003; 72 grower lots (39 Red Delicious, 19 Golden Delicious and 14 Fuji) and 81 grower lots (37 Red Delicious, 19 Golden Delicious and 25 Fuji) were sampled from March to August in 2004 and 2005, respectively.

Over the three year survey, blue mold caused by *Penicillium* spp., gray mold caused by *Botrytis cinerea* and Sphaeropsis rot caused by *Sphaeropsis pyriputrescens* accounted for 32.4%, 29.4% and 18.4%, respectively (Table 4). Sphaeropsis rot was observed in all seven counties where we sampled. Percentage of Sphaeropsis-infected orchards ranged from 32 to 100% with an average of 73%. Percentage of Sphaeropsis rot in the total decayed fruit within a grower lot varied from lot to lot. Instances of severe Sphaeropsis rot were again observed on Fuji and Red Delicious fruit. Our three-year survey for postharvest diseases on apples indicated that gray mold, blue mold and Sphaeropsis rot should be considered major targets for decay control. Bull's eye rot should also be considered a target disease on Golden Delicious.

The incidence pattern of postharvest diseases under different postharvest-handling systems was very similar in all three years (Fig.3). The percentage of gray mold in the total decay was higher on non-drenched fruit than TBZ-drenched fruit, whereas blue mold was more prevalent on TBZ-drenched fruit. There was no difference between non-drenched and TBZ-drenched fruit on Sphaeropsis rot. On TBZ-drenched fruit, blue mold showed the highest incidence followed by either gray mold or Sphaeropsis rot. On non-drenched fruit, gray mold comprised the highest percentage in

the decays followed by blue mold and Sphaeropsis rot. Bull's eye rot comprised less than 5% of the total decays with an exception of non-drenched fruit in 2004. There was no difference in bull's eye rot between non-drenched and TBZ-drenched fruit in 2003 and 2005, whereas non-drenched fruit had a higher percentage of bull's eye rot than TBZ-drenched fruit in 2004.

Table 4. Mean percentages of postharvest fruit rots caused by various pathogens in the total decayed apple fruit sampled from commercial packinghouses in Washington State from 2003 to 2005[a].

Year	Variety	No. of grower lots	Sph rot[b]	Gray mold	Blue mold	Bull's eye rot	Speck rot	Mucor rot	Alternaria rot	Others[c]
2003	Red Delicious	26	23.6	34.0	35.2	3.5	0.7	0.0	0.0	3.0
2004	Red Delicious	39	21.7	23.7	41.4	2.8	6.5	0.4	0.8	2.7
	Golden Delicious	19	18.0	29.9	18.8	30.0	0.2	0.0	1.2	1.9
	Fuji	14	18.8	32.2	30.2	4.9	2.0	0.9	2.8	8.2
	Mean		19.5	28.6	30.1	12.6	2.9	0.4	1.6	4.3
2005	Red Delicious	37	20.2	36.4	27.9	2.8	4.8	0.8	1.3	5.8
	Golden Delicious	19	9.4	22.9	17.5	43.8	0.0	0.3	2.0	4.1
	Fuji	25	6.3	17.7	50.6	5.7	1.5	2.1	3.7	12.4
	Mean		12.0	25.7	32.0	17.4	2.1	1.1	2.3	7.4
	Overall mean[d]		**16.9**	**28.1**	**31.7**	**13.4**	**2.2**	**0.6**	**1.7**	**5.4**

[a] Approximately 50 decayed fruit from each grower lot were collected from cull bins, dump tanks or sorting tables depending on the packinghouses available for sampling during packing or pre-sizing operations. Each grower lot represents the fruit from one unique orchard.

[b] Sph rot=Sphaeropsis rot caused by *Sphaeropsis pyriputrescens*.

[c] Includes Aureobasidium rot, Moldy-core rot, Coleophoma rot, Cladosporium rot, Phacidiopycnis rot, black rot, Cytospora rot, and other minor rots caused by unidentified fungi.

[d] Overall mean is the average of all varieties in all three years.

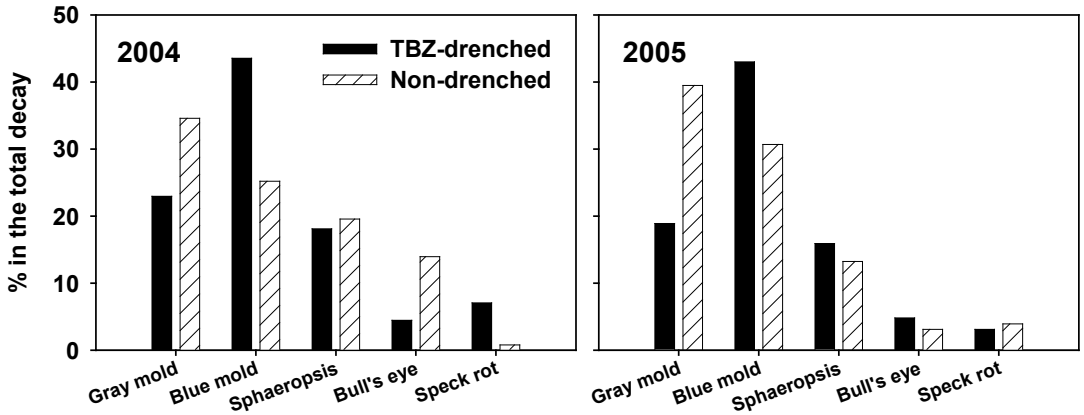

Fig. 3. Comparison of occurrence of common postharvest diseases between TBZ-drenched and nondrenched grower lots in 2004 and 2005.

Chemical control of Sphaeropsis rot

In 2004-06, we evaluated pre- and postharvest fungicides for control of Sphaeropsis rot. The results are summarized as follows. Ziram applied at two weeks before harvest provided inconsistent results in trials conducted in a commercial orchard (in the first year it was effective but not effective the following year). In a trial conducted in a research block of Red Delicious ziram was effective to control Sphaeropsis rot (Fig. 4). Timing of infection may affect its efficacy.

In the experiment conducted in 2005-06 season, Pristine and Topsin applied at seven days before harvest significantly reduced Sphaeropsis rot on apple fruit that were inoculated with the Sphaeropsis fungus at either five or two weeks before harvest (Fig. 4). Pristine and Topsin reduced Sphaeropsis rot by 53-71% and 39-59%, respectively.

Timing of infection of fruit by the Sphaeropsis fungus may affect the effectiveness of preharvest fungicides for control of Sphaeropsis rot. This needs to be further evaluated.

Although the Sphaeropsis fungus infects apple fruit in the orchard, the infections remain latent at harvest and decay symptoms develop only during storage. One question we have been trying to address is whether or not the current decay-control practices are effective to control Sphaeropsis rot. In 2004-05 and 2005-06 seasons, Red Delicious apples from a commercial orchard with a history of severe Sphaeropsis rot were drenched with Mertect and stored in CA for about seven months. We found that Mertect applied as a pre-storage drench treatment was consistently effective to control Sphaeropsis rot (Fig. 5).

In 2005 and 2006 we evaluated postharvest fungicide-drench treatments for control of Sphaeropsis rot. The 2005 experiment was conducted on commercially harvested Fuji fruit. The natural infection of fruit by the Sphaeropsis fungus was low in 2005. We did not obtain reasonable data to separate differences among the treatments. In 2006 we inoculated apple fruit in the orchard and then treated with postharvest fungicides at harvest. The experiment is still in progress. Results will be forthcoming.

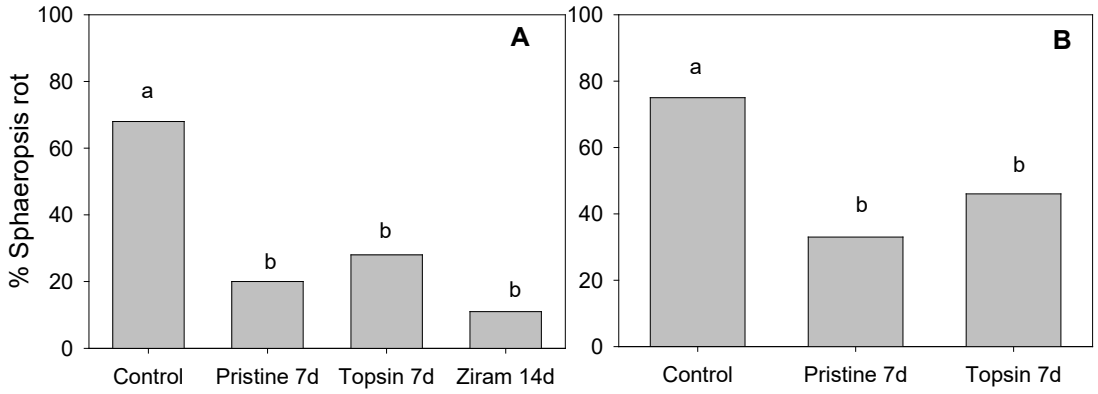

Fig. 4. Control of Sphaeropsis rot by preharvest fungicides. Fruit were inoculated at five weeks (A) and two weeks (B) before harvest. Ziram was applied at two weeks before harvest, and Pristine and Topsin were applied at one week before harvest. Fruit were stored in CA at 32ºF for nine months at which time decay development was evaluated.

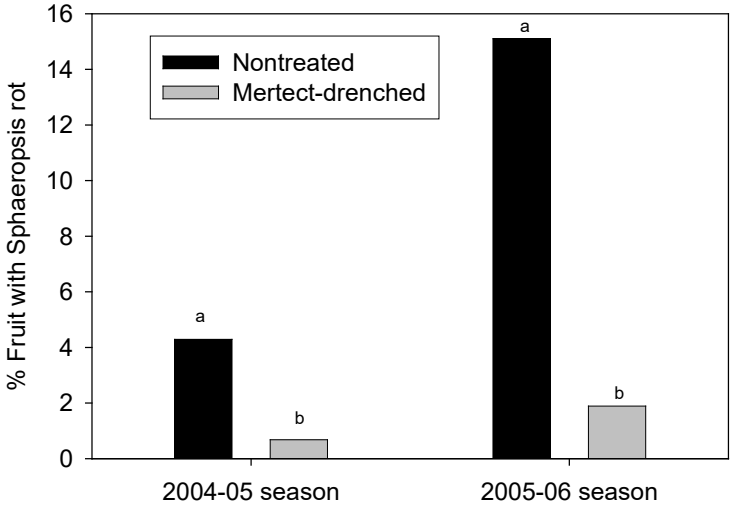

Fig. 5. Control of Sphaeropsis rot by a pre-storage drench with Mertect. Fruit of Red Delicious were from a commercial orchard with a history of Sphaeropsis rot, drenched with Mertect at a commercial facility and stored in CA for seven months.

FINAL PROJECT REPORT
WTFRC Project Number: PH-04-446 (WSU Project No. 13C-3661-7368)

Project Title: Holistic Approach to Decay Management

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Cooperators: Fruit packinghouses

Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	31,000	41,152	42,313
Benefits	12,710	16,486	16,953
Wages	5,000	5,000	5,000
Benefits	800	550	550
Supplies	10,000	10,000	9,000
Travel	2,000	2,000	2,000
Total	61,510	75,188	75,816

Objectives:

7. Evaluate effectiveness and timing of preharvest fungicides in controlling postharvest decay and their effects on fruit finish.
8. Evaluate various pre- and postharvest integrated programs for control of postharvest diseases.
9. Establish baseline sensitivity of major postharvest pathogens to new postharvest fungicides and assess the potential risk of fungicide resistance development in the orchard/storage system.
10. Evaluate new technologies for decay control.

Significant findings:

a. One of the significant accomplishments of this study is the discovery of the effectiveness of Pristine used as a preharvest treatment applied within two weeks before harvest for control of postharvest gray mold and blue mold. Pristine was labeled in April 2005 for use on pome fruits. Based on our findings from this study, I have made recommendations to the industry on the use of Pristine as a preharvest strategy for control of postharvest diseases.
b. Several other preharvest fungicides were also evaluated as preharvest treatments for control of postharvest gray mold and blue mold. In the trials conducted in the last three years, in the worst scenario (fruit were wounded and inoculated with pathogens) Topsin M applied within one week before harvest reduced gray mold by 41-61% and blue mold by 64-75%. Ziram applied at two weeks before harvest reduced gray mold by 94-97%, but its residue on the fruit was not sufficient to protect wounds from infection by *Penicillium expansum*. Elevate was effective to control gray mold but not blue mold. Registration of Elevate for use on apple is pending.
c. Thiram is no longer available. Findings from this study suggest that Pristine, Topsin M and Ziram can be effective alternatives for control of postharvest diseases, particularly gray mold.
d. In a trial on Fuji conducted in a commercial operation, fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking compared with non-drenched fruit. The underlying mechanisms for this phenomenon are unknown yet. These observations and the magnitude of this impact on fruit finish justify further research.
e. The fruit that were treated with Pristine in the orchard and did not receive any postharvest fungicides at packing had a significantly lower level of decay (47% reduction) compared with the nontreated fruit, indicating that Pristine still had residue protection at five months after harvest. However, the residue protection was diminished when the fruit were moved to room temperature.
f. In 2004-06, three postharvest fungicides (Scholar, Penbotec and TBZ) in various combinations as either a drench or an online treatment were tested for control of blue mold. We observed that, when applied as pre-storage drench treatments, Penbotec and Scholar had very good residue protection at packing even after the fruit were stored in CA for five and seven months.
g. Bins in commercial orchards at harvest were heavily contaminated by *Penicillium expansum*, with 4,895 to 17,809 spores/cm^2 on the interior sides of the bins; 26-54% of the *P. expansum* recovered from interior sides of the bins were resistant to TBZ, and 14-31% of the *P. expansum* isolates recovered from the underside bottom of the bins were resistant to TBZ. Bins contaminated with TBZ-resistant *P. expansum* were a major source for buildup of inoculum of TBZ-resistant *P. expansum* in the drench-tank water, in which 68-84% of the *P. expansum* isolates were resistant to TBZ. Interestingly, 1-24% of the *P. expansum* isolates recovered from apple fruit on the trees near harvest in the orchards were resistant to TBZ. It appeared that there was great variation in TBZ-resistant *P. expansum* recovered from apple fruit on the trees in the orchards. Orchard practices may have impact on TBZ-resistant populations of *P. expansum* on apple fruit in the orchards.
h. Based on three assays (mycelial growth, spore germination and germ-tube elongation), we have established baseline sensitivity patterns of *P. expansum* to the two new postharvest fungicides, Scholar and Penbotec. The information will be used for monitoring sensitivity shifts in populations of the pathogen.
i. Fludioxonil-resistant mutants of *P. expansum* were highly resistant to fludioxonil but remained sensitive to pyrimethanil. However, pyrimethanil-resistant mutants also were resistant to

fludioxonil. Pyrimethanil-resistant mutants derived from TBZ-S also became resistant to TBZ. Six phenotypes of fungicide resistance in *P. expansum* were detected, and all pyrimethanil-resistant mutants were triple resistant to all three postharvest fungicides. A fitness cost was associated with fludioxonil-resistant mutants in both saprophytic and pathogenic phases.

j. None of the three postharvest fungicides was able to provide satisfactory control of pyrimethanil-resistant mutants on apple fruit at both 32ºF and 68ºF.

k. Our results indicate that pyrimethanil has a high risk in the development of resistance in *Penicillium expansum*. Multiple resistance to all three postharvest fungicides could become a practical problem if *P. expansum* develops resistance to pyrimethanil. The findings from our study suggest that a program to monitor the shift in sensitivity of *P. expansum* to pyrimethanil and fludioxonil is needed and that strategies of using pre- and postharvest fungicides to avoid or delay the development of resistance to pyrimethanil need to be implemented.

l. In the commercial situation, a fungicide treatment applied by thermofogging to the fruit in a storage room may be delayed for 1-3 days after harvest. One experiment was conducted to look at the kick-back activity of pyrimethanil applied by thermofogging. A 1- to 2-day delay of the thermofogging treatment significantly compromised the effectiveness of the treatment, particularly for blue mold control.

m. In a trial conducted on commercially harvested fruit, the thermofogging treatment significantly reduced the total decay in storage bins as well as gray mold and blue mold. However, the level of decay resulting from natural infections in that year was low (1.1%).

Methods:

Preharvest fungicides were evaluated for control of postharvest gray mold (*Botrytis cinerea*) and blue mold (*Penicillium expansum*) on Red Delicious and Fuji apples. Fungicides were applied within two weeks before harvest. After harvest, fruit were immediately wounded and inoculated with either *B. cinerea* or *Penicillium expansum*. Fruit were tray packed and stored in cardboard boxes in air at 32ºF. The percentages of fruit that developed gray mold and blue mold were recorded, and lesion diameters were measured after 8-12 weeks of storage.

Three postharvest fungicides alone or in various combinations as either drench or online treatments were evaluated for control of decay before packing as well as after packing.

Isolates of *P. expansum* were collected from various apple-related sources, including fruit in the orchard, bins at harvest, soil or organic debris on the bottom of bins, drench solutions, etc. Isolates were identified to species. Isolates of *P. expansum* from various sources were tested for resistance to thiabendazole. Fludioxonil-resistant and pyrimethanil-resistant mutants of *P. expansum* were generated in the laboratory. These mutants were used to examine potential cross resistance of new postharvest fungicides with other fungicides.

Trials to evaluate the efficacy of thermofogging pyrimethanil for control of postharvest diseases were conducted on Red Delicious. Commercially harvested fruit as well as fruit inoculated with pathogens were included in the study.

Results and discussion:

<u>Preharvest fungicides for control of postharvest gray mold and blue mold</u>

Several trials were conducted during 2004-06 to evaluate preharvest fungicides for control of postharvest gray mold and blue mold of apples. In these trials, several preharvest fungicides were evaluated. The major target disease in these trials was gray mold, but we also evaluated fungicide effects on blue mold. All tests were conducted in the worst scenario, in which the apple fruit were wounded to simulate punctures at harvest and inoculated with pathogens. All fruit were stored in RA at 32ºF for 8-12 weeks, at which time decay development was evaluated. The results from the last three-year study are summarized as follows:

In the trial conducted on Fuji in 2004, all fungicides were applied at seven days before harvest. Pristine provided excellent control of both gray mold and blue mold from infection of wounds and reduced gray mold and blue mold by 96% and 78%, respectively. Sylgard used as an adjuvant did not improve control. Pristine+Sylgard did not affect fruit quality compared with the nontreated control.

Topsin M reduced gray mold and blue mold by 41% and 75%, respectively. Elevate and Captevate (Elevate plus Captan) reduced gray mold infection by 57% and 78%, respectively, in comparison with the nontreated control. Neither fungicide was effective to control blue mold. Elevate and Captevate are not yet labeled for use on pome fruits. Thiram reduced gray mold and blue mold infections by 38% and 22%, respectively.

In the trial conducted on Red Delicious apples in 2005, Pristine applied at 7 and 14 days before harvest reduced gray mold by 68-78% and blue mold by 70% in comparison with the nontreated control. Ziram applied at two weeks before harvest significantly reduced gray mold but not blue mold. Topsin M applied at three and seven days before harvest reduced gray mold by approximately 44% and blue mold by approximately 65%.

In the trial conducted on Fuji apples in 2005, Pristine applied at one and seven days before harvest was equally effective and reduced gray mold by 93-99% and blue mold by 87-95% as compared with the nontreated control. Topsin M reduced gray mold by 61% and blue mold by 64%. Thiram reduced gray mold but not blue mold.

In the trial conducted on Red Delicious in 2006, Pristine applied at 7 and 14 days before harvest reduced gray mold by 83-85% and blue mold by 41-46% in comparison with the nontreated control. Ziram reduced gray mold by 94% but not blue mold. The two-year results on Ziram indicate that Ziram residue on the fruit is able to protect wounds from infection by gray mold but not blue mold and that a higher residue level may required for protecting wounds from infection by blue mold.

One of the significant accomplishments of this study is the discovery of the effectiveness of Pristine used as a preharvest treatment for control of postharvest diseases. Pristine was labeled in April 2005 for use on pome fruits. Based on our findings from this study, I have made recommendations to the industry on the use of Pristine as a preharvest strategy for control of postharvest diseases. One of the active ingredients in Pristine is a strobilurin fungicide. Because other strobilurin fungicides may also be used during the fruit-growing season in the orchard for resistance management, DO NOT make more than four applications of strobilurin fungicides per season, including Pristine applied as a preharvest treatment for control of storage rots.

Pre- and postharvest fungicides for control of postharvest decay – commercial orchard trials

We also evaluated pre- and postharvest fungicides on commercially harvested fruit. On the 2004 crop, the amount of decay resulting from natural infections in the nontreated control was low. There were no significant differences in decay among the treatments except the fruit treated with Elevate in the orchard and drenched with Mertect and DPA, which had a higher level of decay compared with other treatments. Interestingly, the fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking compared with non-drenched fruit (results were presented in the 2005 report).

In a separate trial, three postharvest fungicide-drench treatments significantly reduced the amount of decay. About 3% decay developed on nondrenched, packed fruit, whereas no decay developed on Scholar- or Penbotec-drenched fruit at seven days post-packing at room temperature. This indicates that Scholar and Penbotec might have some residue protection at packing even when they were applied as pre-storage drench treatments. These observations are consistent with our findings from controlled experiments (see “integrated postharvest fungicide programs” below). In this trial the fruit that had been drenched with fungicides and DPA tended to have higher levels of lenticel marking compared with non-drenched fruit. The underlying mechanisms for this

phenomenon are not yet known. The magnitude of this impact on fruit finish needs to be further evaluated.

Pre- and postharvest fungicides integrated programs

We have demonstrated that Pristine applied as a preharvest treatment is effective to control gray mold and blue mold on Red Delicious and Fuji in storage bins prior to packing. A further question we tried to address was how to use Pristine residue protection in combination with fungicides used at packing to control blue mold originating from infections of wounds at packing. In 2005 and 2006, we conducted trials on Fuji. The 2006 experiment is in progress. The results from the 2005-06 experiment are summarized in Table 2.

In this study, Pristine was applied to Fuji apples at seven days before harvest and stored in CA for five months, at which time the fruit were washed and subjected to the packing process. After packing the fruit were stored at 32ºF for eight weeks. The fruit that were treated with Pristine in the orchard and did not receive any postharvest fungicides at packing had a significantly lower level of decay (47% reduction) compared with the nontreated fruit (Table 1), indicating that Pristine still had residue protection at five months after harvest. However, the residue protection was diminished when the fruit were moved to room temperature. Scholar and Penbotec applied at packing provided full protection even against a TBZ-resistant strain of *P. expansum*, whereas TBZ did not control TBZ-resistant *P. expansum*.

Table 1. Integration of preharvest Pristine and postharvest fungicides applied at packing for control of blue mold on Fuji apples caused by TBZ-resistant *Penicillium expansum*, 2005-06 season

Preharvest treatment	Fungicide applied at packing 5 months post drenching	8 weeks at 32ºF post inoculation	1 week at room temp after cold storage
		% infected fruit	% infected fruit
Nontreated	No Fungicide	100	100
	Scholar	0	0
	Penbotec	0	0
	TBZ	100	100
Pristine	No Fungicide	52.5	97.5
	Scholar	0	0
	Penbotec	0	0
	TBZ	68.8	100

Integrated postharvest fungicide programs

In 2004-05, three postharvest fungicides in various combinations as either a drench or an online treatment were tested for control of blue mold. We observed that Penbotec and Scholar had some good residue protection at packing even when they were applied as pre-storage drench treatments. The results were presented in the previous report submitted to Commission in July 2005. Based on these preliminary observations, for the experiments conducted in 2005-06 and 2006-07 seasons we ran the fruit through a research packingline after storage.

The results from the 2005-06 experiment are summarized in Table 2. As we observed previously, when Penbotec and Scholar were applied as drench treatments prior to storage, the residues of these two fungicides seemed to be stable in treated Red Delicious fruit in CA storage conditions. Even after seven months in cold storage, the residues of Penbotec and Scholar in the drenched fruit still protected wounds from infection by *Penicillium expansum*. It appeared that Penbotec had better residue protection than Scholar. TBZ residue in drenched fruit did not provide a satisfactory protection after seven months of CA storage, even against a TBZ-sensitive strain of *Penicillium*

expansum (data not shown). An additional online treatment with either Penbotec or Scholar provided an excellent protection of the fruit from infection by either TBZ-R or TBZ-S strains of *P. expansum*.

This study was repeated on the 2006 crop. The fruit are currently in CA, and various tests will be conducted in spring 2007. Results from this study will be forthcoming.

Table 2. Integration of pre-storage fungicide-drench treatments and online treatments at packing for control of blue mold conducted in 2005-06.

Drench treatment applied prior to storage	Fungicides applied at packing 7 months post drenching	8 weeks at 32ºF post packing	1 week at room temp after cold storage
		% infected fruit	% infected fruit
Nontreated	No fungicide	91.3	93.8
	Scholar 8 oz/100 gal	0	0
	Penbotec 32 fl oz/100 gal	0	0
	TBZ 16 oz/100 gal	98.8	98.8
TBZ 16 oz/100 gal	No fungicide	98.8	98.8
	Scholar 8 oz/100 gal	0	1.3
	Penbotec 32 fl oz/100 gal	0	0
Scholar 8 oz/100 gal	No fungicide	2.5	16.3
	TBZ 16 oz/100 gal	16.3	33.8
	Penbotec 16 fl oz/100 gal	0	1.3
Penbotec 16 fl oz/100 gal	No fungicide	0	0
	TBZ 16 oz/100 gal	0	1.3
	Scholar 8 oz/100 gal	0	0

Management of fungicide resistance in postharvest pathogens

Sources of TBZ-resistant Penicillium expansum. In 2004 and 2005, we collected *Penicillium expansum* isolates from decayed fruit sampled from orchard floors and decayed fruit from a commercial packinghouse. Resistance of these isolates to TBZ was tested (Table 3).

In 2005 and 2006, we collected isolates of *Penicillium* spp. from various apple-related sources, including fruit in the orchard, bins at harvest, soil or debris on the bottom of bins, drench solutions, etc. We have obtained hundreds of isolates of *Penicillium* spp. and identified them to species. Isolates of *P. expansum* were tested for resistance to TBZ. The goal of this study is to understand the sources of TBZ resistance of *P. expansum* in the apple-production process from orchard to storage. This would help us implement strategies to minimize the likelihood of development of resistance to new postharvest fungicides. The results are summarized in Table 4. Bins in commercial orchards at harvest were heavily contaminated by *Penicillium expansum*, with 4,895 to 17,809 spores/cm^2 on the interior sides of the bins; 26-54% of the *P. expansum* recovered from interior sides of the bins was resistant to TBZ, and 14-31% of the *P. expansum* isolates recovered from the underside bottom of the bins was resistant to TBZ. Bins contaminated with TBZ-resistant *P. expansum* were a major source for buildup of inoculum of TBZ-resistant *P. expansum* in the drench-tank water, in which 68-84% of the *P. expansum* isolates was resistant to TBZ. Interestingly, 1-24% of the *P. expansum* isolates recovered from apple fruit on the trees near harvest in the orchards was resistant to TBZ. It appeared that there was great variation in TBZ-resistant *P. expansum* recovered from apple fruit on the trees in the orchards. Orchard practices may have impacts on TBZ-resistant populations of the pathogen on apple fruit in the orchards.

Table 3. Sources of TBZ-resistant strains of *Penicillium expansum* collected in 2004-05

Origin	Pre-storage treatment	Collection year	Total isolates	TBZ-resistant isolates (%)
Orchards		2004	303	2.64
Packinghouses	TBZ-drenched	2004	75	69.3
		2005	45	91.1
	Non-drenched	2004	22	0
		2005	48	2.1

Table 4. Sources of TBZ-resistant *Penicillium expansum* from apple-related sources

Collection year	Source	Spore load	Number of isolates of *Penicillium* spp. sampled	*P. expansum* isolates (%)	TBZ-R isolates of *P. expansum* (%)
2005	Apple fruit at harvest	116/fruit	231	72.7	24.4
	Inside the bin at harvest	4895/cm^2	334	21.9	26.0
	Underside bottom of the bin		219	18.3	31.4
	Drench-tank water	814/ml	239	31	67.7
2006	Apple fruit at harvest	46/fruit	110	80.0	1.4
	Inside the bin at harvest	17809/cm^2	158	29.8	54.4
	Underside bottom of the bin		64	56.3	13.9
	Drench-tank water	498/ml	56	35.7	84.2

Baseline sensitivity of P. expansum to fludioxonil (Scholar) and pyrimethanil (Penbotec). We collected *P. expansum* isolates from apple-related sources across the state, and 120 isolates were selected for baseline sensitivity study. Sensitivities of these 120 isolates to thiabendazole, fludioxonil and pyrimethanil were determined, and the distribution of baseline sensitivity was established. The results were presented in a previous report to the Commission. In summary of the results, the current population of *P. expansum* is sensitive to both new fungicides. Of the 120 isolates, one is much less sensitive to fludioxonil, but it does not cause a practical resistance problem. The baseline sensitivity distribution will be used to monitor the shift in sensitivity of the pathogen to the fungicides.

Risk assessment of resistance to fludioxonil and pyrimethanil. We now have two new postharvest fungicides, Scholar (fludioxonil) and Penbotec (pyrimethanil). Questions we have been trying to address are what is the risk of development of resistance in *P. expansum* to fludioxonil and pyrimethanil and whether there is risk of cross-resistance or multi-drug resistance in *P. expansum* to the postharvest fungicides. This information would help us implement strategies of using postharvest fungicides. Our goal is to prolong the effectiveness of these fungicides and to avoid or delay the buildup of resistant populations of postharvest pathogens to these new fungicides.

We generated fludioxonil- and pyrimethanil-resistant mutants in the laboratory, used mutants to determine patterns of resistance of *P. expansum* to the three postharvest fungicides, determined fitness parameters of fungicide-resistant mutants, and evaluated whether current fungicides are able to control fungicide-resistant mutants. We made great progress in understanding the risk of resistance to fludioxonil and pyrimethanil. The findings are summarized as follows:

Fludioxonil-resistant mutants of *P. expansum* were highly resistant to fludioxonil but remained sensitive to pyrimethanil. However, pyrimethanil-resistant mutants were also resistant to fludioxonil. More importantly, pyrimethanil-resistant mutants derived from TBZ-S became resistant to TBZ. Six

phenotypes of fungicide resistance were detected, and all pyrimethanil-resistant mutants were triple resistant to all three postharvest fungicides (Table 5).

Fludioxonil-resistant mutants were less pathogenic to apple fruit and produced a much smaller amount of spores on inoculated apples, whereas in general there were no significant differences in virulence and sporulation on apple fruit between pyrimethanil-resistant mutants and the wild parental isolates (Fig. 1 and Fig. 2). Fludioxonil-resistant mutants also were sensitive to osmotic stress (data not shown).

None of the three postharvest fungicides were able to provide satisfactory control of pyrimethanil-resistant mutants on apple fruit at both 32 and 68ºF (Fig. 3).

Our results indicated that a fitness cost was associated with fludioxonil-resistant mutants in both saprophytic and pathogenic phases. Pyrimethanil has a high risk in the development of resistance in *Penicillium expansum*. Multiple resistance to all three postharvest fungicides could become a practical problem if *P. expansum* develops resistance to pyrimethanil. The findings from our study suggest that a program to monitor the shift in sensitivity of *P. expansum* to pyrimethanil and fludioxonil is needed and that strategies of using pre- and postharvest fungicides to avoid or delay the development of resistance to pyrimethanil need to be implemented.

Table 5. Phenotypes and resistance patterns of fungicide-resistant mutants of *Penicillium expansum* to the three postharvest fungicides.

Isolate[a]	Origin	Phenotypes[b]		
		Thiabendazole (TBZ)	Fludioxonil (Scholar)	Pyrimethanil (Penbotec)
W1	wild type	S	S	S
W2	wild type	HR	S	S
FR1	W1	S	HR	S
FR2	W1	S	HR	S
FR3	W2	HR	HR	S
FR4	W2	HR	HR	S
PR1	W1	LR	LR	R
PR2	W1	LR	LR	R
PR3	W2	HR	LR	R
PR4	W2	HR	LR	R

[a] FR=fludioxonil-resistant mutants; PR=pyrimethanil-resistant mutants

[b] S=sensitive; LR=lowly resistant; R=resistant; HR=highly resistant

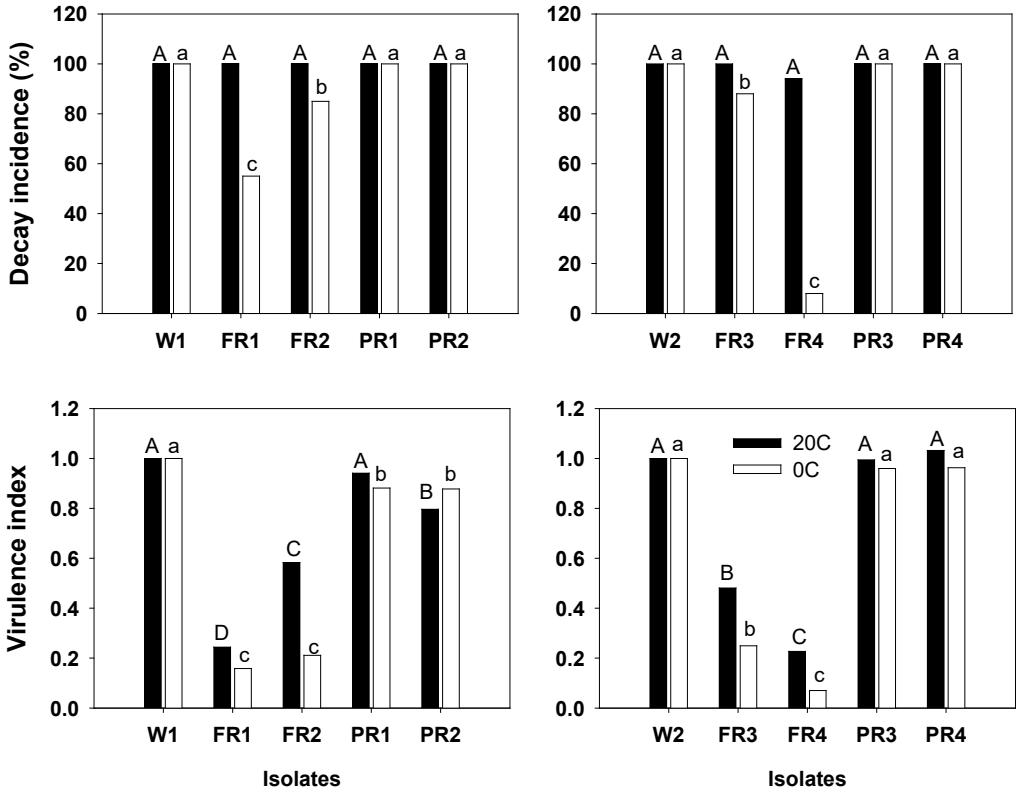

Fig. 1. Pathogenic fitness and virulence of fludioxonil-resistant mutants (FR1 to FR4) and pyrimethanil-resistant mutants (PR1 to PR4) and their wild parental isolates (W1 and W2) of *Penicillium expansum* on apple fruit.

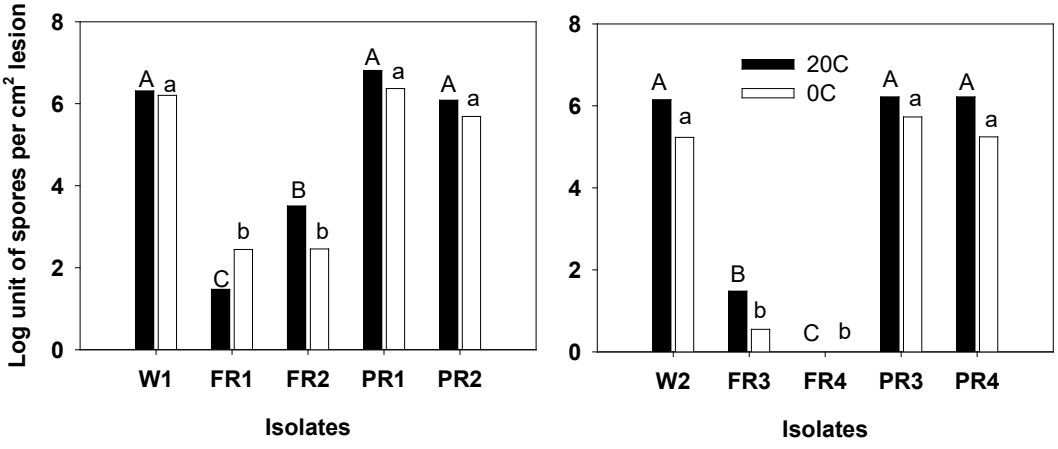

Fig. 2. Sporulation of fludioxonil-resistant mutants (FR1 to FR4) and pyrimethanil-resistant mutants (PR1 to PR4) and their wild parental isolates (W1 and W2) of *Penicillium expansum* on apple fruit.

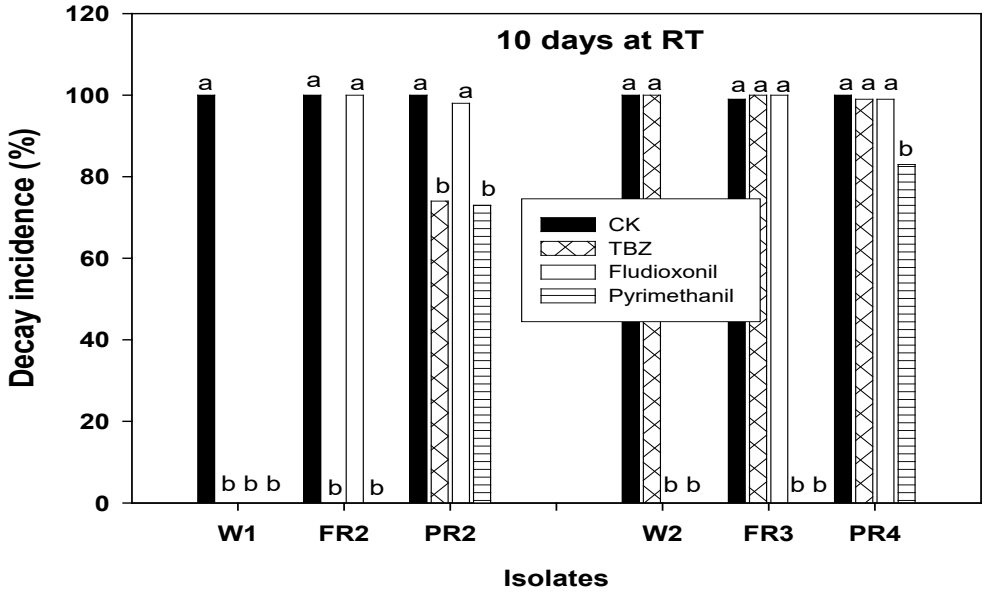

Fig. 3. Effectiveness of thiabendazole (Mertect), fludioxonil (Scholar) and pyrimethanil (Penbotec) for control of fludioxonil-resistant mutants (FR2 and FR3) and pyrimethanil-resistant mutants (PR2 and PR4) and their wild parental isolates (W1 and W2) of *Penicillium expansum* on apple fruit.

New technologies for decay control

Biofumigant fungus Muscodor. In 2004-05, we evaluated biofumigation with the Muscodor fungus for control of postharvest blue mold and gray mold. Results were presented in the 2005 July report. Biofumigation with the Muscodor fungus at both rates significantly reduced gray mold compared with the *Botrytis*-inoculated control. However, fumigation with Muscodor fungus for seven days at 37ºF did not provide satisfactory control of blue mold, though both incidence and severity of blue mold were significantly reduced by the biofumigation in comparison with the *Penicillium*-inoculated control. In preliminary experiments, we observed phytotoxicity problems (lenticel browning) on Gala apples that were fumigated with Muscodor at high rates (46 and 100 g/box) at 37ºF for 10 days. The effort was discontinued because the company claimed that the inoculum of the biocontrol fungus used in 2005 had some problems. The company is trying to improve the formulation of the biofumigant inoculum.

Thermofogging. On 2005 and 2006 crops, we conducted thermofogging trials to evaluate the efficacy of fogging fungicides for control of postharvest diseases. The trials were conducted on organic Red Delicious. The 2006 trial is still in progress. The results from the 2005 trial are summarized here.

In the commercial situation, a fungicide treatment applied to the fruit in a storage room may be delayed for 1-3 days after harvest. In 2005, the first experiment was to look at the kick-back activity of pyrimethanil applied by thermofogging. After harvest, apple fruit were inoculated with either Botrytis or Penicillium, and part of the fruit received the thermofogging treatment with pyrimethanil at 0, 1, 2, and 3 days after inoculation. Delay of the thermofogging treatment significantly compromised the effectiveness of the treatment, particularly for blue mold control (Fig. 4). However,

the sizes of decay on treated fruit were much smaller than those on decayed fruit from the nontreated control (data not shown).

The second trial we did in 2005 was to look at the effectiveness of thermofogging pyrimethanil for control of decays on commercially harvested fruit. Eight bins of fruit from a commercial organic orchard were treated and 8 not treated. Fruit were stored in CA. Decay was evaluated at seven months after harvest. The thermofogging treatment significantly reduced the total decay in storage bins as well as gray mold and blue mold (Table 6). However, the level of decay resulting from natural infections in that year was low (1.1%).

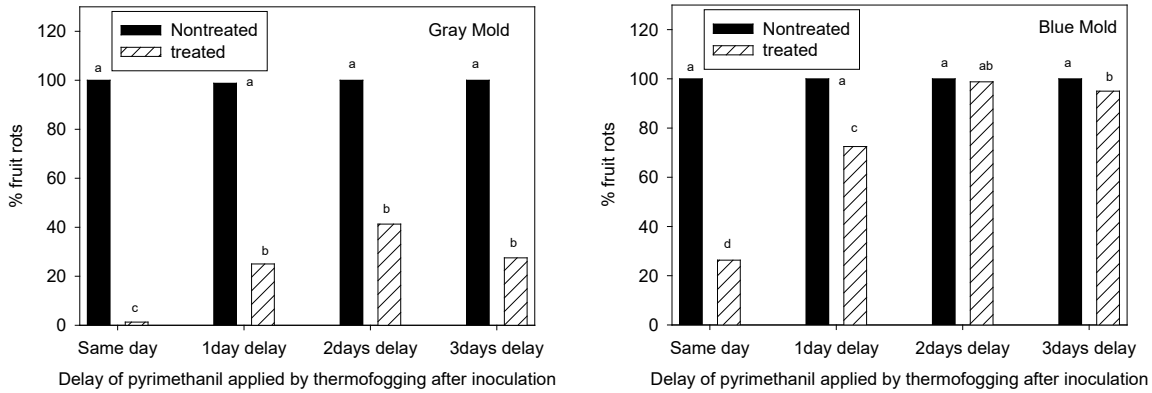

Fig. 4. Effects of delay in application of thermofogging treatment on gray mold and blue mold resulting from infections of wounds. Apple fruit were inoculated with the pathogens at harvest, and part of the fruit was thermofogged with pyrimethanil at 0-3 days after inoculation.

Table 6. Effectiveness of thermofogging pyrimethanil for control of postharvest diseases in Red Delicious apples.

Treatment	Total decay in the bins (%)	Gray mold in the bins (%)	Blue mold in the bins (%)
Nontreated	1.11 a	0.60 a	0.31 a
Thermofogged	0.28 b	0.14 b	0.03 b

FINAL PROJECT REPORT
WTFRC Project Number: PH-05-507

Project Title: Development of an auxiliary cold storage component

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Budget History:

Item	Year 1: 2006
Salaries	0
Benefits	0
Wages	19,700
Benefits	1,970
Equipment	0
Supplies	230
Travel	0
Insects	1,300
Fruits	1,200
Miscellaneous	0
Total	24,400

Objectives:

1. Measure mortality of codling moth larvae at three different physiological stages (non-diapause feeding late instars, diapause-destined feeding late instasrs, and diapausing mature larvae) durations of regular air cold storage at 1.1 °C.

2. Develop mathematical models that describe mortality of physiological stage.

Significant findings:

- All non-diapausing larvae were dead within twelve weeks.
- Diaspause-destined feeding larvae were within seven weeks.
- The survival rates between these two stages were not statistically different and the best descriptive mathematical model was: $\ln y = 4.5719 + -0.1253x^{1.5}$, $r^2 = 0.9967$, where y is the percent live and x is the week in storage.
- At least a half of the diapausing larvae survived after eleven weeks, although there was a slight trend towards decreasing survival with cold storage time.
- Diapause occurs outside the fruit and is the mechanism for overwintering.

Introduction:

Taiwan is the third largest importer of United States (U. S.) fresh apples (Taipei Times, 2005). The apples are primarily from Washington and California (The Fruit Growers News, 2002), with 'Fuji' as the predominant cultivar, comprising 80% of those from Washington (Jimenez, 2004). After over 25 years of apple exports to Taiwan, live codling moth larvae were found for the first time in 2002, leading to a temporary ban on U. S. apples (The Fruit Growers News, 2002). To resume exports, the U. S. agreed to increase the numbers of apples inspected and, if live codling moth larvae were found in three consignments, all apple imports from the U. S. would cease (Taipei Times, 2005).

The Systems Approach (SA) is being expanded to meet these increasingly severe phytosanitary regulations for apples. The SA involves the cumulative effect of commercial operations to reduce the risk of possible pest infestation, followed with validation by intense inspection. One area that can be exploited is the cold storage component. Cold storage is already used against the apple maggot [*Rhagoletis pomonella* (Diptera: Tephritidae)] (Hallman, 2004) and the oriental fruit moth [*Cydia molesta* (Lepidoptera: Tortricidae)] (Hansen, 2002) for apples exported to Mexico. A better understanding of the impact of cold storage on codling moth larvae would strengthen this component and improve the overall utility of the SA.

Cold storage (55 d at 2.2 °C or 36 °F) for control of codling moth eggs is a component of the current quarantine treatment against codling moth for apples exported to Japan (Hansen et al., 2000). Thus, 1.1 °C (or 34 °F), the temperature 'Fuji' apples are frequently stored, may also be effective against codling moth larvae. Toba and Moffitt (1991) reported no survivors among 142,000 codling moth larvae after 13 weeks at 1.5 to 2.0% O_2 and < 1% CO_2 and held at 0 °C. However, they based the efficacy of their study on the lack of adult emergence rather than larval mortality.

Furthermore, the insecticidal effect of cold storage may vary due to the physiological condition of the codling moth larvae at the time of harvest when they are undergoing preconditioning for diapause, an inactive state which allows the larvae to overwinter within their cocoons (Newcomer and Whitcomb, 1924). Diapausing larvae do not feed and are freeze-tolerant (Brown, 1991). Cold exposures may be less effective against diapausing destined larvae, but no studies have been done to determine the effect of commercial cold storage, if any. Thus, base line information for all three physiological stages are necessary to understand the cold storage component.

Here we measured larval survival for cumulative durations of regular air (RA) at 1.1 °C, the temperature used for cold storage of 'Fuji' apples, the major export variety. Feeding larvae of both diapaused-destined and nondiapausing were examined separately. Mathematical models were developed to describe the mortality rates at cold temperature exposures and durations for complete control were calculated.

Materials and methods:

The treatments were conducted in a refrigerated room at the USDA-ARS- Yakima Agricultural Research Laboratory (USDA-ARS-YARL) in Wapato, Washington. For each time-temperature exposure, 50 'Fuji' apples were infested by hand with four larvae each, and then held overnight to allow for fruit penetration before placed in cold storage (1.1 °C). Treatment exposures were: 0 (control), 3, 5, 7, 9, 11, 12, 13, 14, 15, 16, 17, and 18 weeks. The shortest period, 3 weeks, represented the time for transoceanic shipment. Weekly exposures were necessary for the long time periods because treatment exposures ceased when no survivors were observed in two successive weeks.

Three distinct groups of larvae were examined for each cold storage exposures: feeding non-diapausing, feeding diapause destined, and non-feeding diapausing. All were from the rearing colony at USDA-ARS-YARL (Hansen and Anderson, 2006). Diapause destined larvae were induced by altering the rearing conditions, which included lowering the ambient temperatures from the normal regime of 25.5 ± 1.5 °C to 16.5 ± 0.5 °C and reducing the photoperiod from 16 h light: 8 h dark to 8 h light: 16 h dark (Hansen and Anderson, 2006). Feeding diapause destined were collected after three weeks and infested the apples as fourth instars (larval stage after three molts). Diapausing larvae were obtained as fifth instars after five weeks under these temperature and photoperiod conditions. Nondiapausing feeding larvae were acquired in the third week of normal rearing conditions and these were used to infest apples as fifth instars. Late instars were used for evaluating the three physiological stages because cold tolerance increases with larval age (Yokoyama and Miller, 1989).

For each treatment observation, infested fruits were removed from cold storage and held overnight at room temperature (≃20 °C), dissected the following day to procure the larvae, and viability determined. Any larval movement indicated survival. There were four replicates for the non-diapausing larvae, three replicates for the diapause-destined larvae, and two replicates for diapausing larvae.

Data were summarized by using Microsoft® Excel 2002 Spreadsheet (Microsoft Corp., Redmond WA). Data were analyzed by using PROC TTEST with SAS® (Release 6.12, SAS Institute, Cary, NC). Mathematic models describing larval mortality from cold exposures were developed from TableCurve 2D V. 5.01 for Windows (SYSTAT Software Inc., Richmond, CA).

Results;

Both non-diapausing and diapause-destined codling moth larvae were susceptible to cold storage (Table 1). All non-diapausing larvae died by the twelfth week whereas all diapause-destined were dead in the seventh week. The diapause-destined were younger and may have been less tolerant to the cold. Although statistically significant, the average difference in the initial infestation rate (Week 0) between the two groups was less than 2%. There were no further statistical differences in the weekly survival between these types of larvae.

The best simple mathematical model that described mortality in non-diapausing larvae with cold storage was

$$1/y = 0.0104 + 0.00003x^3,\ r^2 = 0.9985,$$

where y is the percent live and x is the week in storage. However, the residuals of this model showed that it consistently over estimated mortality after seven weeks in cold. The simple model that had the best predictions for the long term was

$$\ln y = 4.5719 + -0.1253x^{1.5},\ r^2 = 0.9967,$$

which had the same variables. It was inconsistent with the early storage times (before Week 7), but became more accurate with time. This should be a good predictor of codling moth mortality from cold storage.

Many of the diapausing larvae failed to infest the fruits and stayed outside of the apples (Table 2). Their survivorship slowly decreased during cold storage so that 66.5% were alive by the eleventh week. Larval mortality within fruits remained stable. This test was discontinued because of the high survival rate.

Discussion:

Although the cold storage killed all feeding larvae by the seventh week of cold storage, survival of diapausing larvae was anticipated. To complete the diapause stage in nature, mature fifth instars leave the fruit and quickly seek sites to spin cocoons, such as in bark or on debris lying on the ground (Putman, 1963; Simpson, 1903). By definition, feeding larvae are not diapausing larvae. In our study, we artificially tried to infest fruits with apparent diapausing larvae, but only succeeded with ~ 25% of them, which probably were in transition from feeding non-diapause to diapause and chewed into apples because of the lack of suitable cocoon sites. The literature reports no incidence of codling moth larvae diapausing within the feeding tunnels inside fruits. Similarly, because diapause allows the larvae to overwinter, even at below freezing temperatures, we expected the larvae to survive our cold storage treatment. A slight mortality dose response was observed over time, but these larvae were not in well protected sites like they would have found in orchards. However, Garlick (1948) reported the annual winter mortality of diapausing larvae in an insectary to range between 16 to 36% over a five year period. For feeding larvae, Yokoyama and Miller (1989) observed that older instars were more tolerant to 0 C° than younger instars, although mean survival for fifth instars was still 67.2% after three weeks, a value similar to our observations (Table 1).

The SA is a practical method for insect control. Jang and Moffitt (1994) described the SA as the integration of commercial practices used in production, harvest, packing, and distribution which cumulatively meet the requirements for quarantine security. Each component of the SA need not be efficacious by itself, but that the effect is additive, so that even with variability among the components, the entire process still results in either complete efficacy (100% mortality) or low likelihood of a mating pair (Landolt et al., 1984). Acceptance of the SA requires a change in institutional philosophy. Instead of using probit-9 (99.9968% mortality per 1 million pest individuals) as the standard for quarantine security, the cumulative effect of many components result in effective quarantine security.

The SA to quarantine security has the greatest promise in maintaining fresh fruit and vegetable exports in the face of increasing phytosanitary barriers around the world. This procedure is now used by the vast majority of importing countries for codling moth and other pests in Northwest tree fruits. The SA has been used to export citrus fruits to Japan from fruit fly-free zones in Florida (Simpson, 1993) and Mexican avocados (*Persea americana*) into northeastern U. S. (Animal and Plant Health Inspection Service, 1997). Deciduous tree fruits from the Pacific northwest U. S. are other likely commodities. Thus, high quality products can be distributed to foreign lands without fear of spreading quarantine pests.

The SA for meeting quarantine requirements for codling moth is based on: insect pest control measures in the orchard before harvest in order to eliminate pests in harvested fruit; initial inspection of the fruit upon arrival at the packinghouse; postharvest grading, sorting, and packing procedures, with emphasis upon removal of insect-infested or damaged fruit; and inspection and certification of packed fruit (Moffitt, 1989; Jang and Moffitt, 1994). A high degree of quarantine security can be provided for codling moth on apples, sweet cherries (*Prunus avium*), and nectarines (*Prunus persica*) using such a system (Curtis et al., 1991; Moffitt, 1989; Vail et al., 1993). Sorting and culling along the packing line have been effective in removing cherries infested by surface pests (Hansen et al., 2003b), apples infested by codling moth (Hansen and Schievelbein, 2002; Knight and Moffitt, 1991;), and surface pests from apples (Hansen et al., 2003a).

Previous studies indicate that cold temperatures can be efficacious against codling moth larvae. Morgan et al. (1974) reported > 67.5% mortality in codling moth larvae infesting 'Delicious' apples when held 5 weeks at 0.5 °C RA. Moffitt and Burditt (1989) killed > 35,000 codling moth eggs at red-ring stage after 55 d at $\leq$ 2.2 °C in RA. Moffitt and Albano (1972) reported an increase in codling moth mortality after 60 days for CA (or "controlled atmosphere" where CO_2 ranges between 0.8 to 1.6% and O_2 ranges between 2.2 and 3.0% [Moffitt, 1971]) over RA cold storage. Knight and Moffitt (1991) found only dead larvae after CA and RA cold storage. Simmons and Hansen (1999) found > 97% mortality of third and fourth instar codling moth after four weeks in cherries packed with 10% O_2 and 2% CO_2 and held between 1.0 and 2.5 °C. Toba and Moffitt (1991) observed no adult emergence among 40,000 fifth instar codling moth when held in a commercial CA (1.5 to 2.0% O_2, < 1% CO_2) after 13 weeks at 0 °C. Other apple pests that have been controlled by cold temperatures are: the oriental fruit moth (Hansen, 2002); mealybugs [*Pseudococcus affinis* (Homoptera: Pseudococcidae)] (Hoy and Whiting, 1997); apple maggot (Glass et al., 1961); and plum curculio [*Conotrachelus scutellaris* (Coleoptera: Curculionidae)] (Glass et al., 1961).

Our data show that feeding codling moth larvae can be controlled by cold storage at commercial temperatures used for 'Fuji' apples. In addition to field pest management programs to reduce codling moth populations, verified for intense inspection, cold storage can be used as a supplemental component to the SA for codling moth in apples. Young larvae that may escape detection can still be controlled within seven weeks of cold storage at 1.1 °C. Even at five weeks, the probability of larval survival is reduced to less than 20%. Hence, the marketability of 'Fuji' apples to Taiwan can be sustained with the option of using short term cold storage.

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Table 1. Comparisons by Student's *t* test of the weekly percent (mean ± SE) live codling moth larvae between non-diapausing (four replicates per treatment) and diapaused destined (three replicates per treatment) in 'Fuji' apples held at 1.1 °C for 13 weeks.

	% Live		
Week	Non-diapause	Diapause destined	*t*
0	95.7 ± 0.3	97.6 ± 0.1	4.53**
3	53.6 ± 11.4	67.7 ± 7.4	0.94
5	19.9 ± 5.3	12.6 ± 0.6	1.16
7	9.1 ± 4.4	0	2.08
9	2.7 ± 1.8	0	1.55
11	3 ± 0.3		
12	0		
13	0		

Initial infestation was at four larvae/fruit with 50 fruits per replicate.
** Significant at $P < 0.01$.

Table 2. Weekly percent (mean ± SE) of live diapausing codling moth larvae in 'Fuji' Apples and all found within the container holding the fruits at 1.1 °C for 11 weeks.

	% Live	
Week	In fruit	Total
0	28.6 ± 3.3	98.5 ± 1.5
3	20.7 ± 2.4	84.2 ± 4.9
5	25.0 ± 1.1	88.5 ± 1.0
7	29.0 ± 5.4	81.9 ± 1.3
9	29.4 ± 6.3	74.8 ± 10.0
11	22.7 ± 3.8	66.5 ± 4.1

Initial infestation is at four larvae/fruit with 50 fruits/replicate and two replicates per treatment.

FINAL PROJECT REPORT
WTFRC Project Number: PH05-506

Project Title: Acetic Acid Vapors to Decontaminate Bins & Storage Rooms

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Co-operators: Okanagan Similkameen Cooperative Growers Association, Terry Zeller
BC Fruit Packers, Dan Worley

Budget History:

Item	**Year 1:** 2004-05	**Year 2:** 2005-06	**Year 3:** N/A
Salaries	19,500	19,500	
Benefits			
Wages			
Benefits	2,925	2,925	
Equipment	775	775	
Supplies	600	600	
Travel	700	700	
Miscellaneous	500	500	
Total	25,000	25,000	

Objectives: (Year one)

1. Determine if acetic acid vapors would be effective in eliminating the various post harvest pathogens (*Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata*) from wooden bins and various surfaces in cold storage rooms.
2. Use acetic acid vapors as a phytosanitary treatment to eliminate *Erwinia amylovora* from the surface of harvested mature apples.
3. Corrosion study of various metals to see the effect of acetic acid vapors on them at various rates, duration of application, and the effect of washing exposed metals following fumigation.
4. Compare the effect of acetic acid vapors to commercial products (i.e. chlorine wash, Storox (Pace International)) to decontaminate bins and storage rooms.

Significant findings: (Year One)

1. A rate of 6 mg/l or higher of acetic acid is effective in eliminating post harvest pathogens from the surface of bins and storage room surfaces.
2. Acetic acid can reduce *Erwinia amylovora* from the surface of harvested mature apples. Unfortunately, the rates required also produces phytotoxic response in the fruit.
3. Acetic Acid is corrosive on copper when the relative humidity is above 75%. The corrosive effects can be reduced by rinsing the exposed metal with water, immediately after fumigation and maintaining the relative humidity below 70%.
4. Acetic acid is comparable to Storox for sterilizing CA rooms, but acetic acid is more effective in sterilizing bins.

Objectives: (Year Two)

1. Survey of the contamination levels of *Penicillium* spp. on the floors and walls of cold storage rooms in British Columbia.
2. Fumigate small (100 m^3) to medium (2700 m^3) sized cold storage rooms using specially developed techniques to assess efficacy on several room types.
3. Investigate the possibility of using steam or high pressure hot water to sanitize cold storage rooms.
4. Investigate the sanitation of bins with acetic acid to control *Penicillium* spp. and codling moth.
5. Investigate the relationship between relative humidity and effectiveness of acetic acid on pathogens and corrosion.

Significant findings: (Year Two)

1. The CA room survey showed a variable level of *Penicillium* spp spores depending on the composition of the wall, plywood and fire retardant fibre walls had the highest level of *Penicillium* spp. contamination.
2. Acetic acid treatment of CA rooms was effective in reducing the amount of *Penicillium* spp. present on the walls to zero.
3. Steam/pressure washing of pallets was very effective in reducing the amount of *Penicillium* spp.
4. Acetic acid is an effective bin sanitation material, averaging 72 to 100% reduction in *Penicillium* spp. on bin surfaces.
5. Acetic acid is effective in killing codling moth larvae and pupa but only at a rate of 40 mg/l and if the larvae were in plastic containers.
6. Relative humidity must be above 50% for acetic acid to be effective in reducing the pathogen spore load and below, 65% to minimize the corrosion in CA rooms and bins.

Methods / Results/ Discussion (Year Two)

Objective 1. (Survey of wall contamination in CA storage)

Methods Wall swab samples were taken in various CA rooms from 2 packing houses in the North Okanagan (Table 1) and 5 packing houses in the South Okanagan (Table 2) between March and June 2005. The walls of these CA rooms were composed of different materials ranging from galvanized metal, painted plywood, styrofoam, concrete foam, white or grey fire retardant mineral fibre (white/grey FRF). Samples were taken by dipping a sterile swab into a sterile tube containing 10 ml of sterile distilled water (SDW) and swabbing over an area of 10 cm^2 by using a template. For a single wall area, 5 swabs were taken across the wall and placed into the same tube. For the walls covered with the white or grey fire retardant fibre, 5 sample areas were collected from across the wall by removing a 2 cm^2 area each time and placing it into a sterile tube. Each tube containing the swabs was vortexed for 30 seconds before plating. Aliquots of 100 µl were then pipetted on to 100 ml Petri plates containing lactic acid potato dextrose agar (LPDA). This was repeated three times for each sample. The plates were incubated at 20°C for 5 days or until the fungal colonies could be identified and counted.

Results

Table 1. Results of wall contamination survey in North Okanagan in 2005.

Packing House CA Rooms in North Okanagan (*Penicillium* spp. only)							
Location	Room	Material	CFUs/cm^2	Location	Room	Material	CFUs/cm^2
Roanoke	61	Plywood	1.1	Winfield	510	Plywood	188
	82	Plywood 04	0		511	Plywood	17,556
	83	Plywood 00	1,113		520	Plywood	1.5
	85	Plywood 00	1,112		508	Plywood	2
	64	Styrofoam	0.5		510	Concrete F	45
	72	Stainless	0.5		511	Concrete F	82
	61	White FRF	3,083		520	Concrete F	261
	63	White FRF	45,083		508	Concrete F	3
	83	White FRF	32,083		525	White FRF	30
	61	White FRF	50		526	White FRF	290
	85	Grey FRF	92				

Table 2. Results of wall contamination survey in South Okanagan in 2005.

Packing House CA Rooms in South Okanagan (Penicillium spp only)							
Location	Room	Material	CFUs/cm^2	Location	Room	Material	CFUs/cm^2
Summerland	232	Plywood	7.6	Oliver	115	Plywood	28
	234	White FRF	503		107	Plywood	1.3
Naramata	253	Plywood	0.2		118	White FRF	9.3
	258	Plywood	0.1	Osoyoos	144	Plywood	0.1
	259	Plywood	3		144	White FRF	2,534
	258	White FRF	77		149	White FRF	257
	259	White FRF	276	Keremeos	129	Plywood	1.5
	253	Concrete F	14.4		129	Concrete F	29.1
					125	Plywood	1.0
					125	Concrete F	2,145

Discussion

Various fungal spores were identified in this study, *Penicillium* spp,, *Mucor*, *Alternari*a, and some yeasts. On most of the surfaces checked in this survey, only *Penicillium* spp. was found, which is the most important pathogen of stored apples. The level of *Penicillium* spp. on the various surfaces ranged from 0 to 45,000 colony forming units (CFUs)/cm^2 (Table 1 & 2).

Objective 2. (Fumigation of storage rooms)

Methods All surfaces were swabbed, as described in Objective 1above, before and after fumigation. In Keremeos, BC, the CA room air was sampled with a Burkard portable air sampler (Burkard Manufacturing Co Ltd, Rickmansworth , England). A 100 ml Petri plates containing exactly 27 mls of acidified PDA (LPDA) was used to collect the air contaminants and the sampler was run for 1 minute. This device passes 20 litres /min of air across the plate. There were 3 replicated samples per room.

Fumigation Method Once the FRED (Fumigation, Rapid Evaporation Device) was placed in the CA room, relative humidity (RH) was adjusted by evaporating the amount of distilled water (DW) required to bring the room RH up to a minimum of 50% to a maximum of 65%. Temperature was adjusted by the addition of heat provided by small room heaters, ceiling lights and fans. Temperature was raised to a minimum of 19 ^{0}C (66.2^{o}F) to 26 ^{0}C (78.9^{o}F). The relative humidity, temperature, and the amount of acetic acid used per room is shown in table 3. Once the room RH and temperature was established, the acetic acid was added to the FRED. The room was sealed and locked to prevent entry and the FRED turned on. The acetic acid was evaporated in approximately 45 minutes. The level of acetic acid was monitored with a Gas Chromatograph. The room remained sealed overnight and the room vented in the morning. When it was safe to enter, the corrosion blocks were checked for possible corrosion and the defrost cycle on the cooling system was run to remove any acetic acid residue.

Table 3. Date, location, CA room size used is this study.

Date fumigated	Location- room	Room Size m^3 / ft^3	Bin Capacity	Temp ^{o}C/^{o}F	RH	Acetic Acid added	
						litres	mg/l
17 Aug 05	Kelowna - 61	2267 / 80,040	1522	25.5 / 77.9	64%	20	8.8
15 Sep 05	Keremeos - 129	1768 / 62,500	1260	19.9 / 67.8	59%	20	11.3
06 Jun 06	Kelowna - 85	2720 / 96,050	1876	19.7 / 67.5	53%	21	7.7
15 Aug 06	Kelowna - 63	2720 / 96,050	1876	23.0 / 73.4	64%	20	7.4
22 Aug 06	Keremeos - 125	1768 / 62,500	1260	22.0 / 71.6	54%	20	11.3

Results

Table 4. BC Fruit Packers (Roanoke CA Rooms, Kelowna, BC)

Room- Composition	Penicillium spp CFUs/cm^2		% Reduction
	Before fumigation	After fumigation	
61 Plywood	0.9 ± 0.1	0.4 ± 0.2	55.6
61 Plywood	0.6 ± 0.2	0.3 ± 0.1	50
61 White Fire Retardant Fibre (WFRF)	13,783 ± 3,271	3.3 ± 5.8	99.9
61 Grey Fire Retardant Fibre (GFRF)	16.7 ± 10.4	10 ± 0.0	40
61 Floor	1.7 ± 1.0	0.1 ± 0.1	94
85 Plywood	1093 ± 136	18.4 ± 1.9	98
85 Plywood	63 ± 30	12 ± 0.9	80
85 Gray Fire Retardant Fibre (GFRF)	10 ± 0.0	0.0 ± 0.0	100
85 Gray Fire Retardant Fibre (GFRF)	11.7 ± 3.0	0.0 ± 0.0	100
85 Floor	941 ± 86	7.0 ± 2.3	99
63 Galvanized metal	0.9 ± 0.6	0.2 ± 0.0	78
63 Galvanized metal	1.1 ± 0.3	0.1 ± 0.1	91
63 White Fire Retardant Fibre (WFRF)	3,912 ± 1,330	0.0 ± 0.0	100
63 White Fire Retardant Fibre (WFRF)	4,692 ± 1,976	0.0 ± 0.0	100
63 Floor	5 ± 0.8	0.0 ± 0.0	100
63 Over Head Pipes	509,000 ± 74,800	0.0 ± 0.0	100

Table 5. Okanagan Similkameen Cooperative Growers Association (Keremeos)

Room/wall composition	*Penicillium* spp., CFUs/cm^2		% Reduction
	Before fumigation	After fumigation	
129 Plywood	0.7 ± 0.3	0.0 ± 0.0	100
129 Plywood	2.3 ± 0.5	0.0 ± 0.0	100
129 White Fire Retardant Fibre (WFRF)	14.1 ± 1.7	0.0 ± 0.0	100
129 White Fire Retardant Fibre (WFRF)	33.5 ± 2.7	0.0 ± 0.0	100
129 Air Sample (per 20 l of air)	1.3 ± 0.6	0.7 ± 1.2	50
125 Plywood	0.9 ± 0.5	0.0 ± 0.0	100
125 Plywood	1.1 ± 0.1	0.0 ± 0.0	100
125 White Fire Retardant Fibre (WFRF)	4,260 ± 191	0.0 ± 0.0	100
125 White Fire Retardant Fibre (WFRF)	30 ± 5.0	0.0 ± 0.0	100
125 Floor	2.0 ± 0.3	0.0 ± 0.0	100
125 Air Sample (per 20 l of air)	24 ± 1.5	0.3 ± 0.6	98.8

Discussion

For the CA room treatment in Kelowna, fumigation was most effective on the white/grey fire retardant fibre (W/GFRF). Acetic acid reduced the contamination from 14,500 *Penicillium* spp. CFUs/cm^2 to zero, and the overhead pipes from over 500,000 spores/cm^2 to zero. (Table 4).

For the rooms at Keremeos, BC, the *Penicillium* spp. was reduced by 100% on all surfaces (Table 5). The spore load in the air was reduced to less than 1 spore per 20 litres of air. For the five CA rooms treated, the overall effect was a reduction of *Penicillium* spp. on all surfaces especially those areas that would be hard to clean. The corrosion blocks showed no sign of corrosion.

Objective 3. (Steam pressure washer for sanitizing bins and CA rooms)

Methods

A Hotsy model 555SS hot-water pressure washer (Englewood, Colorado, USA) was used to surface sanitize a small (8.4 m^3/295 ft^3) cold storage room. The unit provides 2.2 gph (8.33lpm) heated water up to 100^{0}C at 1300 psi (89.66 bars).

All surfaces were swabbed as in the method discussed for Objective 1.

Results/Discussion

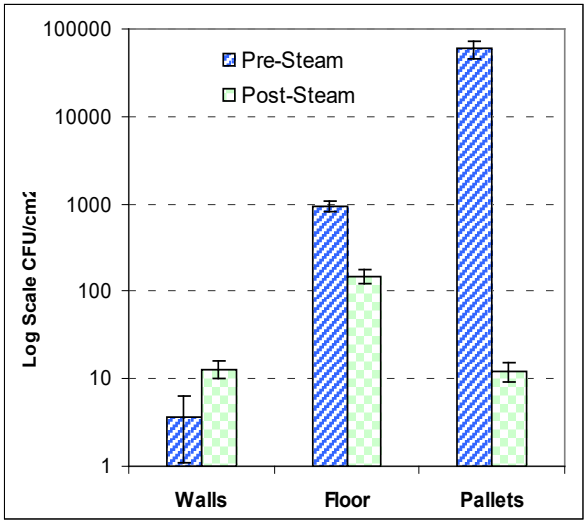

Figure 1. *Penicillium* spp. CFUs per cm^2 before and after steam treatment

Table 6. CFUs per cm^2 before and after steam treatment and percent reduction.

	Pre-Steam	Post-Steam	% reduction
Walls	3.7	13	0
Floor	944	150	84
Pallet	59,333	12	99.98

Figure 1 and table 6 show the results of the steam treatment. The steam-pressure washer treatment was effective in reducing the spore load on the floors (84% reduction) and most effective on the pallets (99.98% reduction). The walls showed an increase and this is due to low initial counts and possible splashing of spores from the floor during the steam/heat treatment.

A disadvantage of using this system for cleaning a cold room is the amount of time required to clean the room and the amount of water needed (2.2Gph). For the cleaning of pallets or bins, steam/pressure washing would be ideal for the most contaminated ones.

Objective 4. (Acetic acid fumigation of bins and codling moths)

Methods -Bin Fumigation

Stacks of 3 bins were placed randomly throughout a CA room. The various sides of the bins within the stack were swabbed before and after fumigation as described above. This included the bins inside of the stack to test whether acetic acid vapours would penetrate into the stacked bins at a sufficient concentration to sanitize the bin surface. These fumigations were conducted in conjunction with the room fumigations mentioned in Objective 2.

Methods - Codling Moth Fumigations.

Trays of codling moths were obtained from Sterile Insect Release (SIR) program, Osoyoos, BC. The larvae were raised on the standard diet and were at the 4^{th} or 5^{th} instar. Fifty larvae per treatment were removed from the diet and placed into wooden blocks or 1 ounce plastic solo cups. The larvae were allowed to spin up overnight. The next day the blocks/cup and 8 to 10 pupae were placed into the 23 litre fumigation chambers and treated with different levels of acetic acid vapours. Additional acetic acid was added during the length of the fumigation to maintain a minimal level of acetic acid vapours in the air.

Results/Discussion (Bin fumigations)

Table 7. Results of the bin fumigations in Kelowna BC.

Room/Bin/position swabbed	*Penicillium* spp. CFUs/cm^2		% Reduction
	Before Fumigation	After Fumigation	
61 Bin 1 Inside	51± 2.7	0.6 ± 0.4	98.8
61 Bin 1 Outside	2.5 ± 0.4	1.1 ± 0.9	56
61 Bin 2a Outside Top	0 ± 0.0	0.2 ± 0.2	0
61 Bin 2b Inside Top	115 ± 8.3	0.9 ± 0.2	99
61 Bin 2c Outside Middle	50 ± 10	19.2 ± 16.7	61.6
61 Bin 2d Inside Middle	120 ± 10	0.3 ± 0.3	99.8
61 Bin 2e Inside Bottom	28 ± 3	17.2 ± 3.3	38.6
61 Bin 2f Outside Bottom	28 ± 2.5	0.1 ± 0.2	99.6
61 Bin 3a Inside	3.4 ± 1.7	0.0 ± 0.0	100
61 Bin 4a Inside	21 ± 3	0.0 ± 0.0	100
61 Bin 4b Outside	0.1 ± 0.3	0.0 ± 0.0	100
61 Bin 10 Inside	22 ± 11	0.7 ± 0.0	96.8
61 Bin 10 Outside	1.9 ± 0.4	0.3 ± 0.0	84.2
61 Bin 11 Inside	50 ± 20	0.1 ± 0.2	99.8
61 Bin 11 Outside	0.3 ± 0.3	0.3 ± 0.3	0
61 Bin 12 Outside	1.4 ± 1.4	0.1 ± 0.2	92.9
61 Fibreglass Box 1 Inside	25.3 ± 2.3	0.3 ± 0.3	98.8
61 Fibreglass Box 2 Inside	10.7 ± 4.7	0.2 ± 0.3	98.1
85 Bin 1	19 ± 5	0.2 ± 0.2	98.9
85 Bin 2a	69 ± 2	26 ± 1.7	63
85 Bin 2b	8 ± 5	24 ± 2.2	0
85 Bin 2c	16 ± 1.5	12 ± 1.2	23
85 Bin 3	4 ± 3.4	0.0 ± 0.0	100
85 Bin 4	1.5 ± 0.0	0.0 ± 0.0	100
85 Bin 5	151 ± 11	0.0 ± 0.0	100
85 Bin 5a	23 ± 7	13 ± 2.7	45
85 Bin 5b Inside	16 ± 13	13 ± 0.9	17
85 Bin 5b Outside	4.4 ± 5	5 ± 0.2	0
85 Bin 6	31 ± 5	0.0 ± 0.0	100
63 Bin 1a Outside	1.9 ± 0.8	0.0 ± 0.0	100
63 Bin 1b Inside	8.9 ± 4.1	0.0 ± 0.0	100
63 Bin 2a Outside	1.7 ± 0.9	0.0 ± 0.0	100
63 Bin 2b Inside	4.3 ± 0.0	0.0 ± 0.0	100
63 Bin 3a Outside	2.8 ± 1.3	0.2 ± 0.4	93
63 Bin 3b Inside	28.2 ± 0.8	0.0 ± 0.0	100

Table 8. Results of the Bin fumigations at Keremeos, BC.

Room/Bin/position swabbed	*Penicillium* spp. CFUs/cm^2		% Reduction
	Before Fumigation	After Fumigation	
129 Bin 1a Outside	0.2 ± 0.2	0.0 ± 0.0	100
129 Bin 1b Outside	0.6 ± 0.2	0.0 ± 0.0	100
129 Bin 2a Outside	16.1 ± 1.6	0.0 ± 0.0	100
129 Bin 3a Inside	8.8 ± 0.8	0.0 ± 0.0	100
129 Bin 3b Outside	1.6 ± 1.6	0.0 ± 0.0	100
129 Bin 4a Outside	1.3 ± 0.7	0.0 ± 0.0	100
129 bin 4b Inside	10.9 ± 0.8	0.0 ± 0.0	100
129 Bin 5 Inside	51.4 ± 10.9	0.0 ± 0.0	100
129 Bin 6 Inside	6.8 ± 0.5	0.2 ± 0.2	97
125 Bin 1a Outside	1.9 ± 0.2	0.0 ± 0.0	100
125 Bin 1b Inside	17.6 ± 1.2	0.0 ± 0.0	100
125 Bin 2a Outside	0.8 ± 0.8	0.0 ± 0.0	100
125 Bin 2b Inside	3.4 ± 0.2	0.0 ± 0.0	100
125 Bin 3a Outside	2.7 ± 0.7	0.0 ± 0.0	100
125 Bin 3b Inside	12.2 ± 0.4	0.0 ± 0.0	100

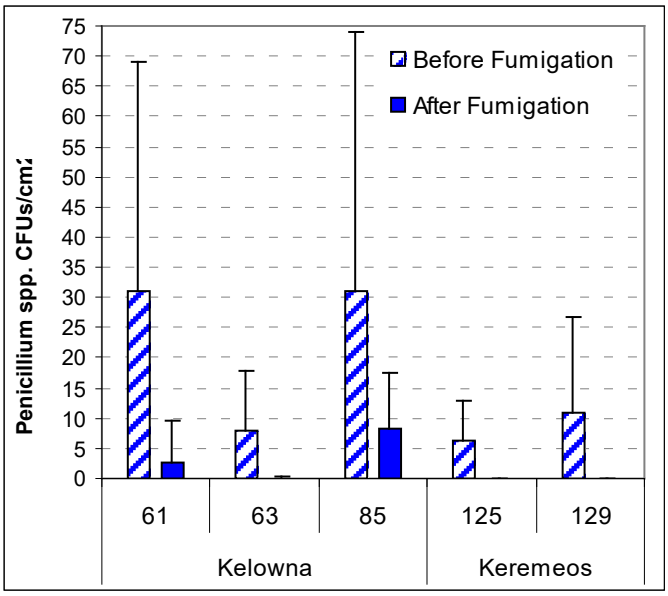

Figure 2. Overall averages of *Penicillium* spp. CFUs/cm^2 on all bins.

Of the bins treated there was an average reduction of *Penicillium* spp./cm^2 from 72 to 100% (figure 2). The reduction of 100% was obtained at Keremeos, BC due to the higher mg/l rate of acetic acid used at that location and a lower pre fumigation CFU/cm^2.

Results/Discussion Codling Moths

The fumigation of the wooden blocks containing the codling moth larvae caused a problem due to the absorption of the acetic acid by the unpainted wooden blocks. There were repeated additions of acetic acid at various times during the fumigation (figure 3). This was done to keep the acetic acid vapours at a high enough concentration to kill the larvae/pupae. Plastic solo cups did not absorb the acetic acid and were therefore at a higher concentration resulting in 100% kill of all the larvae and pupae (figure 4). The results indicate that acetic acid can be used to kill larvae and pupae

in bin, but the rate of 40mg/l is 4 times the rate used in the Bin/room fumigations. The use of this higher rate would require a separate room. More research into this aspect is necessary.

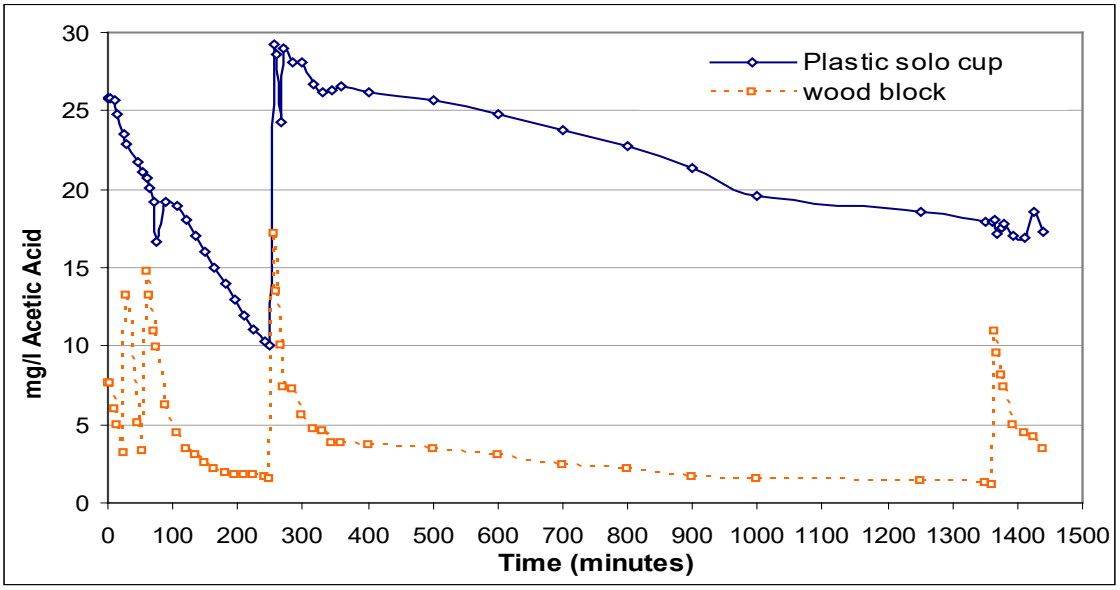

Figure 3. The effect of wood vs plastic on acetic acid concentration during the fumigation of the codling moth larvae/pupae. Note that there were 4 additional injections of acetic acid for the wooden blocks and only one for the plastic solo cups, resulting in a higher mg/l of acetic acid in the chamber.

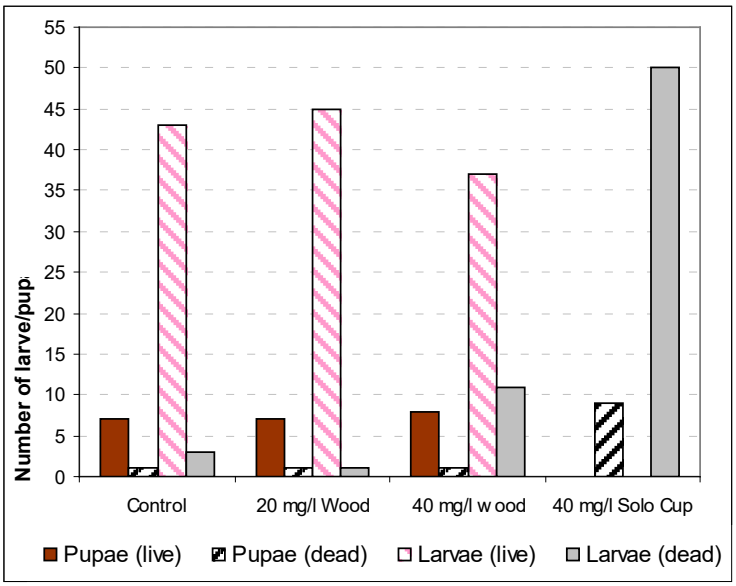

Figure 4. Acetic acid fumigation of codling moth larvae and pupae. Note that there was 100% kill of both larvae and pupae in the plastic solo cups.

Objective 5. (Effect of relative humidity on acetic acid fumigation)

Methods

Inoculum. Fungi from *P. expansum* isolate 1790 and *B. cinerea* isolate B-27 were used. The number of conidia per ml of inoculum was adjusted to ~ 1.5 x 10^4 conidia per ml by diluting with sterile distilled water as needed. A 2 cm by 2 cm sheet of a clear map overlay material were cut, each inscribed with 3 circles of ~ 4 mm in diameter, clipped onto Styrofoam blocks using paper clips, washed with SDW and 95% ethanol and allowed to air dry in a laminar flow hood. Once dried, ~ 10 µl of the inoculum was placed onto the center of each inoculation circle and allowed to air dry.

Fumigation. The dried Styrofoam blocks were then placed into 23 l fumigation chambers. The relative humidity of the chambers was adjusted to the desired percentage. Measured amounts of 99% Glacial Acetic Acid (Fisher Scientific, Ottawa, Ontario) was added to create treatments of ~ 0.0 (control), 4.0, 6.0 and 8.0 mg / litre of air. Temperature and RH readings in the fumigation chambers were taken every 5 minutes. Acetic Acid concentration was monitored using a Model 910 Gas Chromatograph (GC) (Questron Technologies Corp, Mississauga, Ontario). Fumigations were performed for 120 minutes in duration and the plastic sheets with inoculated sides down, were placed on quarter strength PDA plates and incubated for 18 to 24 hours at ~ 20º C.

Evaluation of Spore Death. After the 18 to 24 hour incubation period, the inoculated circles were viewed using a compound light microscope (Carl Zeiss, Germany), and the number of ungerminated spores and germlings within the inoculation circles were counted. The plates were then checked again for growth at 48 hours or more.

Statistical Analysis. For each trial, 12 to 15 replicates per treatment were fumigated and then rated for germination. The average germination rates were calculated using all the replicates of each treatment and the standard error of the means were also calculated.

Results

Table 9. *Botrytis cinerea* mean germination percentages with increasing relative humidity and acetic acid concentration.

Relative Humidity	Control	4mg AA/ l	6mg AA/l	8mg AA/l
25%	62.6 ± 1.5%	26.6 ± 2.3%	5.9 ± 1.0%	0.00 ± 0.00%
30%	70.5 ± 2.7%	1.8 ± 0.8%	0.06 ± 0.06%	0.02 ± 0.02%
40%	71.1 ± 2.1%	0.04 ± 0.04%	0.00 ± 0.00%	0.00 ± 0.00%
50%	62.1 ± 2.6%	0.07 ± 0.07%	0.00 ± 0.00%	0.04 ± 0.04%

Table 10. *Penicillium expansum* mean germination percentages with increasing relative humidity and acetic acid concentration.

Relative Humidity	Control	4mg AA/ l	6mg AA/l	8mg AA/l
25%	61.1 ± 1.6%	33.1 ± 2.5%	17.8 ± 3.67%	3.5 ± 2.1%
30%	60.2 ± 1.8%	21.2 ± 2.2%	6.8 ± 1.96%	0.00 ± 0.00%
40%	58.5 ± 1.0%	1.4 ± 0.3%	0.2 ± 0.07%	0.05 ± 0.03%
50%	66.4 ± 2.1%	0.03 ± 0.03%	0.0 ± 0.0%	0.0 ± 0.0%

Discussion

The control (0 mg AA/l) all resulted in germination percentages that were quite consistent among the species, with the *B. cinerea* germination percentages ranging from 62.1% to 70.5% (Table 1), and the *P. expansum* germination ranging from 58.5% to 66.4% (Table 2). In both cases, germination percentages were not 100% and this could be due to several reasons. Firstly, not all conidia produced by a sporulating culture are viable. Secondly, it is possible that not all the viable spores have germinated by the time the plates were counted which was after an incubation period of 18 – 20 hours for *B. cinerea* and 20 – 24 hours for *P. expansum*. Some of the spores germinated quite early as opposed to some of the other spores, and such germlings were already developing extensive mycelia making counting very difficult if the germlings were allowed to incubate any longer.

From the results of the fumigations at different RH's, it can be seen that there is a relationship between relative humidity and acetic acid concentration. As the relative humidity increases, lower concentration of acetic acid was required to obtain 100% control. It could be possible that in the range of RH% and AA concentrations used in this study, a decrease in one of these two variables could be roughly compensated by increasing the other variable. For example, fumigations performed at a low relative humidity could still cause significant spore death if the acetic concentration was high, while at higher relative humidity fumigations, low acetic acid concentrations still resulted in germination percentages of 0%.

In the first year of this study we had found that when the relative humidity was greater than 75%, copper corrosion increased as the acetic acid concentration increased. Hence, it is desirable to use a low acetic acid concentration in combination with a relative humidity below 65% to minimize corrosion, and still allow effective control of pathogenic spores. From the data obtained through this study, it appears that a relative humidity of ~ 50% in combination with an acetic acid rate of 6 mg AA/l will provide the necessary toxicity to inactivate or kill the conidia of both *B. cinerea* and *P. expansum*.

FINAL PROJECT REPORT
WTFRC Project Number: PH-02-240 (Amendment for 2005 project)

Project Title: Molecular techniques to study apple and pear pathogens in CA storage

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Cooperators: BC Fruit Packers, Dan Worley
University of British Columbia – Okanagan, Kandis Pohl and Dan Durall

Budget History:

Item	**Year 1:**	**Year 2:** N/A	**Year 3: N/A**
Salaries	20,000		
Benefits			
Wages	3,522		
Benefits			
Equipment			
Supplies	3,478		
Travel			
Miscellaneous			
Total	27,000		

Objectives:

1. Study epidemiology of fungal pathogens on storage room walls using molecular techniques for identification. Additionally determine if these fungi are pathogenic on apple and resistant to fungicides such as thiabendazole.

2. Develop a PCR assay for two new postharvest diseases (Phacidiopycnis and Sphaeropsis rot) known to occur in Washington and British Columbia.

Significant findings:

- Thirty nine *Penicillium* isolates were identified and sequenced from a survey conducted of different Controlled Atmosphere (CA) rooms in the Okanagan Valley

- Eleven of the 20 *Penicillium* spp. isolated from the CA walls were capable of decaying apples and seven of the 20 isolates were found to be resistant to thiabendazole (TBZ).

- Twelve *Phacidiopycnis* and 27 *Sphaeropsis* isolates were identified using molecular techniques that relied on unique DNA sequences from samples of a packinghouse survey of diseased winter pears conducted in January 2006.

- Polymerase chain reaction (PCR) primers were developed for both *Phacidiopycnis* and *Sphaeropsis*.

- *Phacidiopycnis* was identified in the orchard from canker-like areas and diseased branches of pear fruit trees.

Objective 1. Identification of the *Penicillium* pathogens using molecular techniques.

Methods:

1.1 Initial collection

Wall swab samples were taken from CA rooms in the seven major packinghouses in the Okanagan Valley between March and June 2005. The packinghouses had walls that were made of a number of different materials ranging from metal, painted plywood, styrofoam, concrete foam, white fire retardant mineral fiber and a dark fiber material. Samples were taken by dipping a sterile cotton swab or Q-tip into a sterile tube containing 10 ml of sterile distilled water (SDW) and swabbing over an area of 10 cm^2 by using a template. For one wall, five swabs were taken across the wall and placed into the same tube. For the walls covered with the fiber material five sample areas were collected across the wall, with the material being removed from a 2 cm^2 area and placed into one tube. To plate out the samples each tube containing the swabs were vortexed for 30 seconds before plating. A 100 µl aliquot was taken from each sample and then pipetted onto 100 ml Petri plates containing acidified potato dextrose agar (APDA) in triplicate. These were then incubated at 20ºC for five days or until the fungal colonies could be identified and counted. For those samples taken from the fiber material, 10 ml of SDW was added to each tube, which was then vortexed, plated and incubated as above. An initial selection of 100 *Penicillium* isolates was made from these plates taking into account different locations, wall materials and *Penicillium* morphology. Thirty-nine of the 100 isolates were further purified by single-sporing them for use in the molecular identification studies.

1.2 Molecular identification

The isolates were grown on 60 mm plates containing Potato dextrose agar (PDA) at 20ºC, for one to two weeks. Approximately 0.5 ml of wet fungal tissue was placed in a 2 ml extraction tube. Extraction of total DNA was accomplished using a FastDNA kit (BIO 101 Inc., Vista, CA) and eluted in 100 µl volumes. PCR amplification was carried out using the parameters in Sholberg et al. (2005) with a GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA). The modifications made to the method of Sholberg et al. (2005) were the use of forward primers Bt-LEV-Up4 and Pex-ITS-35f in conjunction with reverse primers Un28S22, Pex-Bt-384r and Pen_other-233r. Three separate PCR reaction mixtures including known samples of *P. expansum*, *P. commune* and *P. solitum* were included as positive controls. A negative control of the PCR reaction mixture lacking DNA was also included. Purification using a Wiaquick purification kit (Qiagen, Mississauga, Ontario) and electrophoresis using a Mass ladder (Gibco-BRL, Burlington, Ontario) was carried out using the method in Sholberg et al. (2005). The primers Bt-LEV-Up4 and Bt-LEV-Lo1 (De Jong et al., 2001) along with the Big Dye Terminator sequencing mix (Applied BioSystems, Foster City, CA) were used to sequence the β-tubulin region of the *Penicillium* spp. in separate reactions. The sequences were then imported into SeqMan Pro sequence analysis software Lasergene 7.1 (DNSTAR Inc., Madison, WI) for contig assembly and visual editing. Ambiguities identified in the sequence data were resolved by comparison with reverse complimentary sequences.

1.4 Pathogenicity test.

Fifteen Gala apples were used per isolate (5 apples replicated 3 times); each apple was wounded in triplicate with a sterile nail (3.0 mm diameter). Fruit was then inoculated by pipetting 20 µl of a 1 x 10^4 (conidia/ml) spore suspension of each isolate into each wound. The fruit was then incubated at 20^oC for 6 and 15 days, decay area was then measured and recorded using electronic calipers. Each of the decay areas were measured twice and the readings averaged. Only those isolates which caused decay on apples were tested for fungicide resistance.

1.3 Fungicide Resistance

Potato dextrose agar (PDA) amended with thiabendazole (TBZ) was used to test the sensitivity of the *Penicillium* isolates. Stock solutions of commercial grade thiabendazole (Mertect SC; Syngenta Crop Protection Canada Inc., Guelph, Ontario) were made by suspension in sterile distilled water (SDW) with different concentrations of active ingredient (µg ml = ppm) (Table 1). The stock solutions were then added to the PDA which had been autoclaved and cooled to 50°C to obtain the test concentrations. The media was then poured into 60 mm plates.

The stock solutions of the commercial grade thiabendazole at 500 g active ingredient /l were made as follows:

Stock solution A: 10 ml of TBZ (500,000 ppm)
Stock solution B: 1/10 dilution of stock A (50,000 ppm)
Stock solution C: 1/10 dilution of stock B (5000 ppm)
Stock solution D: 1/10 dilution of stock C (500 ppm)

Table 1: Amount of thiabendazole stock solution added to 200 ml of autoclaved PDA agar.

Required concentrations (ppm)	Amount added to agar (ml)	Stock solution
0	-	-
1	0.4	D
5	0.2	C
10	0.4	C
50	0.2	B
100	0.4	B
500	0.2	A
1000	0.4	A

A 7 mm diameter plug was then taken from the margin of a 10-14 day old pure culture and placed in the center of each plate. Each concentration was replicated twice. Plates were incubated at 20°C for 4 days; mean colony diameters were measured and calculated to determine resistance.

Results and Discussion:

Penicillium expansum and *P. solitum* are important postharvest pathogens of apple, in this study they were found to occur on all the wall materials in the CA rooms as well as in the packing line and defrost water. The *P. cyclopium* was predominantly found on the fire retardant fiber material and occasionally on the other materials. Using molecular techniques the isolates were identified as 8 *P. expansum*, 11 *P. solitum*, 2 *P. commune*, 15 *P. cyclopium*, 1 *P. cyclopium-like*, 1 *P. melanoconidium* and 1 *P. polonicum* (Table 2).

The pathogenicity tests showed that of the 6 *P. expansum* isolates tested all were able to decay fruit at 6 and 15 days when compared with the positive control and *P. melanoconidium* which is not usually associated with fruit decay was also comparable with the control. One out of the 9 *P. solitum* isolates tested was found to cause decay at only 15 days while the others caused only mild decay at 15 days. One out of the 3 *P. cyclopium* isolates caused decay to the same level as the control (Fig.1 and Table 3).

Of the 20 isolates tested 4 were found to be resistant and 3 moderately resistant (50% growth reduction at 1000 ppm) to TBZ. These isolates were shown only to cause mild decay at the 15 day rating. All the *P. expansum* isolates tested were found to be sensitive to TBZ. Of the 9 *P. solitum* isolates tested only 1 was sensitive and the rest were found to be either resistant, moderately resistant or sensitive at the 500 ppm level (Table 3).

Table 2: *Penicillium* isolates with their locations and identifications.

Isolate	Location	Source	Material[1]	sequence ID
P-2	Kelowna	Room 82	dark fiber	*P. solitum*
P-3	Kelowna	Room 61	fire retardant fiber	*P. commune*
P-5	Kelowna	Room 72	metal/steel	*P. expansum*
P-8	Summerland	Dump tank	water	*P. expansum*
P-9	Summerland	Dump tank	water	*P. cyclopium*
P-10	Summerland	Packing line	water	*P. expansum*
P-11	Summerland	Packing line	water	*P. solitum*
P-12	Kelowna	Room 63	fire retardant fiber	*P. cyclopium*
P-13	Kelowna	Room 63	fire retardant fiber	*P. expansum*
P-17	Rutland	Room 57	fire retardant fiber	*P. solitum*
P-22	Kelowna	Room 61	fire retardant fiber	*P. cyclopium*
P-23	Kelowna	Room 83	fire retardant fiber	*P. cyclopium*
P-24	Kelowna	Room 61	dark fiber	*P. cyclopium*
P-26	Kelowna	Room 63	fire retardant fiber	*P. cyclopium*
P-28	Kelowna	Room 61	dark fiber	*P. cyclopium*
P-31	Kelowna	Room 64	metal/steel	*P. commune*
P-33	Kelowna	Defrost tank	water	*P. expansum*
P-35	Kelowna	Dump tank	water	*P. melanoconidium*
P-37	Kelowna	Room 63	pipes	*P. cyclopium*
P-39	Rutland	Room 54	fire retardant fiber	*P. expansum*
P-41	Winfield	Room 525	fire retardant fiber	*P. solitum*
P-43	Winfield	Room 526	fire retardant fiber	*P. solitum*
P-45	Winfield	Room 510	plywood	*P. cyclopium*
P-48	Winfield	Room 611	concrete foam	*P. cyclopium*
P-51	Winfield	Room 508	plywood	*P. solitum*
P-55	Naramata	Room 253	plywood	*P. solitum*
P-56	Summerland	Room 232	plywood	*P. cyclopium-like*
P-58	Naramata	Room 258	concrete foam	*P. cyclopium*
P-61	Oliver	Room 107	plywood	*P. cyclopium*
P-63	Oliver	Room 104	metal/steel	*P. cyclopium*
P-64	Oliver	Room 115	plywood	*P. solitum*
P-72	Osoyoos	Room 144	fire retardant fiber	*P. polonicum*
P-76	Osoyoos	Defrost tank	water	*P. solitum*
P-84	Winfield	Room 511	spore trap	*P. solitum*
P-87	Kelowna	Room 61	spore trap	*P. cyclopium*
P-91	Kelowna	Room 82	spore trap	*P. solitum*
P-96	Kelowna	Room 83	spore trap	*P. cyclopium*
P-97	Kelowna	Room 83	spore trap	*P. expansum*
P-100	Kelowna	Room 60	plywood	*P. expansum*

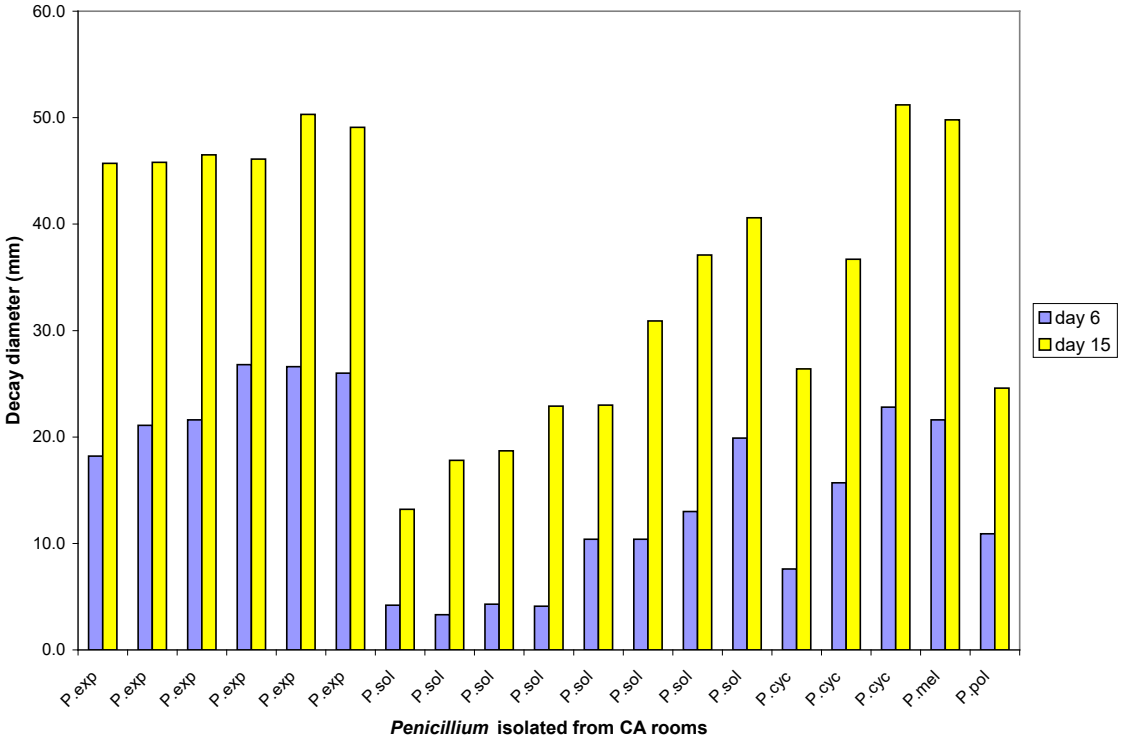
[1]Samples were taken by dipping a sterile cotton swab or Q-tip into a sterile tube containing 10 ml of sterile distilled water (SDW) and swabbing over an area of 10 cm^2 by using a template. For plywood and metal walls 5 swabs (10 cm^2)/ wall into 1 tube. For fiber walls 5 samples (2 cm^2) into 1 tube.

Table 3: *Penicillium* isolates screened for decay on 'Gala' apples and tested for thiabendazole fungicide resistance.

Isolate	Sequence ID	Pathogenicity		Level of fungicide resistance (ppm)
		Day 6	Day 15	
Group 1[1]				
Water	-	3.0 d[3]	5.5 h	-
1790 (positive control)[2]	*P. expansum*	21.3 b	50.6 ab	1000 (R)[4]
P-8	*P. expansum*	18.2 c	45.7 b	5
P-33	*P. expansum*	21.6 ab	46.5 ab	5
P-10	*P. expansum*	21.0 b	45.8 b	1
P-2	*P. solitum*	4.2 d	13.2 ef	1000 (R)
P-51	*P. solitum*	4.3 d	18.7 cd	1000 (MR)[5]
P-64	*P. solitum*	4.1 d	22.9 c	500
P-17	*P. solitum*	4.1 d	11.42 f	500
P-11	*P. solitum*	3.3 d	17.8 de	1
P-9	*P. cyclopium*	22.8 a	51.2 a	5
P-35	*P. melanoconidium*	21.6 ab	49.8 ab	1
Group 2[1]				
Water	-	15.9 c	35.2 cd	-
1790 (positive control)	*P. expansum*	27.0 a	47.7 a	1000 (R)
P-39	*P. expansum*	26.8 a	46.1 ab	5
P-97	*P. expansum*	26.6 a	50.3 a	1
P-100	*P. expansum*	26.0 a	49.1 a	1
P-55	*P. solitum*	10.4 def	23.0 f	1000 (R)
P-43	*P. solitum*	19.9 b	40.6 bc	1000 (R)
P-41	*P. solitum*	13.0 d	37.1 cd	1000 (MR)
P-91	*P. solitum*	10.4 def	30.9 de	1000 (MR)
P-48	*P. cyclopium*	7.6 fgh	26.4 ef	50
P-45	*P. cyclopium*	15.8 c	36.7 cd	1
P-72	*P. polonicum*	10.9 de	24.6 ef	1000 (R)

[1] Group 1 isolates were tested in January 2006 and group 2 isolates were tested in March 2006. The groups were analyzed separately to account for any differences in storage time of the fruit.
[2] Isolate 1790 is *P. expansum* previously isolated from decayed fruit and identified as TBZ resistant.
[3]Numbers followed by the same latter in each column are not statistically different at the p=0.05 level.
[4] R = resistant where colony growth reduction at 1000 ppm is not more than 25% compared to growth at 0 ppm.
[5] MR = moderately resistant where colony growth reduction at 1000 ppm is between 50-60% compared to growth at 0 ppm.

Fig.1 Screening of the *Penicillium* isolates on 'Gala' fruit incubated at 20°C and rated at 6 and 15 days. Decay diameter was the mean of 15 fruit (3 wounds/fruit).



Objective 2. Develop a PCR assay for two new postharvest diseases (Phacidiopycnis and Sphaeropsis rot) known to occur in Washington and British Columbia.

Methods:

2.1 Packinghouse survey

Winter pears were surveyed in British Columbia in January 2006 for *Phacidiopycnis* and *Sphaeropsis* rot. Two CA rooms of 'Anjou' pears at a Kelowna packinghouse were examined during this study. Room 67 contained 841 bins (366,564 kg) from 14 different growers and room 73 held 912 bins (398,876 kg) from 7 different growers. During pear packing only fruit with stem or calyx end rot were sampled from the cull bins. The decayed area was wiped with 95% alcohol and a small piece of the infected tissue from each pear was removed and placed on acidified potato dextrose agar (APDA) plates. These plates were then incubated at 20°C for 5-7 days and the resulting fungus identified to genus using a dissecting microscope.

2.2 Field isolations

Infection of the fruit by these organisms is known to occur in the field so two orchards were examined for any signs of these fungi on dead bark, twigs and canker-like areas. Small sections of the canker-like areas and diseased branches from the orchard were surface sterilized, placed on to APDA plates and incubated at 20°C for 5-7 days.

2.3 Single spore cultures

From the microscope identifications a selection of *Phacidiopycnis* and *Sphaeropsis* cultures were single spored before DNA extraction to remove any possibility of cross contamination. From previous work by Liu and Xiao (2005) for the *Phacidiopycnis* spp., and Xiao and Rogers (2004) for the *Sphaeropsis* spp. it was determined that in order for both species to produce pycnidia 12 hour cycles of alternating light and dark at 20°C were needed. Pycnidia were collected by gently scraping them off the surface of the cultures and put into a 1.5 ml eppendorf tube with a few drops of 10% pear juice for *Phacidiopycnis* or sterile distilled water for the *Sphaeropsis*. The pycnidia were then crushed using a 1.5 pellet pestle (VWR International Ltd). Spores released from the pycnidia were streaked onto 60 mm PDA plates, incubated at 20°C for 12-24 hours from which the single spore cultures were made.

2.4 DNA and sequence identification

DNA was extracted using a Fast DNA extraction kit (BIO 101 Inc., Vista, CA) for all samples. Polymerase chain reaction (PCR) amplification of the internal transcribed spacer region (ITS) was made by using the universal primers UN18S-42 and UN28S-22. Samples were amplified using low or limiting levels of primers so that no clean up of PCR product was needed prior to using it as a sequencing template. For all samples the primers UN18S-42 and UN28S-22 along with the Big Dye Terminator sequencing mix (Applied BioSystems, Foster City, CA) were used to sequence the ITS region in separate reactions. This method was repeated but using the Bt-LEV-Up4 and Bt-LEV-Lo1 (De Jong et al., 2001) primers to all the *Phacidiopycnis* and some of the *Sphaeropsis* samples for the initial PCR and sequencing mix to sequence the β-tubulin region. The sequences were then imported into SeqMan Pro sequence analysis software Lasergene 7.1 (DNSTAR Inc., Madison, WI) for contig assembly and visual editing. Ambiguities identified in the sequence data were resolved by comparison with reverse complimentary sequences.

DNA obtained from Washington State isolates were also sequenced as described above and compared to our sequences.

2.5 Primer design

Using the Primer 3 analysis program a number of primers were designed and made up by Invitrogen Canada Inc, (Burlington, Ontario). These primers are able to target specific differences in the β-tubulin region between the *Phacidiopycnis* and *Sphaeropsis* so when DNA is extracted from decayed fruit and used in a PCR reaction with these primers it should be possible to identify the causal agent without additional PCR and sequencing.

Results and Discussion:
Packinghouse survey:

From room 67, 41% of the rots were caused by *Phacidiopycnis* and 35% by *Botrytis* and from room 73, 37% were caused by *Phacidiopycnis* and 41% by *Botrytis*, consequently a significant amount of the stem and calyx end rot previously thought to be caused by *Botrytis* was found to be *Phacidiopycnis* spp. *Sphaeropsis* spp. was also isolated from rooms 67 and 73 at 2.4% and 1.1% respectively (Fig.2).

Phacidiopycnis spp. was isolated from the orchard from small canker-like areas and diseased branches from pear trees. The field isolations were made in May, June and October 2006 and it was observed that the *Phacidiopycnis* was re-isolated in conjunction with *Botrytis* showing that it is active in the orchard for a number of months. There may be some connection with the *Botrytis* however; a

more extensive field survey would be required to ascertain the level and length of field infections. Effective control measures such as good orchard sanitation to remove over wintering inoculum and the use of effective fungicides should be considered in orchards that harbor the disease.

Sequence identification:

Both *Phacidiopycnis* and the *Sphaeropsis* sequences matched 99-100% with the isolates from Washington State showing that they are *Phacidiopycnis pyri* and *Sphaeropsis pyriputrescens* (Fig 3). Six primers were designed for the *Phacidiopycnis* and four for *Sphaeropsis*. These primers will be used in future studies for accurate identification of these fungi.

Fig.2 Survey results showing the identity of fungi and percent rot isolated from calyx and stem end rots of 'Anjou' pears in 2006.

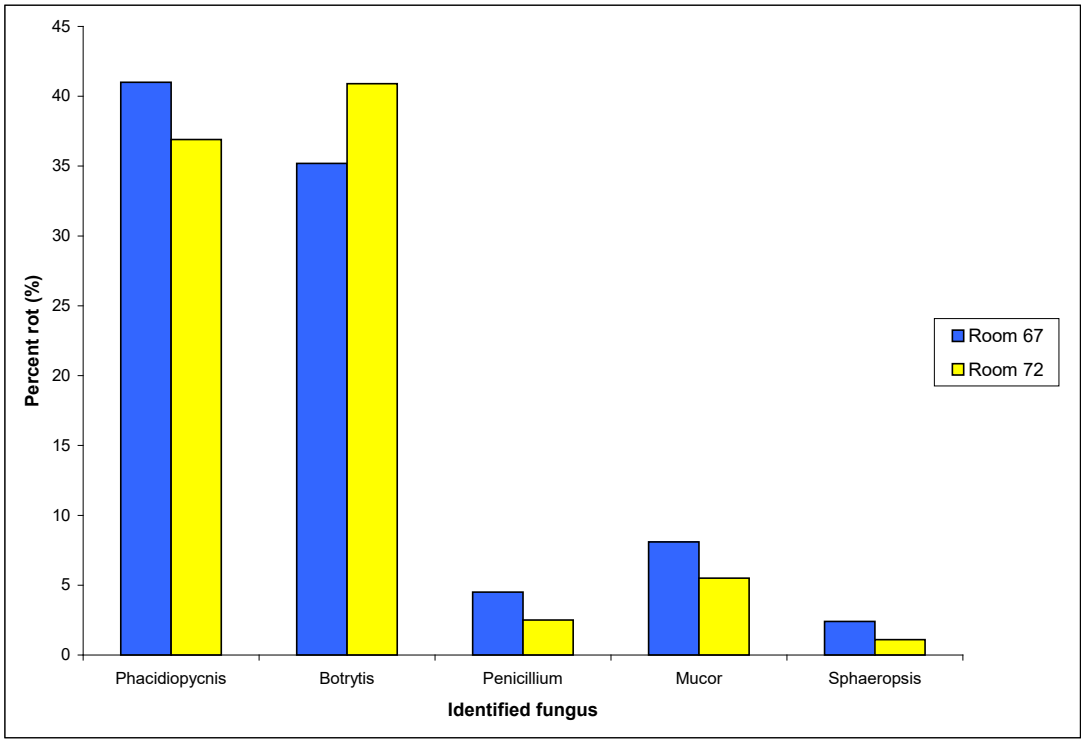


Fig.3 *Phacidiopycnis* and *Sphaeropsis* β-tubulin DNA gene sequences compared to one another showing differences between bases. WI - Washington isolates; Pha - *Phacidiopycnis* isolates; SRS - *Phacidiopycnis* isolated from the orchard; Sph - Sphaeropsis isolates; Major differences between the sequences are in bold type.

WI 2271: CCCACCCTCTGT**T**TACA**A**TACC**AT**TGTTGCTTTGGCGGCCCCGTCGCA**A**GACAACCGGC**T**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**T**CATAT**TG**TCA**T**TGTCGTCTGAGTA

Pha 2300: CCCACCCTCTGT**T**TACA**A**TACC**AT**TGTTGCTTTGGCGGCCCCGTCGCA**A**GACAACCGGC**T**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**T**CATAT**TG**TCA**T**TGTCGTCTGAGTA

SRS 2383: CCCACCCTCTGT**T**TACA**A**TACC**AT**TGTTGCTTTGGCGGCCCCGTCGCA**A**GACAACCGGC**T**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**T**CATAT**TG**TCA**T**TGTCGTCTGAGTA

WI 2373: CCCACCCTCTGT**C**TACA**G**TACC**TC**TGTTGCTTTGGCGGCCCCGTCGCA**G**GACAACCGGC**C**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**C**CATAT**CA**TCA**G**TGTCGTCTGAGTA

Sph 1206: CCCACCCTCTGT**C**TACA**G**TACC**TC**TGTTGCTTTGGCGGCCCCGTCGCA**G**GACAACCGGC**C**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**C**CATAT**CA**TCA**G**TGTCGTCTGAGTA

Sph 2381: CCCACCCTCTGT**C**TACA**G**TACC**TC**TGTTGCTTTGGCGGCCCCGTCGCA**G**GACAACCGGC**C**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**C**CATAT**CA**TCA**G**TGTCGTCTGAGTA

Sph 3362: CCCACCCTCTGT**C**TACA**G**TACC**TC**TGTTGCTTTGGCGGCCCCGTCGCA**G**GACAACCGGC**C**CCGGCTGGTCAGCGGCCGCCAGAGGACTCCAAAAC**C**CATAT**CA**TCA**G**TGTCGTCTGAGTA

.

References:

De Jong S.N., Lévesque C.A., Verkley G.J.M., Abeln E.C.A., Rhae J.E. and Braun P.G., 2001. Phylogenetic relationships among *Neofabraea* species causing tree cankers based on DNA sequencing of the ITS nuclear rDNA, mitochondrial DNA and β-tubulin gene. Mycol. Res. 105, 658-669.

Liu Q. and Xiao C.L., 2005. Influence of nutrient and environmental factors on conidial growth of *Potebniamyces pyri*. Phytopathology 95:572-580.

Sholberg P.L., Harlton C., Haag P., Lévesque C.A., O'Gorman D. and Seifert K., 2005. Benzimidazole and diphenylamine sensitivity and identity of *Penicillium* spp. that cause postharvest blue mold of apples using β-tubulin gene sequences. Postharvest Biology and Technology. 36:41-49

Xiao C.L. and Rogers J.D., 2004. A postharvest fruit rot in d'Anjou pears caused by *Sphaeropsis pyriputrescens* sp. nov. Plant Disease 88:114-118.

FINAL PROJECT REPORT
WTFRC Project Number: AE-04-428 (WSU Project 3643-8366)

Project Title: The importance of dispersal in biological control and IPM

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	20,487	21,306	15,215
Benefits	6,146	6,392	5,170
Wages	11,000	11,000	6,667
Benefits	1,760	1,760	733
Equipment	0	0	0
Supplies	3,200	3,200	3,200
Travel	3,200	3,200	2,303
Miscellaneous			
Total[a]	45,793	46,858	33,288

[a]Years 1 and 2 split with Pear Commission (2/3 apple, 1/3 pear); year 3 separated into two projects with different names and budget numbers.

Objectives:

1. Examine the area of influence ("active space") of a rose/strawberry garden used to bolster parasitism of leafrollers.
2. Examine the movement of insect pests from areas of high population density to surrounding managed areas.

Significant findings:

- Rose gardens influence at least 7.2 acres of surrounding orchard.
- The movement of *C. florus* from the gardens to the surrounding orchard is not well synchronized with pest leafroller abundance in the orchard; it is likely driven by population dynamics of strawberry leafroller in the gardens themselves. More work on this area is required.
- The number of CM moving into a managed orchard from an adjacent abandoned area drops off quickly with distance but can still encompass the entire managed block. Damage tends to be more restricted to the border, but pockets of damage do occur throughout the managed area.
- The use of Surround® on the border between the abandoned area and the managed orchard greatly diminished damage and moth capture in the managed section. This may be a good tactic to reduce damage from migrating moths.
- Movement of both CM and OBLR appears to be directional and is likely based on prevailing wind conditions. We found that a low level population in one orchard may contribute more moths to an adjacent orchard with high levels of the pest than the converse.
- CM moved >1200 feet within three days of the application of our markers.

Objective 1. Examine the area of influence ("active space") of a rose/strawberry garden used to bolster parasitism of leafrollers.

We monitored two sites this year, one at Wenatchee Valley College (WVC) and one on the south side of Frenchman Hills. At the Frenchman Hills site, we only collected six *Colpoclypeus florus* (two positive for the soy marker) from early May to late August, and all of those collections occurred in May. Because of the low catch, the discussion will focus on the WVC site. At both sites, we used the methods developed last year where we used fine netting placed over the garden and then applied soy flour using a fertilizer spreader. This had the advantage of heavily coating the leaves and foliage of the strawberries and roses as well as the netting (the holes in the netting were much larger than the parasitoids) so that emerging parasitoids were well marked. We then used our traps, which consisted of a infested shoot placed through a hole in a sticky card and into a 100-ml vial of water. The shoots and sticky cards were changed each week. Both the larvae and the sticky cards were examined for *C. florus*; if any were found, they were tested with ELISA to see if there was any soy protein present.

At WVC, the garden was adjacent to a block of organic Fuji apples and all trapping was done in the Fuji block. The block had extremely low leafroller population levels in the orchard. We collected low numbers of *C. florus* in early May but had two large peaks during the season. The first peak occurred on 30 June and the second from 2 August to 14 August (Fig. 1). The first peak occurred when most of the leafrollers were in instars 1-3 (only 5% were in instars 4-6 which is the time the parasitoids can successfully attack the leafrollers), but the second peak occurred when virtually all PLR were in the susceptible stages (instars 4-6). However, the population of PLR was decreasing at this time.

Our markers were detected only during the two large peaks described above. For those three collections, we found that an average of 40% of the parasitoids were marked, indicating they were coming from the strawberry/rose garden. When we examined only the positive parasitoids, we collected them as far as 316 feet from the edge of the soy treated areas, with 75% being collected within roughly 225 feet. If we use the 316-foot figure, the area of influence is a minimum of 7.2 acres. We did catch multiple individuals at the furthest distance so the effect of the rose garden is likely to be larger. Interestingly, there is not a uniform decrease in *C. florus* with distance from the garden; instead, the positively marked parasitoids appeared to follow the edge of the orchard and were more commonly collected 40+ meters (≈130 feet) from the garden (Fig. 2).

Fig. 1. Trap capture of *C. florus* over time at WVC orchard in 2006 with the relative number of PLR instars 4-6 as predicted by the PLR model.

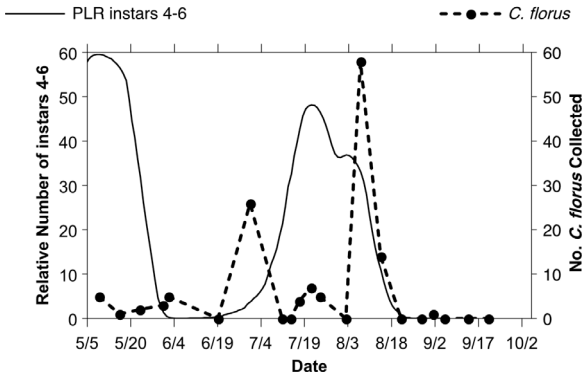
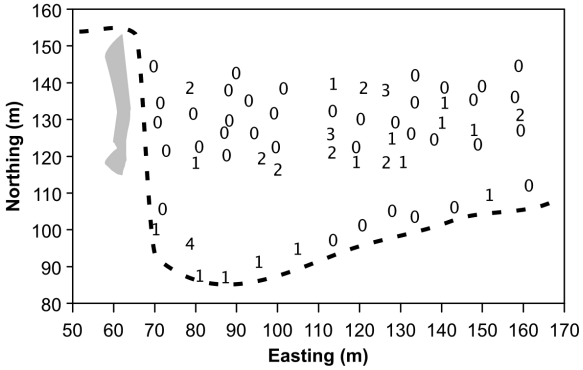


Fig. 2. Map of trap locations and soy treated area (gray area). Numbers indicate locations and numbers of soy positive *C. florus;* dotted line indicates edge of orchard.



While some of the population trends we observed were undoubtedly influenced by the spray program (and overspray on the garden), particularly in the early season and midseason, our data suggest that further investigation of the interrelationships between strawberry leafroller, PLR or OBLR in the orchard and *C. florus* is warranted. The fact that our first peak in trap catch occurred well before suitable hosts in the orchard were present suggests that *C. florus* movement into the orchard occurs when available strawberry leafroller hosts within the garden are rare and are likely not driven simply by the presence of pest leafrollers in the orchard. The data from 2005 was more restricted in time, but the peak in late season occurred when the relative number of larvae in instars 4-6 was extremely low, again suggesting a poor synchrony with the PLR population. It is clear that without a better understanding of the relationship of *C. florus* and the strawberry leafroller in the strawberry/rose gardens, the utility of the gardens as a supplement to BC of our pest leafrollers within the orchard will always be difficult to predict or understand.

Objective 2. Examine the movement of insect pests from areas of high population density to surrounding managed areas.

This year we performed four studies under this objective, three with codling moth and one with OBLR. Two of the codling moth experiments were able to measure the movement of CM from abandoned blocks to adjacent managed blocks. In both blocks, the distance was roughly 40 feet from the managed sections. The other CM experiment was two large orchards that were again separated by a road (≈40 feet) but both sides were managed, with one having a large CM population and the other a relatively low population level. The OBLR study was to investigate movement from a young heavily infested cherry orchard to an infested adjacent apple block in late season.

CM movement from Abandoned to Managed Orchards:

Methods: We applied our egg protein marker to about 1.5 acres of the abandoned orchard blocks immediately adjacent to the managed areas. All sprays were applied at roughly 25% adult emergence

in the second generation on 18 July. Both of the managed blocks were not under mating disruption and were under a conventional CM program. The first block (Y1) was about 4 acres in size and planted with Gala apples and the adjacent abandoned orchard was comprised of Red Delicious apples. The second block (Y2) was 8 acres and was predominately Fuji with some Red Delicious apples intermixed; the abandoned block was Red Delicious. We placed one row of pheromone traps in the egg-treated area (eight traps) and 60 in each of the managed orchards. All traps were baited with Trécé's Combo/DA lure which attracts males and a lower number of females; all moths were identified to sex and processed by ELISA to determine whether they had picked up the protein marker in the abandoned areas. We geocoded (using GPS) every trap so that we could determine the exact distances flown from the edge of the protein marked block.

We also evaluated fruit injury in both the abandoned areas (in the center of the treated areas) and throughout the managed orchards just before harvest. All samples were geocoded so that we could determine the exact location of all samples with respect to the abandoned areas. Our sampling density was roughly 25 trees per acre (60 fruit/tree) with the edges adjacent to the abandoned orchards being sampled more heavily (every row for the first five tree rows, samples ≈40 feet apart) to determine exactly how far damage extended into the managed orchards.

***Analysis**:* We analyzed the moth catch data using the total marked moths caught as well as separated by sex. Because we geocoded all samples, we calculated how far the marked moths flew and plotted the data as distance from the edge of the marked areas. Similarly, we analyzed the fruit damage in the same fashion.

***Results**:* We captured 3,372 moths in the Y1 plots over a two-week period with 2,398 being caught in the eight traps in the abandoned area and 974 in the managed area. Of those caught in the managed area, 230 (24%) were positive for the egg marker indicating that they definitely had visited or originated from the abandoned orchard. However, our abandoned area had 1,102 (46%) marked moths. The percentage marked is quite low compared to other studies we have run, which suggests that a large number of moths originated deeper in the abandoned area than our treated zone. If this is true, then the number in the managed areas originating from the abandoned area was higher than 24% (roughly 2x higher likely) because moths from deeper in the abandoned areas bypassed our marked area and would not have acquired the mark.

When we examined only the marked moths, we found that they were in the highest numbers closest to the abandoned orchard but were found at virtually all distances, both males and females (Fig. 3). Roughly 29% of all the marked moths caught within the orchard were females. When plotted as the percentage trap catch vs. distance from the abandoned orchard, both sexes had a similarly shaped drop off with distance (Fig. 4a).

The damage in the plot fit a similarly shaped curve as the dispersal into the orchard, where the damage was highest closest to the border and

Fig. 3. Trap catch of marked moths in the Y1 orchard. Gray area is the abandoned orchard adjacent to the managed orchard. Top Males only, bottom, females only.

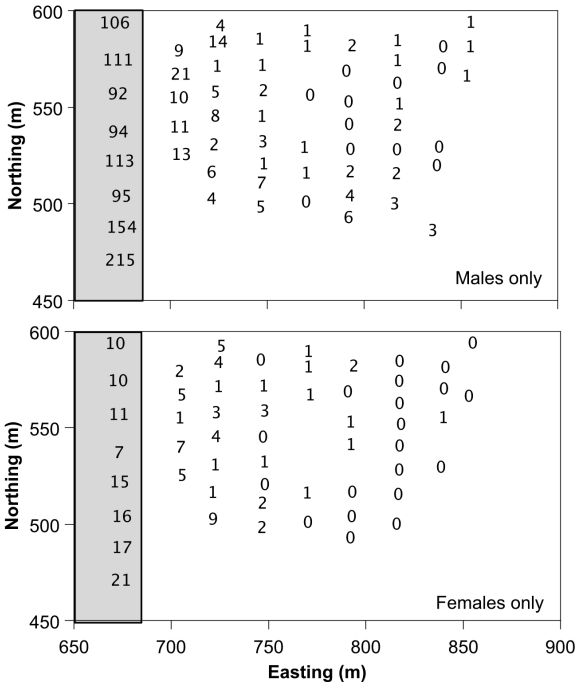
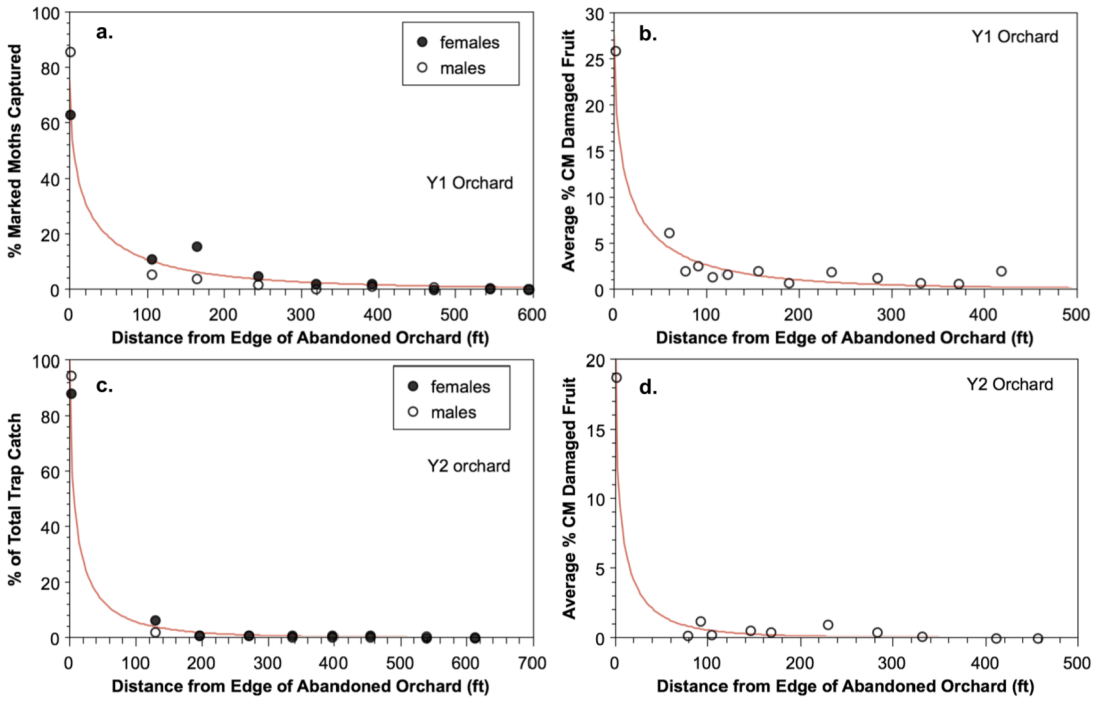


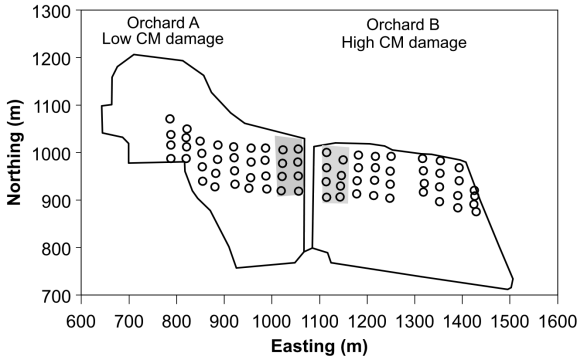
Fig.4. Percentage marked moths and fruit damage versus distance from abandoned area for orchards Y1 and Y2. Solid lines are predicted values based on non-linear regression. Orchard Y2 was sprayed with Surround before we treated the area.



decreased as we moved away from the edge (Fig. 4b). We also found that the damage tended to be slightly higher on the top and bottom of the orchard where it appeared that moths flew around the edge for a short distance.

In the Y2 plots, we captured 1,177 moths in eight traps in the abandoned area and 193 in the 60 traps within the orchard. In the abandoned area, 654 moths were marked, and within the orchard only 42 (21%) were marked. Considering the magnitude of the trap catch within the abandoned area and the relatively short distance (40 feet), the low trap catch surprised us, especially after the numbers we found in the Y1 plots. However, the big difference was that the Fuji block was sprayed with Surround® for sunburn protection and previous studies by Tom Unruh have shown that CM tend to avoid Surround treated areas. The drop off with distance of moth capture was considerably quicker than we saw in the Y1 plot (that was not treated with surround) (Fig. 4a vs. Fig. 4c). Damage in the plot dropped off very quickly, similar to the way that trap catch dropped off with distance from the abandoned area (Fig. 4d), again faster than in the Y1 orchard (Fig. 4b vs. 4d). These data suggest that even a single treatment of Surround on a few border rows may be a good management tactic adjacent to a source of high levels of CM (*e.g.*, bin piles, abandoned orchards, etc.).

Fig. 5. Trap locations (circles) and treated areas (gray boxes) at the two adjacent managed orchards. Orchard A treated with milk marker, Orchard B with egg marker.



Two managed orchards with different damage levels

***Methods**:* We chose two side-by-side orchards that had differing damage levels the previous year

(Fig. 5). We treated 1.5 acres with our protein markers in each block. Orchard A received a milk treatment and Orchard B an egg treatment. Both treatments were applied on 18 July when roughly 25% of the summer generation had emerged. We placed 36 traps on a regular grid in both orchards and followed the movement for 2.5 weeks, which is roughly the time where our markers have sufficient residue to mark moths that just walked on treated leaves. We used the Trécé DA/combo lures so that we could collect data on male and female movement patterns.

Fig. 6. Trap catch over two weeks for moths that tested positive for the milk marker. The milk treated area is in grey in Orchard A.

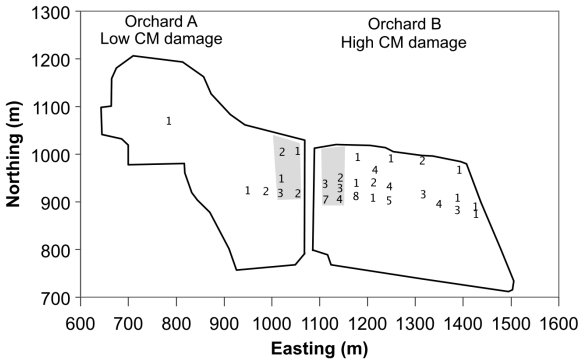
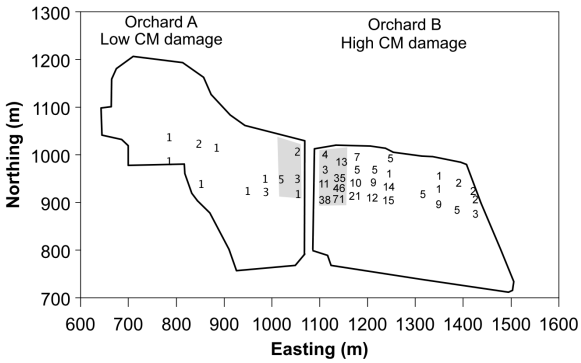


Fig. 7. Trap catch over two weeks for moths that tested positive for the egg marker. The egg treated area is in grey in Orchard B.



***Results**:* Because we treated the two orchard areas with different markers, we could distinguish the origin of the moths. At harvest, Orchard A had the lower levels of damage (0.09% over 361 trees, 60 fruit/tree) and Orchard B had 0.9% damage over 489 trees. We caught 186 moths in Orchard A and 1,394 in Orchard B. However, looking at marked individuals, we found that only 27 from Orchard B were detected in Orchard A, while 63 from Orchard B came from Orchard A (Figs. 6, 7). The trap catch is the opposite of what we would predict by marker efficiency (the egg marker in Orchard B allows marking at roughly 2x the rate of the milk marker used in orchard A). Part of the discrepancy is likely simply a directional movement from east to west; when we examined movement patterns of the marked moths, 12.9% of the milk-marked moths moved east from the treated area, while 16.7% of the egg-marked moths moved towards the east. The reason for the directed movement may be the prevailing wind direction or some other factor.

The damage in Orchard A was low and scattered in a random fashion over the entire orchard. However, in Orchard B, there was a hotspot, which corresponded to one section adjacent to the marked area that was comprised of Gala apples. This area also had the highest trap catch for individuals that were either marked or unmarked.

When we examined the distance dispersal curve for moths marked with the egg marker (origin = Orchard B) it appeared similar to the ones found previously in orchards Y1 and Y2. However, damage could not be analyzed the same way because our treated area was likely not the sole source of the moths; as mentioned previously the major source was likely the Gala area adjacent to our marked area. The moths marked with the milk marker (origin = Orchard A) did not show any marked pattern with distance from the area treated (Fig. 6). We found them throughout Orchard A and Orchard B in a random fashion. In marked moths originating from either area, we collected them at the maximum distance where our traps were placed (>1200 feet), all within the initial three days after treatment.

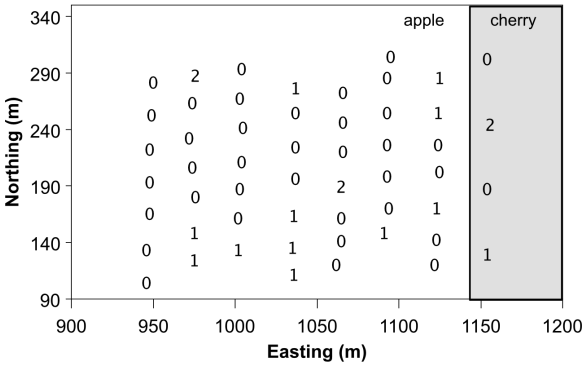
OBLR Movement from Cherries to Apples after Cherry Harvest

***Methods**:* We used a young cherry orchard infested with OBLR adjacent to a large apple block. In this situation, we applied our egg protein to the cherry orchard and placed a grid of traps in the cherry block. We placed 49 pheromone traps in the apples and four in the cherry orchard. We also placed 18 acetic acid traps in the apple orchard in an attempt to collect females, but no moths were captured in

the acetic acid traps. Treatments were applied on 4 August, and trapping continued for three weeks after treatment.

***Results**:* We captured 350 moths total, 15 in the cherry and 335 in the apple block. Of those, only 16 marked adults were captured in the apple block. The distribution of the egg-marked captures was relatively random and uniform throughout the block out to more than 450 feet from the cherry block (Fig. 8).

Fig. 8. Trap catch of egg positive OBLR over 2 week period.



We have run at least one experiment per year trying to examine the importance of movement from cherries to apples in late season. In the first year, where young cherries were between two mature apple blocks, we found that movement from the cherries to the apples was strongly directional and accounted for 30% of the moths caught in one block and less than 4% in the other. Last year, we conducted movement studies between an apple and cherry block but found that very little movement occurred between the two blocks; only five moths were found to have moved from the treated area into the other. This year, our results also show little movement between the cherries and apples.

We were also unable to determine the flight ability of female OBLR because they do not respond to the pheromone, and the acetic acid traps caught no moths. Thus, our comments on flight refer only to males and thus the impact of movement patterns of females can only be inferred from male data. If females disperse in a similar direction, distance and frequency as males, even with the relatively low levels found in our studies, the high reproductive rate of OBLR would allow it to spread over a large area and initiate hot spots the following year.

Based on our studies with both OBLR and CM, it is likely that infestation of adjacent orchards is highly dependent on the spatial arrangement of the two orchards, distance between them and environmental factors such as the direction and intensity of the prevailing wind. It appears that the movement is not necessarily a function of high numbers in one orchard moving to the adjacent clean orchard; it is a two-way interaction, but it is not symmetrical. In some cases, the orchard with the lower density may actually be the source of a greater number of dispersing moths, depending on wind patterns or whatever environmental variable influences directionality in movement. However, even if a lower number of moths come from one direction, their high reproductive rate can result in hot spots that may be important the following year as a source for population buildup.

FINAL PROJECT REPORT
WTFRC Project Number: AE-04-429 (WSU Project 3643-7366)

Project Title: Mechanisms underlying mating disruption

PI:	Vincent P. Jones	**Co-PI(2):**	Jay Brunner
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State/Province/Zip	WA 98801	**State/Province/Zip:**	WA 98801

Budget History:

Item	Year 1: 2004	Year 2: 2005	Year 3: 2006
Salaries	22,724	23,634	24,377
Benefits	7,272	7,563	9,263
Wages	12,800	12,800	12,800
Benefits	2,048	2,048	1,408
Equipment			
Supplies	3,200	3,200	5,700
Travel	3,200	3,200	1,500
Miscellaneous			
Total	51,244	52,445	55,048

Objectives:

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

Significant findings:

- Our data show that CM females discriminate against males older than two days, resulting in a reduction in successful matings even in no-choice situations. CM males did not show significant age-based discrimination.
- OBLR males and females both showed age-based discrimination that resulted in a reduced percentage of successful matings.
- Female CM mated to older males showed significant reductions in reproductive output compared to females mated to young males. The effect was pronounced (20-45% reduction) but smaller than reductions found when female age was increased.
- We developed a method to partition the codling moth and OBLR models to allow us to determine male age distribution at any point in the season. Combined with other studies, we were able to show that male age effects result in a roughly 15% decrease in reproductive rate.
- Our previous studies showed conclusively that the delay in mating for both males and females should be calculated on a calendar date basis.
- We found in lab wind tunnels that CM could not successfully contact a lure when speeds were over 2.2 mph and OBLR were unable to successfully contact a lure over 3 mph.
- Studies in the field showed that under certain circumstances, the reduction in mating caused by wind velocity over 2.2 mph was significant compared to situations with no wind. The effects were highly location and orchard specific.
- Our studies to determine the importance of CM dispersal between MD and non-MD this year were unsuccessful because of weather and location-specific problems in the first generation. The hot spell in May and the rainy weather along with low trap catches at the test orchards resulted in our being unable to complete this objective.

Objective 1

Our data from last year showed that even in no-choice tests female CM discriminated against males that were >2 days old. The study also showed that 6-day-old males were able to successfully pass a spermatophore only 50% as often as males aged 0 or 2 days. In contrast, males showed little age-based discrimination against females, and even 6-day-old females were able to mate at roughly 87% of the rate of newly emerged females (Fig. 1).

In the no-choice trial with OBLR, mating success decreased with age in both sexes similarly compared to individuals paired on the day of emergence. By day 6, males were only 70% as efficient at mating as males paired on the day of emergence and females were about 66% as efficient (Fig. 2).

Fig. 1. Proportion of CM pairs where a spermatophore was successfully passed. Values corrected relative to the success for 0 day old individuals.

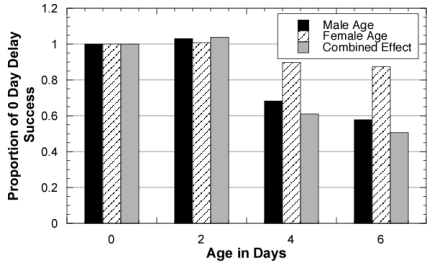
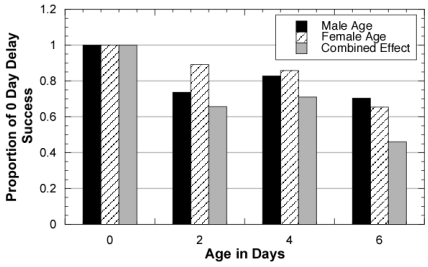


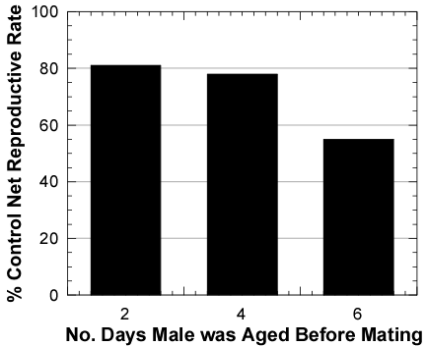
Fig. 2. Proportion of OBLR pairs where spermatophores were successfully passed. Values corrected relative to the success of 0 day old individuals.



Objective 2

Last year we finished our laboratory studies on the effect of CM females mating with males of different ages. We found that the net reproductive rate (average number of daughters produced over the average female life span) dropped as the male age increased past four days (Fig. 3). The reduction in population growth associated with older males being mated to females of a fixed age is similar (but of lower magnitude) to our previous studies where females of different ages were mated with fixed-age (1-day-old) males. In this study, there were two components affecting female reproductive output: (1) as male age increased, the incidence of no eggs produced also increased and (2) there was a change in reproductive rate not associated with number of eggs produced (*i.e.* a decrease in percent egg hatch).

Fig. 3. Effect of male age on female reproductive output. All females were mated at 4 days old.



These data, in conjunction with our previous data on female age at time of mating, clearly show that the ages of both sexes at time of mating are critical to population growth. Combined with our data in Objective 1, it appears that incidences of zero reproduction are likely to be caused at least in part by female rejection of older males. While young males may reject some older females, it appears to be relatively rare.

This year, we concentrated on integrating the effects of male age on female reproductive rate in our model of the effects of delayed mating. We accomplished this by taking the current CM phenology model and partitioning the output so that the number of moths emerging per day could be tracked. This allowed us to take field temperatures from TFREC and our information on field longevity of CM (reported below) and determine the male age distribution of the population at any point in time. This was then combined with our laboratory information on the effect of male age on female reproductive rate. When this was done, we were able to show that male effects were relatively constant (they caused roughly a 15% decrease in female reproductive rate) after the peak emergence of CM (Fig. 4). Before peak emergence, the population was increasing so rapidly that the male population was heavily skewed towards those just emerging so the effects on female reproductive output are relatively minor. After peak emergence, the effects on reproductive output fluctuate only mildly, even at the times between generations.

Objective 3

Last year we finished the original objective and showed with lab studies that the effects of delayed mating occur on a degree-day (DD) basis. In those studies we took newly emerged individuals and placed them for a constant number of DD in either 15 or 30°C chambers. For both OBLR and CM, the reproductive rates were very similar even though the moths placed in the 30°C chambers were aged for two calendar days vs. 10 days at 15°C for the other treatment.

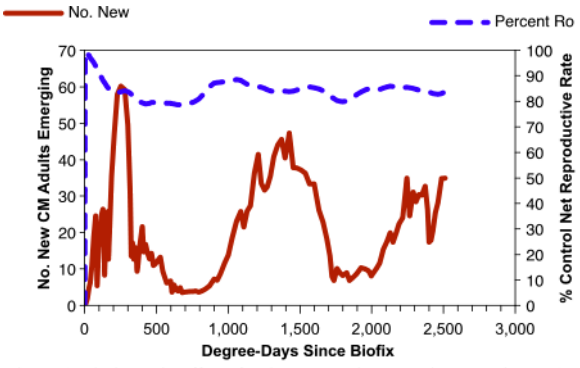


Fig. 4. Graph shows the effect of male age over the season in Wenatchee, WA based on phenology of emergence, temperature driven mortality, and male age on female reproductive rate. Solid line is the phenology of codling moth, dotted line the percentage of the reproductive rate of females mated to males on the day of emergence.

Fig. 5. Mortality rate of CM females in the field at TFREC in 2004-2005.

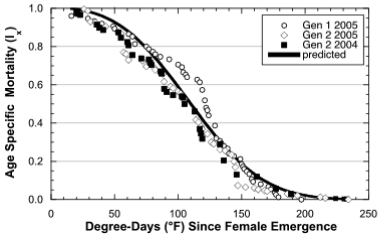


Fig. 6. Shape of the oviposition curve for CM females. Includes those with and without a delay in mating.

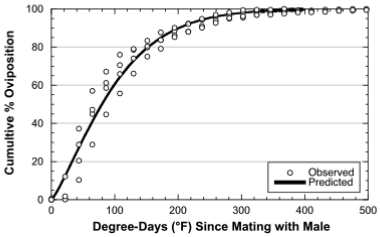
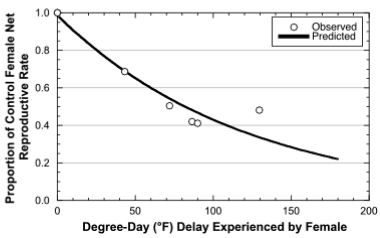


Fig. 7. Effect of delaying female CM mating on net reproductive rate. All rates scaled so undelayed females reproductive rate equals 1.



We also presented information that showed we were able to predict the longevity of adult OBLR and CM moths of both sexes based on DD (Fig. 5). In addition, we showed that the shape of the oviposition curve was easily predictable by the amount of time (measured in DD) since mating (Fig. 6). Finally, we were also able to show that the effect of a delay in mating could be predicted again using DD (Fig. 7).

Last year, we expanded our original objective to look at the effect of wind velocity on mating. This objective has both a laboratory and a field component. In our laboratory wind tunnel, we were able to get information on the males' ability to fly upwind and locate a pheromone lure at different wind speeds. In the field, we placed anemometers in three apple orchards of varying configurations and at different times throughout the spring and summer.

In the lab wind tunnels, we found that male CM were unable to fly against a wind of 3.3 mph; they were all sucked backward into the rear of the tunnel where the fan was housed. If the wind speed was reduced, it was not uncommon for those moths to emerge from the back chamber and fly towards the lure. None of the moths was able to orient and locate the lure above 2.2 mph in our wind tunnel tests. Between 40 and 50% of the moths were able to locate the lure at 1.1-2.2 mph (Fig. 8).

In our laboratory OBLR tests, we did find that a relatively small percentage of male moths (7.7%) was able to orient and successfully contact the lure at 3 mph but none at higher wind speeds. There was a strong drop off in ability to locate the lures as wind speed increased (Fig. 8).

In the field, we tested three apple orchards: Block 16 at the Tree Fruit Research and Extension Center (TFREC), a commercial orchard in East Wenatchee, and a commercial orchard in Quincy. The tests were run at different times of the season so some of the differences could simply be a result of prevailing weather conditions. The biggest differences among the orchards were that the TFREC orchard is planted at 9x18 feet (≈270 trees/acre), the orchard in East Wenatchee is planted at 5x15 feet (≈580 trees/acre), and the Quincy orchard is planted at 5x13 feet (≈670 trees/acre). Additionally, the tree rows at the TFREC and East Wenatchee sites are positioned perpendicular to the prevailing wind direction, and the tree rows at the Quincy site are positioned parallel to the prevailing wind direction. In all situations, one anemometer was placed at the upwind edge of the orchard, and the others distributed throughout the orchard. We moved the anemometers at roughly 1-2 week intervals.

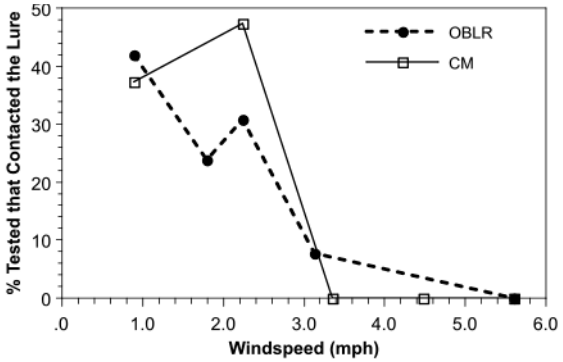


Fig. 8. Effect of wind speed on the ability of OBLR and CM to contact a lure in wind tunnel assays. All moths pre-conditioned to wind velocity for 15 min before flying.

Analysis. We analyzed the data by determining the daily time of sunset and examined the wind speed

for two hours before and two hours after sunset (this corresponds to most CM flight). We recorded the average wind speed at five-minute intervals (TFREC and East Wenatchee) and one-minute intervals at the Quincy orchard. Each day, we recorded the percentage of intervals in the four-hour flight period where the average wind speeds were >2.2 mph (speed above which CM were unable to locate the lure in our wind tunnel experiments).

Results. The TFREC orchard showed the highest wind velocities and the greatest percentage of times where moth flight would be unlikely to lead to mating. During the first period, the percentage of unsuitable times was between 75 and 95% of the entire flight period for anemometers on the edge or close to the edge of the orchard, with 55% being unsuitable for interior locations (Table 1). Over all sensor locations over the period of 14 June to 26 July, 48% of the time the average wind speed during the flight period was >2.2 mph (Table 1).

The orchard in East Wenatchee has a wind break about 150 feet in front of the block. At this orchard, we virtually never (0.3%) exceeded the 2.2 mph threshold for the average wind speed. However, the gust speed was over the threshold 38% of the time, with the greatest frequency in the interior of the orchard (Table 1).

The Quincy orchard is oriented so that the prevailing wind parallels the tree rows. In this configuration, the station on the edge of the orchard had the highest frequency of wind velocity exceeding the 2.2 mph threshold, as would be expected. The second highest total came from the station in the same row but 80 m towards the center of the block.

While it is tempting to assume that the differences in wind speed in the orchard are related to the different tree spacing, we cannot do so because the data were not taken concurrently. This means that some of the differences are likely driven by the seasonal and daily fluctuations occurring at the different times the stations were out, as well as the locations themselves. We expect that the tree density does affect weather readings and hence the percentage of times that the male moths can successfully locate calling females, but the exact contribution of tree density vs. location vs. time of the year cannot be determined from this study.

Of primary importance, our data do show that relatively modest wind velocities can dramatically affect the ability of male CM and OBLR to locate females. If wind velocities correspond to high temperatures over a key portion of the flight, the delay in mating may dramatically lower the reproductive capacity of the population, leading to lower pest pressure. Secondly, our data suggest that the edges of the orchard experience the greatest reductions in mating time because wind velocities are highest there. This is an interesting possibility because it would suggest that in windy

locations the upwind side of the orchard would have a sort of natural delay of mating and may not require heavy border treatments to reduce damage in those areas. Finally, in designing and planting orchards, maximizing wind flow may reduce the overall codling moth pressure by maximizing wind velocity.

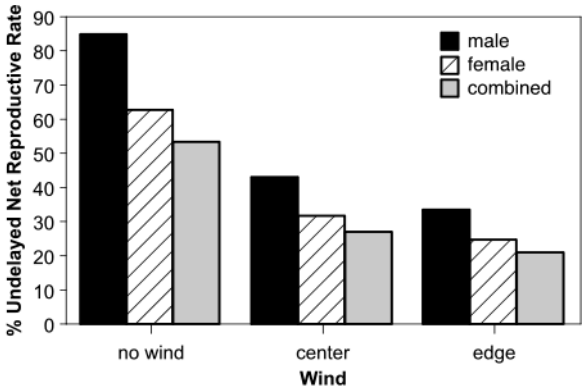


Fig. 9. Comparison of male and female age on net reproductive rate from 14 June to 26 July at TFREC. No wind shows effects of age alone, center indicates effects using wind speed in the interior of the block, edge using wind speed at the edge of the block.

To determine how important the delay in mating caused by wind could be, we used daily wind speed in our TFREC orchard to determine the reduction in female reproduction caused by wind speeds >2.2 mph. This model combined the wind speed information with the survival and age distribution of both males and females to determine the overall impact of the natural delay in mating as a percentage of the net reproductive rate of females that were able to mate on the day of emergence with newly emerged males.

The results of the model were evaluated by first considering the effects by sex separately, then combined, using no wind effects. Secondly, the model was run for the same period in the same fashion but incorporating the proportion of times that flight was inhibited by wind velocity >2.2 mph. The black bars on Fig. 9 show the effects of male age on female reproductive rate, the striped bars the effect of female age at time of mating, and the grey bars the combined effect on a female's reproductive rate during the period of 14 June to 26 July (the period for which we had wind data). Examination of the black bars shows that male effects alone cause a 15% reduction in population growth rate, female effects yield a roughly 37% drop and the combined effects result in a drop of about 47% compared to individuals that experience no delay. The wind effects are rather dramatic for both sexes, and both result in a decrease of >70% in the net reproductive rate for individuals that experience no delay.

Objective 4

Our studies on dispersal over the past two years showed that the scales of our experiments were too small because we still caught multiple marked individuals in the traps that were the greatest distance from our protein-treated areas. Even so, our studies showed that moths flew an average of 390 feet

Table. 1. Percentage of recording intervals occurring 2 hrs before and after sunset that had wind speeds >2.2 mph.

	% Intervals > 2.2 mph				
Dates	**Average[1]**	**Highest Sensor**	**Lowest Sensor**	**Location**	**Tree Spacing**
14 June to 19 June	82.2	95.1	55.6	TFREC	9 x 18
20 June to 12 July	32.1	51.9	6.9	TFREC	
13 July to 18 July	74.4	84	56.9	TFREC	
19 July to 26 July	45	49.4	41.4	TFREC	
27 July to 9 Aug	0.11	0.4	0	E. Wenatchee	5 x 15
14 Aug to 17 Aug	1.04	3.1	0	E. Wenatchee	5 x 13
21 Aug to 9 Sept	5.7	25.3	0	Quincy	5 x 13
[1]Average of all 6 sensors scattered throughout the orchard					

and we still caught multiple marked moths at distances >800 feet away. Our studies did suggest that there might be differences in the average flight distance between plots where Assail or Guthion were used as cover sprays. Parts of the experiments planned this year were to investigate if these differences in flight distance were related to cover sprays applied.

We set up three experiments to examine codling moth dispersal between MD and non-MD areas during the spring. Unfortunately, the warm period in the spring accelerated the emergence and although we were able to apply our markers, rain began and lasted for roughly two weeks on and off and dramatically affected both the flight and the retention of our markers. In addition, in an orchard where we had caught hundreds of moths last year we caught only 23 moths during the first flight this year, with over 140 traps placed. As such, in the second generation, we needed to find new sites and our planned experiments (looking at movement from MD to non-MD and the effects of cover sprays) were impossible to perform. Instead of the planned experiments, we concentrated on examining the role of CM movement from abandoned or poorly managed areas to well managed areas, which was easily testable during a single generation. Those experiments are reported in Objective 3 of our grant on dispersal (AE-04-428).

FINAL PROJECT REPORT
WTFRC Project Number: AE-06-604 (WSU Project 13C-3643-5367)

Project Title: Evaluation of tachinid parasitoids for OBLR in apples

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Budget History:

Item	Year 1: 2006
Salaries[1]	10,046
Benefits	1,707
Wages	6,240
Benefits	686
Equipment	0
Supplies[2]	1,000
Travel[3]	1,000
Miscellaneous	
Total	20,679

[1] Half-time Ag Project Assistant - Nik Wiman.
[2] Supplies include rearing supplies, routine lab and field supplies.
[3] Travel to in-state plots and vehicle costs.

Objectives:

1. Develop rearing methods for *Nemorilla pyste* and *Nilea erecta.*
2. Test the means by which the two tachinid flies locate leafroller hosts.
3. Examine the effect of Esteem® and Intrepid® on the tachinid parasitoids within the host larvae.

Significant findings:

- Both tachinid species can be reared in the lab using colony OBLR larvae as hosts. Colony-reared CM larvae are also suitable hosts for the flies, although tachinid parasitism of CM is unlikely to occur naturally in the field.

- Over the course of this study it was determined that *Nilea erecta* has a different mode of attack than previously reported in the literature; eggs are injected subcutaneously into the caterpillar and are not deposited externally on the cuticle. This suggests that its impact has been underestimated because there are no obvious indications of parasitism on the host caterpillar.

- With *Nemorilla pyste*, the time of attack has a strong influence on parasitism success. Eggs are deposited externally on leafroller larvae. If the eggs have not hatched by the time the molt takes place, they are shed along with the exoskeleton during the molting process and parasitism is unsuccessful. This may not be a problem with *Nilea erecta* because the eggs are inserted beneath the surface of the exoskeleton.

- *Nemorilla pyste* were long lived in the laboratory, and egg production occurred over most of adult female life.

- Flies of both species reared from OBLR treated with sublethal doses of Esteem® and Intrepid® developed more rapidly than flies reared from control larvae and did not demonstrate increased mortality in any lifestage. However, it appears that adult reproductive potential of *Nemorilla pyste* was reduced by exposure to Esteem®.

Objective 1. *Develop rearing methods for Nemorilla pyste and Nilea erecta.*

In 2006, 308 tachinid-parasitized leafrollers were recovered from eight commercial orchards, of which six were conventionally managed and two were organically managed. Parasitoids were collected from field populations of leafroller larvae and from sentinel OBLR larvae placed in the field. Parasitized larvae were distinguished by the presence of tachinid eggs on the larval body or on the cast cuticle or headcapsule of larvae and pupae. Field exposure to insecticides in both conventional and organic orchards may have been the cause of high mortality (62%) among the collected parasitized larvae; just 50 male and 45 female *N. pyste* and 14 male and 6 female *N. erecta* were successfully reared to the adult stage. Field-parasitized larvae were placed individually on pinto-bean diet in small cups. After leafroller pupation and tachinid emergence, tachinid pupae were removed from diet cups and placed in small Petri dishes where flies were reared to the adult stage. Species identifications were made using characters described in a recent taxonomic description and by comparison with voucher specimens identified by Dr. James O'Hara (Diptera Systematics Unit, Agriculture Canada).

Adult flies were sexed and were placed in cages with water and honey-water solutions. Each cage contained up to 20 adult flies at a 1:1 sex ratio. Colony-reared OBLR larvae were placed on shoots of apple foliage inserted into 100 ml water-filled tubes. These small artificial "trees" were placed into the cages and were removed daily so that all leafroller larvae could be checked for

parasitism. We have found that the tachinids will also directly parasitize hosts on artificial diet, without the presence of leaves, although rates of parasitism are somewhat lower. The artificial diet method will be used to keep tachinid colonies going through winter when apple foliage is in short supply. Initially, cages were exposed to indirect sunlight for part of each day to encourage mating, but when the season changed they were placed in an incubator (22˚C, 70% RH, 16L:8D). Magnetic fluorescent lighting in the incubator was supplemented with electronic fluorescent lighting, which flickers at a speed that exceeds flicker fusion rate of higher Diptera (≈250 Hz). More recently, flies are successfully being reared in growth rooms under the same photoperiod and humidity but with the addition of a halogen light source for several hours a day to simulate natural light. Mating of flies in the cages is typically observed during the period when the halogen light is on.

Parasitized OBLR larvae collected from the cages were placed on artificial pinto-bean diet in small cups, and their head capsule width was measured to determine larval instar. Host development was monitored daily until tachinids emerged or, in cases where parasitism was not successful, the adult leafroller emerged.

Results - Nemorilla pyste: Mating in cages was frequently observed two days after adult emergence, and parasitized larvae began to appear roughly three days after mating. The sex of emerging *N. pyste* adults was not related to the number of eggs per host larva or the size of larvae attacked. The maximum number of eggs per leafroller was 12, although the average was 2.1 (± 1.7) eggs among all larvae attacked (n = 567). Notably, the mean number of eggs from wild and sentinel *N. pyste* collections was 1.1 (± 1.01) eggs per larva, and the maximum number was eight. Eggs were typically oviposited dorsally on or near the pronotum and head, but eggs also occurred on other locations. The success of *N. pyste* parasitism increased as a function of the number of eggs oviposited on hosts (Fig. 1). As higher numbers of eggs per host imply greater competition between parasitoid larvae, this result was unexpected. This relationship may actually reflect host suitability, where the most vulnerable hosts are more highly targeted by female *N. pyste.* It is not known whether the eggs on superparasitzed larvae originated from the same or multiple females, but future observations will determine this. Other tachinid species have shown an aversion to attacking previously parasitized hosts, but the higher density of eggs may also be related to the relatively high number of parasitoids and the few OBLR larvae within the small cage.

Adult *N. pyste* were remarkably long lived under laboratory conditions (Fig. 2), and egg production occurred over most of adult female life. Future experiments will address survival of the tachinids in the field, as lab-derived longevity estimates are typically not realistic assessments of field survival due to the provision of nutritional supplements, constant temperatures, and lack of mortality factors that occur in

Fig. 1. Parasitism success of *N. pyste* as related to the number of eggs per host larva.

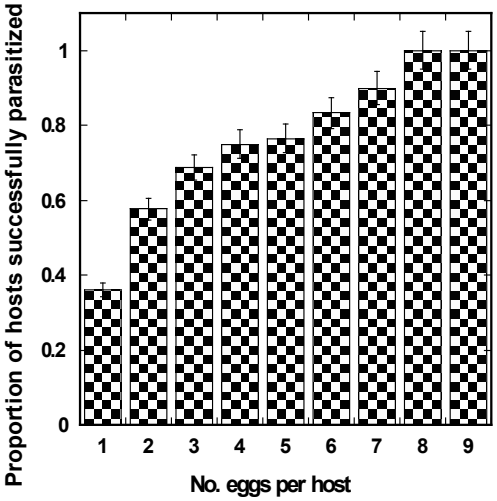
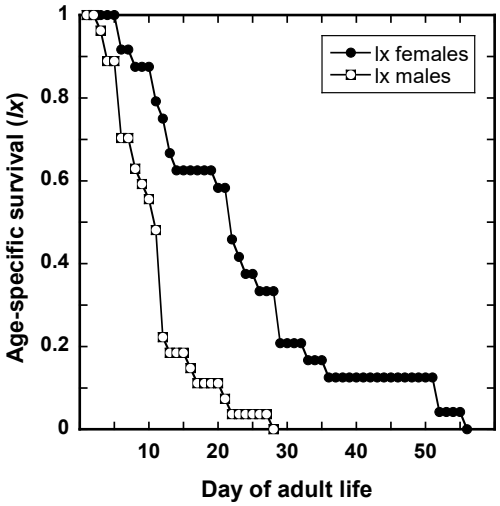


Fig. 2. Proportion of *N. pyste* surviving at different times after adult emergence in the lab.



the field. Parasitism success, which entails death of the host and subsequent emergence of at least one parasitoid, was surprisingly low (51%) in relation to the number of larvae attacked. From a population dynamics perspective, this low rate of success is compensated to some degree by the intermittent emergence of up to three parasitoids per host larva. However, those flies are often smaller and may have lower fitness and reproductive rates than singly emerging flies.

Explanations for the low level of parasitism success may be attributed to laboratory effects or rearing methods, *i.e.*, cage size, adult density, or functional dependence on the number of larvae available to the flies. However, under our hypothesis, the molting schedule of host larvae and the time required for *N. pyste* eggs to hatch determine the window of opportunity for successful parasitism. To determine the day of egg eclosion, parasitized larvae were dissected at different intervals from the time of parasitism. Preliminary results indicate that *N. pyste* eggs may require as many as six days of incubation on the host before hatching. At 22° C, OBLR larvae take four days as fourth instar larvae and nine days as fifth instar larvae (unpublished data). Although more dissection data are needed, our results to date suggest that the success of parasitism in *N. pyste* is highly dependent on the timing of oviposition as it relates to the host molting schedule; if the host caterpillar molts before egg hatch, the egg remains on the cast exoskeleton and parasitism does not occur. Given that leafroller larvae molt at increasingly longer time intervals as they approach pupation, this may partially explain why *N. pyste* females target later instar larvae.

Results – Nilea erecta: Although mating of *N. erecta* was observed in rearing cages, OBLR larvae were not visibly parasitized and therefore most larvae were not reared. This later proved to be a mistake when we found *N. erecta* emerging from OBLR that had been exposed to the adult flies in cages but had no eggs deposited on them. We found this contrary to literature predictions that *N. erecta* would oviposit externally on the host; it appears that *N. erecta* injects its eggs beneath the cuticle of the host. This finding has major implications for collecting and identifying leafroller larvae parasitized by *N. erecta* and explains why so few of this species were collected in 2006. Because larvae with external tachinid eggs were the only wild and sentinel larvae that were reared, larvae that yielded *N. erecta* had also been parasitized by an externally ovipositing tachinid species (*i.e.*, multiparasitism). With no clear external physical indication of *N. erecta* parasitism, experiments with this species will entail rearing of all leafroller larvae exposed to gravid adult females. Although internal incubation of eggs by female *N. erecta* requires greater female investment than externally deposited ones by other species, inserting the eggs may be a more effective mode of attack because eggs cannot be shed by hosts during molts, which appears to be a limitation for *N. pyste*. However, this strategy may also incur certain risks, such as longer exposure of the parasitoid larvae to the host immune system and the risk of pathogen-induced host death via the oviposition puncture. Nonetheless, we hypothesize that our rearing experiments will demonstrate higher rates of parasitism success with *N. erecta* compared to *N. pyste*, but we also expect lower rates of egg production due to higher maternal investment in eggs. These experiments are in progress.

Objective 2. *Test the means by which the two tachinid flies locate leafroller hosts*.

No progress has been made on this objective, but this work will proceed in the spring.

Objective 3. *Examine the effect of Esteem® and Intrepid® on the tachinid parasitoids within the host larvae.*

Insect growth regulator (IGR)-induced pest mortality is often delayed until specific phenological events such as pupation occur, and parasitoids may be exposed to the compounds through utilization of intoxicated hosts during this period. Field observations this year (reported above) suggest that tachinid parasitism of IGR-intoxicated hosts does occur in treated orchards. Exposure

of tachinid flies to Esteem® and Intrepid® is of concern because the larvae of tachinids are internal parasitoids which are exposed and respond not only to the hormones of the host but also potentially to these IGR compounds widely used for leafroller control. The mode of action of the IGR insecticides is to manipulate the level of two of the key hormones involved in the molting process, Juvenile hormone (JH) and molting hormone (MH); Esteem® mimics JH and Intrepid® mimics MH.

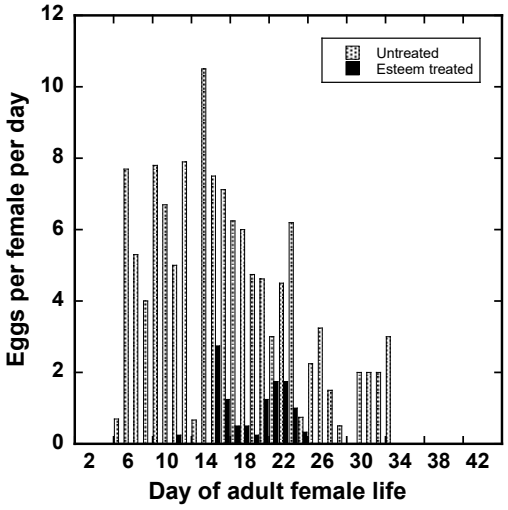
While studies have shown that JH and MH analogs generally have low acute toxicity to fish, birds, and mammals, some JH analogs are known to affect a range of non-target insects, including predators and parasitoids from disparate taxonomic groups. MH analogs such as Intrepid® are purportedly specific to Lepidoptera but may affect adult egg production and mortality in non-target insects. However, the effect of these compounds on internal parasitoids has rarely been considered.

We tested the effects of Esteem® and Intrepid® on the development of *N. pyste* by exposing host OBLR larvae to sublethal doses of the compounds and then allowed treated larvae to be parasitized by the flies. Leaf discs were punched from apple leaves using a 7 mm cork borer, and each was treated by application of 10 µl of solution. Solutions were either water (control) or sublethal rates of Esteem® or Intrepid®. Sublethal doses were defined as those that cause less than 10 to 12% larval mortality. All solutions included 80 ppm of the surfactant Sylgard® 309 to facilitate even dispersal over the disc surface. After solutions on the leaf discs had dried, discs were presented to leafroller larvae that had been starved for 24 hours. Larvae that fully consumed the disc within 24 hours were placed in tachinid rearing cages for parasitism. Parasitized larvae were then placed individually into cups provided with pinto-bean diet, and were reared in growth chambers (22˚C, 70% RH, 16L:8D).

Results - Esteem treatment: While OBLR larvae that were fed leaf discs treated with Esteem® took an average of ≈2 days longer to pupate than control larvae, the immature development time of *N. pyste* was significantly shorter. This result suggests that the parasitoids were directly affected by the treatment of their hosts with Esteem®. The direct effects of the JH analog on *N. pyste* were more important than the indirect effects from hormone-induced physiological changes causing delayed pupation of the host because, although the host responded with increased time as a larva, the parasitoid's development accelerated.

Mortality of *N. pyste* reared from treated larvae was not elevated in any development stage. The effects of Esteem® exposure were most pronounced during the pupal period of the parasitoid, which was significantly shorter (9.2 days) compared to parasitoids reared from untreated larvae (16.9 days). Unfortunately, adult reproductive potential was reduced by exposure to Esteem®-treated hosts (Fig. 3), perhaps because of premature development of reproductive structures or because of the importance of JH in egg maturation. Nine pairs of adult *N. pyste* males and females reared from Esteem®-treated hosts produced just 48 eggs on 30 larvae, of which only six larvae were successfully parasitized, or 0.66 fertile eggs per female over the course of adult life. Apparently both fecundity and fertility were affected by exposure to Esteem®, although more data will be needed to statistically compare reproductive data from tachinids reared from treated and untreated hosts.

Fig. 3. Egg production of adult female *N. pyste* reared from untreated and Esteem-treated OBLR larvae.



Results - Intrepid treatment: Results from early testing of sublethal doses of Intrepid® were remarkably similar to those obtained in the Esteem® tests, suggesting that the compound

affected parasitoids directly. It took significantly longer for intoxicated hosts to pupate and significantly less time for *N. pyste* to emerge from the host body than on non-intoxicated hosts. As with the Esteem® treatments, we also saw a significantly more rapid pupal development period of *N. pyste* (11.42 d) in the Intrepid® treatments compared to controls (16.91 d). Results from adult fertility and fecundity tests are in progress.

CONTINUING PROJECT REPORT **YEAR**: 3 of 3

Project Title: ULV microencapsulated sex pheromones for codling moth control

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Doug Light, USDA, ARS, Albany, CA; Tom Larsen, Suterra LLC, Bend, OR; and Bill Lingren, Trécé Inc., Adair, OK.

Budget 1:
Organization Name: USDA, ARS **Contract Administrator:** Carolyn Yager
Telephone: (509) 454-6576 **Email address:** cyager@yarl.ars.usda.gov

Item	**Year 1: 2005**	**Year 2:** 2006	**Year 3:** 2007
Salaries	14,000	14,000	0
Benefits	2,250	2,250	0
Wages	6,000	6,000	0
Benefits	1,000	1,000	0
Equipment	1,000	1,000	0
Supplies	2,000	2,000	0
Travel	1,750	1,750	0
Miscellaneous	0	0	0
Total	28,000	28,000	0

Footnotes: No-cost extension granted for 2007.

Objectives:

1. Evaluate the effectiveness of season-long ULV applications of a microencapsulated sex pheromone formulation (MEC) for codling moth and compare its performance against Isomate-C PLUS in replicated apple and pear orchards.
2. Evaluate the use of insecticides with ULV applied microencapsulated sex pheromone to control adults.

Significant findings:

- The effectiveness of a 5-spray ULV Checkmate CM F pheromone program was similar to 400 Isomate C PLUS in replicated 10-acre apple plots.
- The addition of either Assail or Asana to a ULV pheromone 5-spray program significantly improved the effectiveness of the pheromone alone program.
- The application of insecticides alone via a low volume application significantly reduced fruit injury.
- Mite populations were low in all treatments except the air blast applications of Asana. The ratio of predator mites to two-spotted mites was higher in all ULV insecticide treatments versus the air blast treatments.
- Laboratory assays suggest that Assail and Warrior are excellent materials to use as ULV treatments for codling moth.
- Traps baited with artificial leaves treated with 50 – 100 microcapsules were attractive for at least 3 weeks.
- The use of a visual detector triggered by tree trunks was used to increase the clumping of capsules on leaves (PULSV).

Methods:

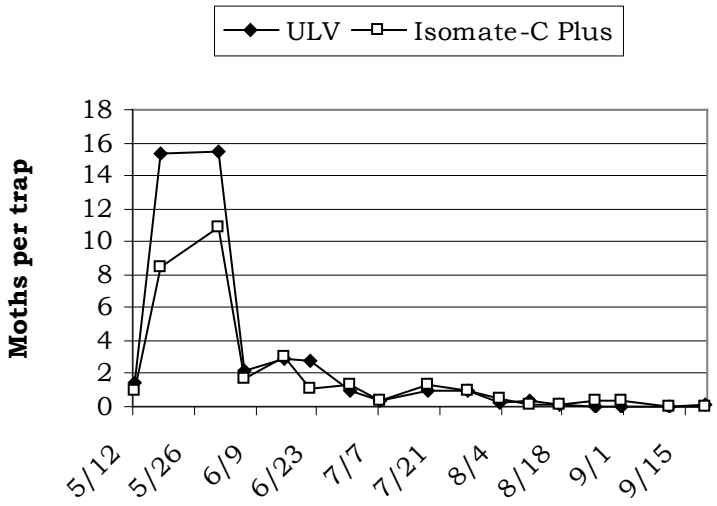
Laboratory studies funded by this project are continuing through April 2007. Continuation of these studies is dependent upon funding of a new proposal. Bioassays are being conducted with insecticides currently registered for use against codling moth to identify which materials could be the most effective if applied in low volume sprays for activity against adults (lethal and sublethal). Plastic cups are sprayed with various concentrations of each material and after drying one virgin pair of moths are placed in each cup. The mating status of females, moth longevity, and number of eggs deposited in cups are measured. Twenty replicates of each insecticide at 9 to 12 rates are being conducted.

Results and discussion:

Grower field trials.

Trials were established in six 20-acre apple orchards situated near Brewster, WA. Orchards were split into two halves and one half was treated with Isomate-C PLUS at 400 dispensers per acre and the other half with five ULV applications (1.25 gallons per acre) of 10 g A.I. Checkmate CM-F (Suterra Inc., Bend, OR) applied every 4 weeks. Orchards were monitored with sex pheromone-baited traps. Orchards were monitored for fruit injury at midseason and prior to harvest. All orchards received a full-season insecticide spray program. Levels of fruit injury were low in all blocks (< 0.2%), likely due to the heavy use of insecticides. Moth counts were somewhat higher in the ULV-treated plots early in the season perhaps reflecting the difficulty of using the ULV approach during this period of frequent rain showers (Fig. 1). In general, moth counts have been higher in the ULV treatment compared with Isomate-C Plus in other years, yet no difference in fruit injury occurred.

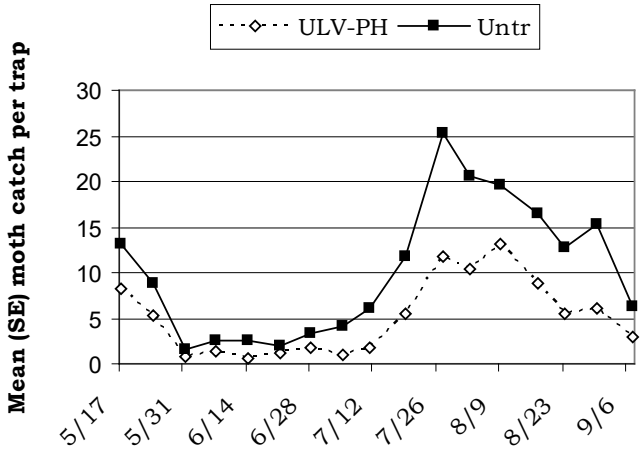
Figure 1. Weekly moth counts in replicated plots treated with either Isomate-C Plus dispensers or five ULV sprays of Checkmate CM F.



Small plot trials.
Studies were conducted in a 40 acre block of 'Red Delicious' with an extremely high population of codling moth overwintering from 2005. The orchard was divided into three sections (office, canal, and warehouse) and due to the difficulty in testing the effectiveness of sex pheromones separate tests were conducted in each section of the orchard. The first study was conducted in the office section of the orchard. This 28-acre section was subdivided into 32 plots and treatments were randomly assigned. Eight replicate plots were treated with five spray applications during the season on 10 and 30 May, 20 June, 17 July, and 14 August. All ULV plots were treated with insecticides with or without sex pheromone (10.0 g A.I.) in 1.25 gallons of water per acre. Sprays were applied with a GF120 sprayer at 20-25 psi. Three insecticides were evaluated with the addition of a microencapsulated sex pheromone (Checkmate CM-F) applied at 10 g. a.i. per acre: Imidan (1.0 lb per acre), Assail (1.7 oz per acre), and Asana (4.0 oz per acre). A second study was conducted in the 8-acre canal subplot again with 8 randomly assigned replicates of four treatments. These included the same insecticides applied as an ULV spray and an untreated control. A third test compared the use of air blast applications of insecticides versus the use of Isomate C+ dispensers at 400 per acre and an untreated control. One replicate of each treatment was included in each of the three orchard sections. Four applications of each of three insecticides were applied at a full rate using 100 gallons of water per acre with an air blast sprayer (Imidan at 5.0 lbs per acre, Assail at 3.4 oz per acre, and Asana at 8.0 oz per acre). These sprays were applied on 25 May, 19 June, 19 July, and 17 August. Fruit injury was assessed in mid-September. Twenty-four hundred fruits were picked by a field crew from the center of each plot and placed in a bin. Eight hundred fruit were then randomly checked from each bin. Statistical analyses were conducted separately for the pheromone alone versus pheromone plus insecticide treatments and for untreated and each of the insecticide alone treated plots. Data for the air blast treatments were compared with the Isomate-treated plots and other untreated plots.

Moth counts were not strongly reduced in the ULV pheromone-treated (office) versus untreated plots (canal) (Fig. 2). Yet, levels of fruit injury were lower among the pheromone-treated versus insecticide treated plots (Tables 1-3).

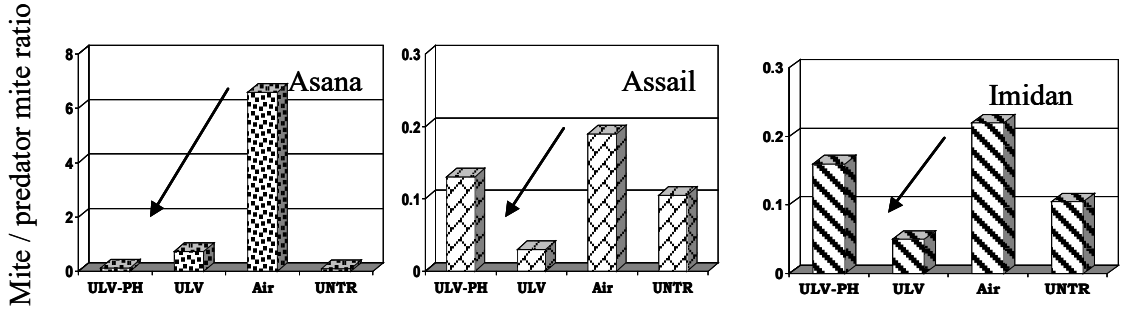
Figure 2. Seasonal moth catches in plots treated with ULV sprays of Checkmate CM F versus untreated plots.



The addition of the insecticides to the sprayable pheromone significantly reduced levels of fruit injury (Table 1). The use of ULV-applied insecticides alone also significantly reduced fruit injury (Table 2). Air blast applications of insecticides significantly reduced fruit injury from the untreated control and versus the Isomate treatment (Table 3). No statistical differences were detected among insecticides in any of these three tests though numerically the Imidan treatments always had the highest mean levels of fruit injury. Unfortunately, due to the experimental design mean levels of fruit injury could not be compared between tests but it is interesting to note that the lowest levels of injury occurred either with a 5-spray ULV program of pheromone plus a reduced rate of Assail (Table 1) or the 4-spray air blast program with either Asana or Assail applied at full label rates (Table 3).

Samples were collected for both mites and white apple leafhopper in these treatments. Leafhoppers at the end of the season were reduced 98% in the Assail versus the untreated controls in both the canal and office plots. Imidan reduced leafhoppers by approximately 75% and Asana reduced population densities by only 50%. Phytophagous mite population densities remained low in all plots during the season; however densities of two-spotted mites were somewhat higher in all three of the Asana-treatments (still < 1.0 mite per leaf). Population densities of predator mites (*Zetzellia mali* and *Galandromus occidentalis*) were generally higher than the phytophagous mites in all samples across treatments. However, there was a slight reduction in the density of predators in the Asana treatments. An interesting trend of lower mite to mite predator ratios was seen with all three insecticides (Fig. 3)

Figure 3. Mite / mite predator ratios in blocks treated either with air blast or ULV spray applications of Imidan, Asana, or Assail.



Further studies are needed with Assail and synthetic pyrethroids to address this potential benefit of the ULV approach on biological control.

Table 1. Comparison of the use of ULV sprays with sprayable pheromone alone versus adding reduced rates of insecticides, five applications were made, Office block, n = 8.

Treatment	Mean (SE) % fruit injury
ULV pheromone only (10 g a.i.)	15.2 (4.7)a
3.0 oz Asana + pheromone	5.5 (1.6)b
1.7 oz Assail + pheromone	3.4 (0.9)b
1.0 lb Imidan + pheromone	9.2 (1.3)ab
ANOVA df = 3, 28	$\boldsymbol{F}$ = 5.02, $\boldsymbol{P}$ = 0.007

Table 2. Comparison of the use of five ULV applications of insecticides alone versus no treatment, Canal block, n = 8.

Treatment	Mean (SE) % fruit injury
Untreated	32.5 (5.1)a
3.0 oz Asana	9.5 (2.0)b
1.7 oz Assail	9.8 (1.5)b
1.0 lb Imidan	13.8 (2.0)b
ANOVA df = 3, 28	$\boldsymbol{F}$ = 14.9, $\boldsymbol{P}$ < 0.0001

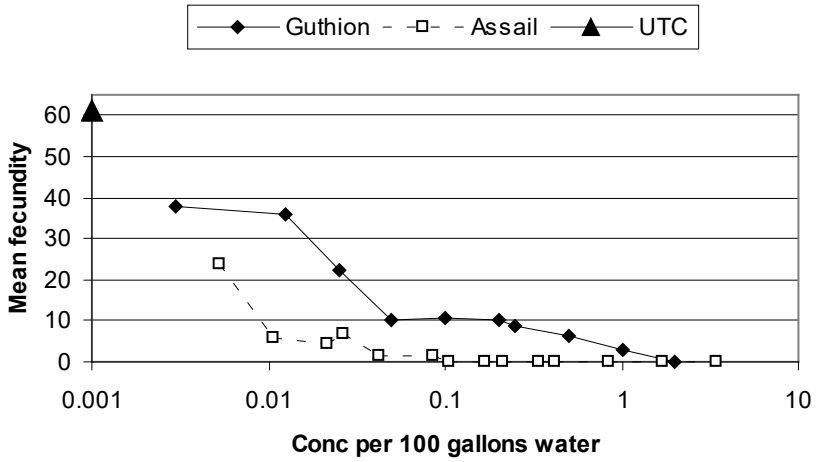
Table 3. Comparison of four applications of three insecticides made with an air blast sprayer versus the use of Isomate C+ alone and no treatment.

Treatment	Mean (SE) % fruit injury
Untreated	82.0 (2.1)a
Isomate only	51.0 (3.1)b
6.0 oz Asana	5.1 (1.4)c
3.4 oz Assail	3.5 (0.2)c
5.0 lb Imidan	7.7 (4.1)c
ANOVA: df = 4, 10	$\boldsymbol{F}$ = 109.0, $\boldsymbol{P}$ < 0.0001

Future studies will evaluate the use of Warrior which is currently the only insecticide whose label allows it to be applied by ground in low spray volumes (2.0 GPA). Fortunately, Warrior looks to be very effective in reducing female fecundity in cup bioassays at extremely low rates. Battalion (deltamethrin) which is purported to be less disruptive of mites will also be tested.

Studies conducted with Asana in 2005 showed that moth exposure to low levels of this material had dramatic reductions in female fecundity even though males could still respond to a pheromone source and adult longevity was only moderately reduced. This was proposed as how the ULV spray applications could be effective in reducing fruit injury by 98% in 2005. Current studies have found that Assail has a similar affect on codling moth and at even lower rates relative to the field rate. When compared to Guthion one can see that Assail strongly suppresses moth fecundity at rates reduced nearly 100-fold from its field rate (Fig.4). Many of the other insecticides tested, such as Success, Intrepid, Esteem, Rimon and Altacor, have had little effect on moth fecundity in these assays and would likely not be effective as an ULV spray application. Further studies with Assail will look at

Figure 4. The effect of insecticide rate for Guthion and Assail on codling moth female fecundity in treated plastic cups.

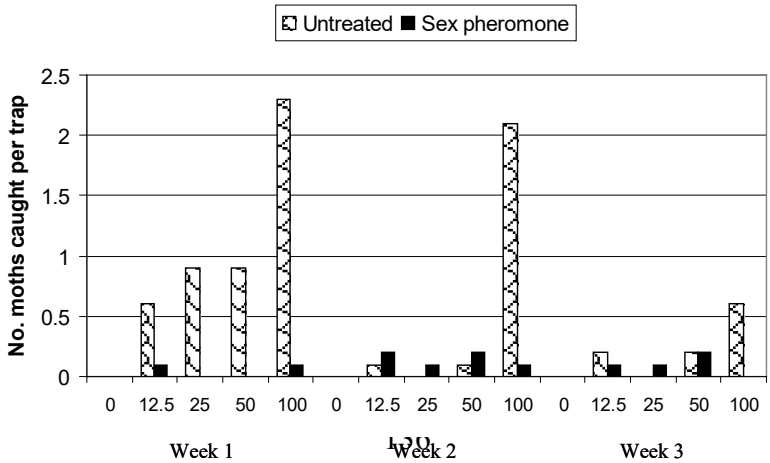


Data collected so far suggests that ULV spray applications may allow these compounds to be used without any associated flare-up of mites. Numerically a five spray program using 8.5 oz of Assail was equivalent to a four spray program using 13.6 oz in 2006. The use of the GF120 sprayer allows growers more flexibility in spraying based on seasonal thresholds and reduces the cost of application as the ATV can be driven at 8 mph through most orchards versus the standard 2 mph of tractor-pulled sprayers. Further testing is needed to assess the optimal rate of these insecticides and to further evaluate their potential effects against secondary pests.

Attraction of Sprayed Leaves.

Previous studies showed that traps baited with artificial leaves treated with 5 or more capsules can catch moths and elicit moth orientation and landing in a flight tunnel. Studies conducted in 2006 found that leaves were attractive for at least 3 weeks (unfortunately the study was terminated by the end of the season). Interestingly, there was some indication that within a block sprayed with sex pheromone, individual leaves become more apparent as point sources during the second and third week (Fig. 5). These data are consistent with results from 2004 and suggest that the success of the ULV approach is due to both disruption and enhanced competition by false trail following. The addition of the bisexual attractant pear ester and the use of insecticides could make this a novel attract and kill approach.

Figure 5. Moth catch in traps baited with artificial leaves sprayed with variable numbers of microcapsules.



PULSV (Pulsed Ultra Low Spray Volume).
We contracted with Edwards Manufacturing (Prosser, WA) to have a long-range (2.5 m) detector attached to an ATV to allow the pulsing of sprays (PULSV) when the trunk of a tree was detected. PULSV was used to concentrate sprays into the upper center of the tree's canopy. This allowed us to reduce the total spray volume per acre depending on the orchard's tree and row spacing (Fig. 6). When we applied the same amount of pheromone per acre in a 16 x 16' orchard (170 trees per acre) this increased the concentration of microcapsules by nearly 3-fold on leaves (Fig. 7).

PULSV or the use of alternate middle sprays can enhance the attractiveness of individual leaves by increasing the density of microcapsules and thus may improve the attract and kill approach. PULSV requires that orchards maintain a reasonable under-tree weed management program.

Figure 6. The reduction in spray volume applied with the PULSV versus the ULV spray application in orchards with different tree and row spacing.

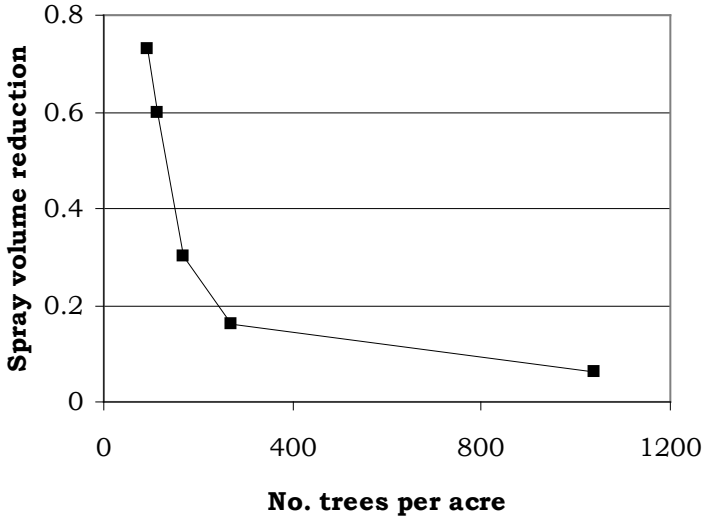
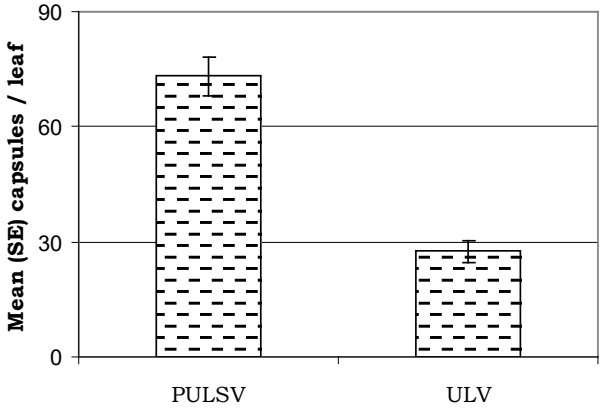


Figure 7. The mean density of microcapsules per leaf deposited with the PULSV versus the ULV spray application.



CONTINUING PROJECT REPORT **YEAR**: 3 of 3

Project Title: Direct control of codling moth with pear ester

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Address: 5230 Konnowac Pass Rd
City: Wapato
State/Province/Zip WA 98951

Cooperators: Doug Light, USDA, ARS, Albany, CA; Bill Lingren, Trécé Inc., Adair, OK; Rick Hilton and Phil VanBuskirk, Oregon State, Medford, OR

Budget 1:
Organization Name: USDA, ARS **Contract Administrator:** Carolyn Yager
Telephone: (509) 454-6576 **Email address:** cyager@yarl.ars.usda.gov

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	8,200	8,200	0
Benefits	1,300	1,300	0
Wages	0	0	0
Benefits	0	0	0
Equipment	0	0	0
Supplies	2,200	1,700	0
Travel	800	800	0
Miscellaneous	0	0	0
Total	12,500	12,000	0

Footnotes: A no-cost extension was granted for 2007.

Objectives:

1. Evaluate the use of a microencapsulated pear ester formulation in combination with Imidan for control of codling moth.
2. Evaluate the use of hand-applied dispensers loaded with sex pheromone and pear ester in small plot and large-scale orchard trials.
3. Evaluate the use of insecticide-treated killing stations (AKISS) baited with combo pheromone / pear ester lures.

Significant findings:

- ❖ A combo 'puzzle-piece' dispenser loaded with sex pheromone and pear ester effectively shut-down traps baited with virgin female moths all season and outperformed the Checkmate dispenser late in the season.
- ❖ The Cidetrak Combo dispenser is not attractive and reduces close-range moth orientation.
- ❖ The CM-DA and CM-DA Combo lures remained effective for at least 7 weeks. The Combo outperformed the Biolure 10X lure in orchards treated with 400 dispensers/acre.
- ❖ AKISS performed poorly in 2006 due to short-lived residual toxicity and attractiveness
- ❖ Moth catches were significantly lower in orchards treated with the puzzle piece than comparable blocks treated with Isomate-C Plus. No difference in fruit injury were found in orchards treated with either dispenser type.
- ❖ A microencapsulated pear ester formualtion effectively reduced codling moth injury when added at rates of 12 – 24 ml/100 gallons to half rates of Imidan.

Methods:

Laboratory studies funded by this project are continuing through April 2007. Continuation of these studies is dependent upon funding of a new proposal. Several types of behavioral studies are being pursued to improve our understanding of how pear ester impacts moth and larval behavior. Flight tunnel tests with adult male moths are examining the influence of adding pear ester to a range of sex pheromone concentrations that are below or above the optimal rate that elicits upwind orientation (enhanced false trail following). In other tests, male moths are flown to an attractive sex pheromone source in the flight tunnel after a brief exposure to high concentrations of pear ester or sex pheromone alone, or to several blends of these compounds (habituation or adaptation). Studies will be started in March with Dr. Light to define the orientation responses (false trail following / habituation) of codling moth larvae to different concentrations and distributions of microcapsules. Subsequent assays will use leaves from several major apple, pear, and walnut cultivars to examine larval behaviors (wandering time and distance) to various rates of the DA-MEC alone and in combination with insecticides.

Results and Discussion:

Cidetrak dispensers – small plots. Randomized plots were established in a heavily-infested grower orchard situated near Wapato. Five replicates of each treatment were included in the study and plots were about 0.25 acres and separated by < 20 m. Five delta traps baited with two virgin female moths and a single trap baited with a sex pheromone lure were placed in the center area of each plot and checked weekly for 14 weeks. Female moths were replaced each week. Moth counts were significantly higher in the untreated plots. No significant differences were found among pheromone treatments. This is likely due to the low number of replications and the variable moth pressure across the study site. However, the data suggested that the Cidetrak Combo dispenser applied at 400/acre provided the most effective disruption of traps (Table 1). In addition, the Checkmate CM dispenser appeared to lose its ability to disrupt the lure-baited but not female-baited traps beginning in mid-July. These preliminary data with the new Cidetrak dispenser are encouraging. In particular, the Combo dispenser appeared to be highly effective all season.

Table 1. Evaluation of Cidetrak dispensers in replicated small plots, Wapato, WA

Treatment	Proportion of virgin female-baited traps catching males		No. males caught per trap type			
			Virgin female-baited		Lure-baited	
	1st gen.	2nd gen.	1st gen.	2nd gen.	1st gen.	2nd gen.
Untreated	0.17b	0.44b	2.1b	15.5b	57.3b	209.8b
Cidetrak pheromone (400/acre)	0.01a	0.13ab	0.04a	0.7a	1.8a	16.0a
Cidetrak Combo (200/acre)	0.0a	0.06a	0.0a	0.6a	2.0a	21.2a
Cidetrak Combo (400/acre)	0.01a	0.06a	0.04a	0.3a	0.4a	2.4a
Checkmate CM (200/acre)	0.0a	0.06a	0.0a	0.6a	4.6a	50.8a

Column means followed by a different letter were significantly different, $P < 0.05$.

Adult responses to Cidetrak dispensers.

Cidetrak dispensers loaded with both sex pheromone and pear ester were not attractive when placed in delta-shaped traps in studies conducted during June within a 0.25 acre plot treated with dispensers (Table 2). Furthermore, moth catch was reduced when traps were placed at the same height as a function of decreasing distance, i.e. 0.1, 1.0, and 2.0 m. Traps placed 2.0 m from the dispenser but 1.0 m lower in the canopy caught fewer moths than traps placed higher and also 2.0 m from dispensers.

Table 2. Influence of trap distance from a combo Cidetrak dispenser in the canopy of an 0.25 acre orchard plot treated with dispensers.

Distance below dispenser (m)	Distance from dispenser (m)	Mean (SE) moth catch per trap
0	0	0.0 (0.0)a
0.1	0.1	0.3 (0.2)a
0	1	1.2 (0.6)a
0	2	2.8 (1.0)b
1	1	0.9 (0.5)a
1	2	1.0 (0.3)a

A second study was conducted to evaluate the attractiveness of Cidetrak dispensers from 12 July to 29 August. Ten replicate traps were baited with either Cidetrak pheromone or Cidetrak combo dispensers. Moths were counted and sexed each week. Moth counts each week were compared with counts in similar traps (n = 2) baited with a pheromone, a pear ester, or a combo lure in another area of the orchard. Few moths were caught in traps baited with the Cidetrak Combo dispenser and counts were higher in traps with pear ester lures (Table 3). Moth catches in traps baited with the pheromone dispenser were about one third of catches with either pheromone or combo lures. These data were enlightening for two reasons. The Cidetrak pheromone dispensers were more attractive than what I found in previous studies with Isomate-C Plus. This suggests that either this dispenser has a lower emission rate or a higher purity of sex pheromone than the industry standard. Second, the lack of a moth response to the traps with the Cidetak Combo dispenser suggests that the addition of pear ester in this dispenser does not increase false trail following but is likely causing adaptation of the antennae or habituation in the central nervous system in male codling moth. Data on the emission rate of aged dispensers will be made available by the manufacturer this winter and are critical in addressing how these dispensers may be impacting codling moth's behavior.

Table 3. Moth catches in delta-shaped traps baited with either standard lures or the Cidetrak pheromone or Cidetrak Combo dispensers.

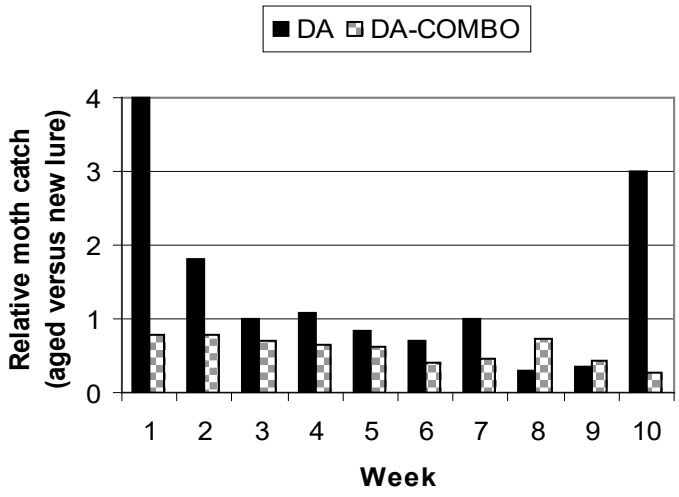
Date	Pheromone lure	Pear ester lure	Combo lure	Pheromone Cidetrak dispenser	Combo Cidetrak dispenser
19 July	93.5	9.5	78.0	27.1b	2.8a
26 July	74.5	4.5	69.0	29.0b	2.8a
3 Aug	99.0	3.0	60.5	23.2b	2.2a
10 Aug	61.0	0	35.0	9.0a	1.0a
17 Aug	22.5	1.5	17.5	9.3a	1.6a
23 Aug	58.5	0.5	35.0	16.0b	1.0a
29 Aug	57.5	4.5	35.0	6.8a	0.8a

ANOVA was conducted only to compare the Cidetrak dispensers.

Field-life of dispensers containing pear ester.

Both the DA and the DA COMBO lures are considered to be long-lived but no studies have addressed their effectiveness over time. I monitored 15 replicates of aged versus new lures of each type for 10 weeks in late summer. The data showed that the DA lure emission rate for new dispensers is likely too high and aged dispensers were more attractive for the first two weeks. Then, out to seven weeks the aged lures were similar in attractiveness to new ones; and less attractive at week 8 and 9. Moth catches in week 10 were very low and the comparison is not valid. The attractiveness of a new combo lure has an initial burst and then the attractiveness of aged lures has a gradual decline out to week 10.

Fig. 1 Relative comparison of field-aged versus new DA and DA-COMBO lures to codling during the second moth flight.

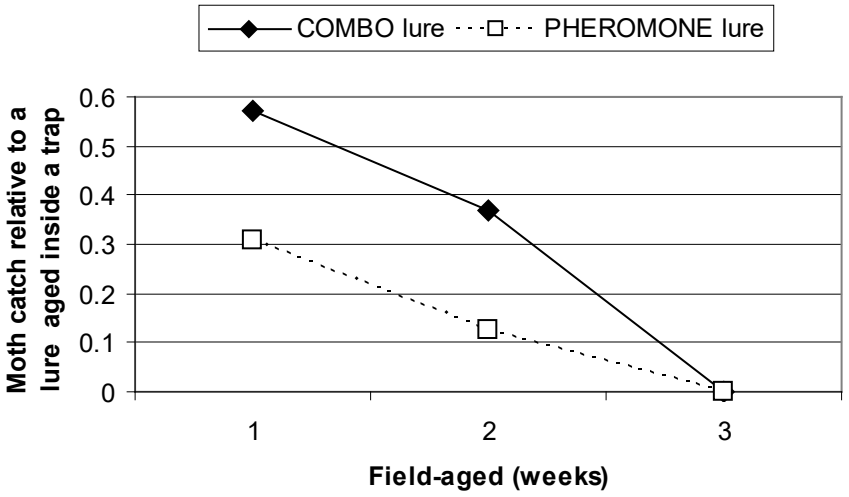


Studies with AKISS

The AKISS is being developed as an effective approach to manage codling moth along orchard borders and near bin piles. The use of these insecticide–treated devices baited with DA-COMBO lures were evaluated in 2006 in both small plots and in orchard trials. Stations were constructed of bird netting treated with a 10% Asana plus sticker residue. Flight tunnel bioassays demonstrated that these stations did not maintain a toxic residue beyond 2-3 weeks. Efforts to retreat the stations using a spray bottle attached to a telescoping pole were fairly ineffective. In addition lure studies found that gray septa loaded with either sex pheromone or sex pheromone and pear ester were attractive for only

a few weeks (Fig. 2). Also observational studies found that the bird netting was not an effective landing source for moths under field conditions. A new approach is planned for 2007 using clear plexiglass panes treated with a commercial foam formulation of deltamethrin and retreated with an adapted paint roller every 2-4 weeks as needed. The combo lures will be placed inside of a sunscreen to extend their longevity.

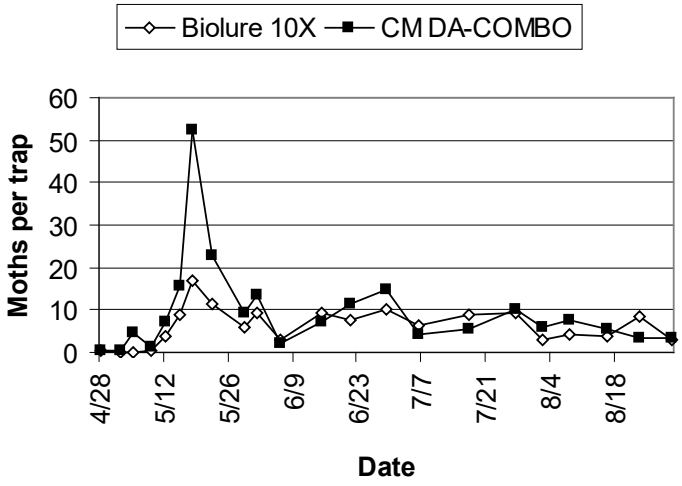
Figure 2. Attractiveness of lures placed in full-sunlight on an AKISS.



Lure Comparisons

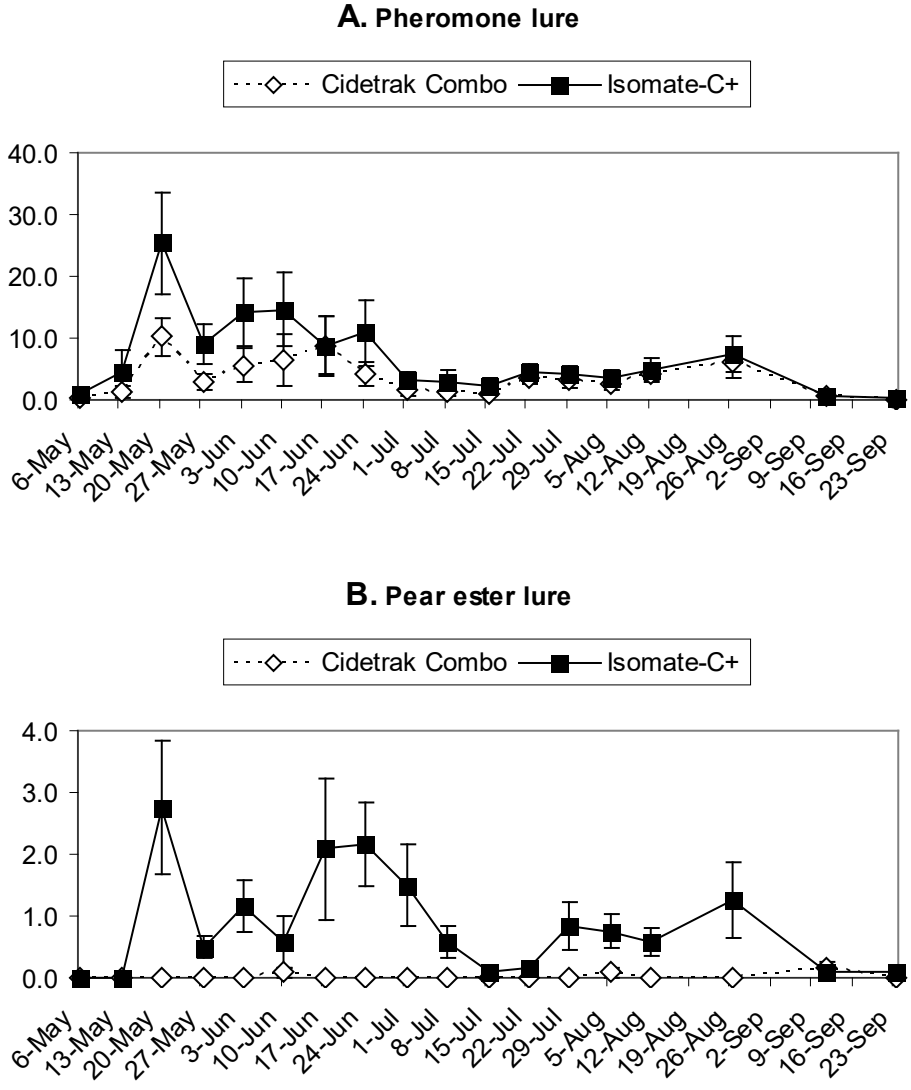
The two most effective lures for monitoring codling moth in sex pheromone-treated orchards in my previous trials have been either the Biolure 10X membrane lure or the Pherocon CM-DA COMBO gray septa lure loaded with moderate rates of sex pheromone and pear ester. However, most of these trials have been conducted in orchards treated with a reduced rate of dispensers (200 – 300 / acre). During 2006 we conducted a comparison of these lures in replicated grower orchards in Brewster all treated with 400 Isomate-C Plus dispensers per acre. The COMBO lure was much more attractive than the Biolure during the peak flight in the first generation (Fig. 3). Both lures were replaced after 8 weeks and caught similar numbers of moths in the second flight. Moth catch was much lower in the second flight due to the use of multiple insecticide spray covers in these orchards.

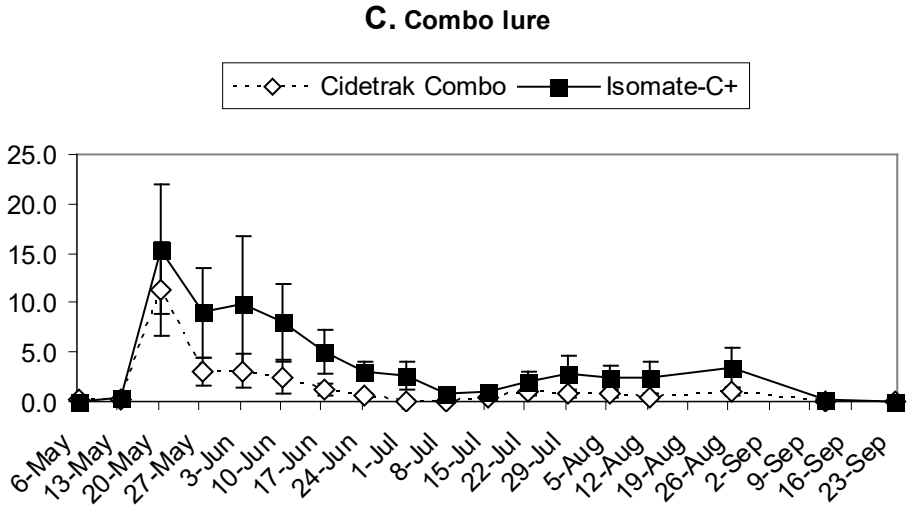
Figure 3. Comparison of two lures for codling moth in sex pheromone-treated orchards.



Orchard trials with Cidetrak.
Three formulations of the new Cidetrak dispenser were evaluated in replicated 5-acre grower orchards and compared with Isomate-C Plus dispensers. Plots were monitored with a pair of traps baited with pheromone lures, DA lures, and DA-COMBO lures. Orchards were also heavily sprayed in 2006 and levels of fruit injury were low (ca. 0.4%) and not different between treatments at harvest. Data only for the standard Cidetrak Combo dispenser versus Isomate-C Plus are shown here. Moth counts were generally higher in the pheromone-baited and Combo-baited traps during the first flight in the Isomate-treated plots. A significant difference in moth catches in the pear ester-baited taps occurred between dispenser types.

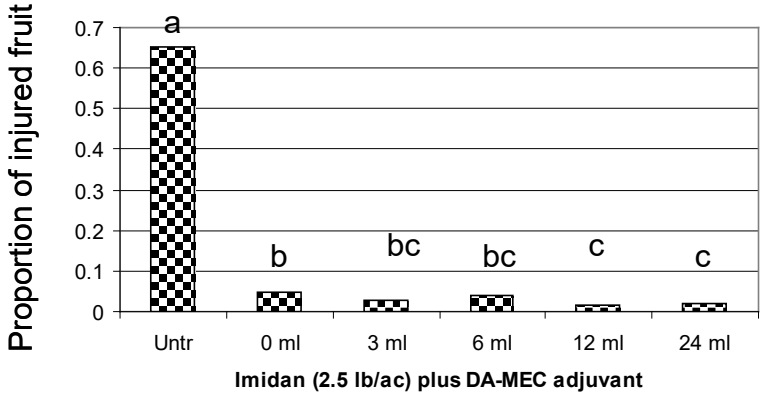
Figure 4. Comparisons of moth catches in traps baited with sex pheromone (A), pear ester (B), or Combo lures (C) in replicated 5-acre blocks treated with either Isomate-C Plus or Cidetrak Combo dispensers (400/acre).





DA-MEC Plus Imidan.
Spray trials were conducted with Imidan 50WP applied at a half rate (2.5 lb/acre) with and without the addition of the DA-MEC adjuvant. Four rates of the DA-MEC were evaluated and 13 replicates of each treatment were included in the study. Fruit injury averaged 65% in the untreated plots and all insecticide-treated plots had significantly lower levels of fruit injury (Fig. 5). The two highest rates of DA-MEC were significantly better than the lowest rate. Similar results were found in trials using Guthion last year.

Figure 5. Spray trials comparing the effectiveness of adding the DA-MEC as an adjuvant to a reduced rate of Imidan.



Summary
A number of very important uses of pear ester to manage codling moth continue to be investigated. The subtlety of the impacts of this kairomone on the behaviors of codling moth adults and larvae are not understood. Further studies that address the mechanisms of its activity are needed to optimize these management approaches.

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AE-06-601 (WSU Project No. 13C-3643-7387)

Project Title: Reinstating integrated mite control in apple orchards

PI: Elizabeth H. Beers
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Budget 1:
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Telephone: 509-335-7667; 509-663-8181 x221 **Email:** (mderos@wsu.edu; (saray@wsu.edu)

Item	Year 1: 2006	Year 2: 2007	Year 3: 2008
Salaries[1]	9,374	**9,754**	
Benefits	904	**917**	
Wages[1]	7,800	**8,400**	8,400
Benefits	780	**966**	966
Equipment			
Supplies[2]	1,500	**1,500**	
Travel[3]	1,923	**2,820**	500
Miscellaneous			
Total	22,281	**24,357**	9,866

[1] Master Graduate Student (Ag Project Assistant): 1 semester (each) is being requested from WSCPR and WTFRC; benefits include health insurance and 1.5% medical aid. Funds are also being requested for summer wages for Master Graduate Student ($15/hrx560 hrs); benefits are calculated at 11.5%.

[2] Supplies are for field plot and bioassays, including those for marking and spraying plots, sampling mites, and mite rearing and bioassay materials. Cell phones are authorized under this project.

[3] Travel (WTFRC) is lease of WSU motor pool vehicle during field season (including gas and mileage) for travel to off-station plots; travel (for WSCPR) is personal vehicle mileage travel to Wenatchee from Pullman during the academic year.

Objectives:

1. Determine the effect of multiple-season applications of newly registered insecticides used for codling moth control (Assail, Calypso, Rimon) on integrated mite control.

2. Determine the effect of sulfur-containing pesticides on the components of integrated mite control.

3. Determine the additive effect of varying numbers and classes of potentially disruptive materials on integrated mite control.

Proposed schedule of accomplishments:

1. Large-scale field plots were set up in the spring of 2005. They will be monitored for 3 years (2005-2007).

2a. Year 1: test methodology for bioassays. Years 2-3: Complete bioassays, summarize results.

2b. Field studies will be conducted in years 1-3. Treatments will vary depending on grower/consultant input and results of previous trials.

3. Small-plot trials will be conducted in years 1-3. Treatments will vary depending on grower/consultant input and results of previous trials.

Significant findings:

1. In the large-scale commercial orchard trials, 5 out of 6 orchards experienced a mite outbreak in one or more of the treatments Rimon caused an elevated tetranychid mite level in five orchards, Assail in two, and Calypso in three.

2. Additive effects were found where the newer codling moth materials were used in conjunction with apple thinning materials. Only treatments containing a codling moth insecticide plus lime-sulfur plus carbaryl caused elevated mite levels; treatments with just the codling moth insecticides or CM insecticides+lime sulfur did not. Lime-sulfur and carbaryl used with the OP standard (Imidan) also caused higher mite levels, indicating that the two thinning materials alone are sufficiently disruptive to cause flare-ups.

3. All sulfur-containing treatments suppressed rust mites in relation to the check. Lime-sulfur had the greatest detrimental effect on rust mites, with flowable sulfur intermediate; ATS had the least effect. All sulfur treatments suppressed predatory mites also and to about the same degree.

Methods:

1. Large-scale field plots: Large (1-4 acres) plots for each treatment will be set up in commercial orchards using three potentially disruptive codling moth materials (acetamiprid [Assail], thiacloprid [Calypso], and novaluron [Rimon]) and a non-disruptive standard organophosphate program (either azinphosmethyl [Guthion] or phosmet [Imidan]). Individual grower orchards (8-10 orchards distributed throughout central Washington) will serve as replicates. [Note: the same treatments will be applied to the same blocks for a second year to determine multiple season or carryover effects]. Phytophagous mites (tetranychid and eriophyid) and their natural enemies will be sampled at regular intervals throughout the season. Calculated parameters will be cumulative mite days, a measure of

population density over time. Comparisons among treatments and blocks will be made using analysis of variance (ANOVA) for randomized complete block (RCB) design.

2a. Laboratory studies: The effect of sulfur compounds on mortality and fecundity (and, in the case of predators, prey consumption) of mites will be examined. Three mite species (twospotted spider mite, European red mite, and western predatory mite) will be exposed (topical/residual) to sulfur compounds used commonly in tree fruit (lime-sulfur, micronized sulfur, and ammonium thiosulfate). Mortality will be assessed after 24 h. Fecundity of mites exposed to residues of the compounds will be evaluated over a 10-day period, using a cohort of 24- to 48-h-old females. Predatory mites will be starved for 24 h, after which prey consumption will be measured (with or without presence of sulfur compound residues) over the next 24-h period using eggs of twospotted spider mite as a prey source.

2b. Field studies: Naturally occurring populations of rust mites, predatory mites, and tetranychid mites will be treated with sulfur compounds in small-plot trials (1-3 trees) with 4-6 replicates. Mites will be sampled before and at intervals after treatment. Pre-treatment counts will be used to randomize treatments within blocks.

3. Small-plot field trials: Various combinations of products potentially disruptive of IMC will be assessed in small plots. Different products will be applied to small plots (3-9 trees) replicated 3 to 4 times at typical times and frequencies during the season. Programs will start with a base of codling moth control program (neonicotinyl, Rimon, organophosphate) and add increasing numbers of other disruptive materials (lime-sulfur used as a fruit thinner, carbaryl used as a fruit thinner, micronized sulfur used as a fungicide). Mite samples (pest and predatory) will be taken at intervals throughout the season. Cumulative mite days (CMD) will be calculated for each mite species and treatment.

Results and discussion:

1. Large-scale field plots.
Tetranychid mite densities were elevated in one or more treatments in five out of six orchards in 2006. Only the Butte orchard (Chelan Butte) had no measurable increase in mites throughout the season. This is in contrast to the results from 2005, where only one orchard in five experienced high mite levels. Peak densities in 2006 in the affected orchards occurred in July and August and ranged from 20-80 mites/leaf (Fig. 1). The Assail treatment was associated with elevated mite populations in two orchards, with a smaller peak <10 mites/leaf) in a third orchard. The Calypso treatment had elevated mite levels in three orchards, ranging from 10-20 mites/leaf. Five of the Rimon-treated plots had elevated levels of mites, while only one of the OP-treated plots had a severe mite outbreak Even this single incidence of a mite outbreak in the OP plot is unexpected since OPs are typically not disruptive to integrated mite control; the orchard in which it occurred also experienced the highest level of mite populations in 2005, and all treatments in 2006 had high mite levels. The OP plot was next to a dusty road, which may have contributed somewhat to the high mite populations.

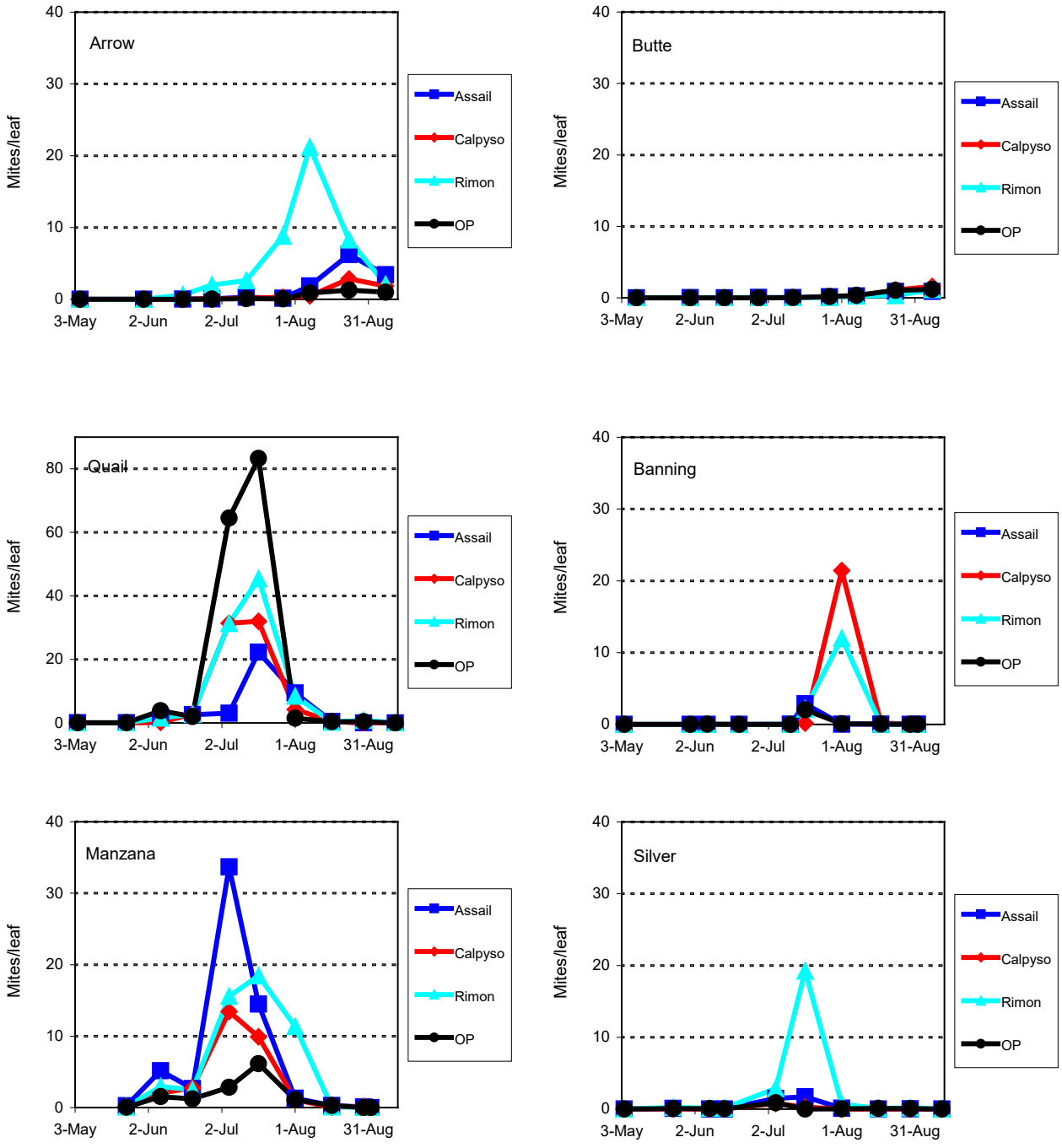

Fig. 1. Tetranychid mite densities in six commercial orchards under various codling moth regimes.

The trends in predatory mite densities are less clear than those of the tetranychid mites. In the Arrowhead block, Assail and Calypso had moderate predator densities, while the high tetranychid densities occurred in the Rimon treatment. In the Banning orchard, predatory mite densities in the Rimon treatment were the highest of the four treatments. The Manzana orchard had a late-season recovery of predatory mites in all plots, but this did not prevent the early July outbreak of tetranychid

mites in the Assail, Calypso and Rimon treatments. The Quail Ridge orchard also had a late-season recovery of predatory mites, but there was little or no numerical response on the part of predators in July when mite populations peaked. The Silver Hawk orchard had moderate densities of predators in mid-July, and this may have helped suppress the tetranychid mite populations which occurred at about the same time.

Apple rust mite densities varied considerably among the test orchards. Arrowhead, Manzana and Quail Ridge had very low densities all season; Butte and Silver Hawk had a moderate peak on July or August, and the Banning orchard had the highest levels, peaking in late July. The variability among orchards greatly exceeded the variability among treatments, and no consistent treatment effect was noted.

No codling moth fruit damage was recorded in four out of six orchards, and levels were low in the remaining two. There were no statistical differences in fruit damage among treatments, in part because of the between-orchard variability. Fruit damage was highest in the Calypso treatment, although this was driven mainly by the high number of stings found in that treatment at the Manzana ranch.

2. Field studies (effect of sulfur on rust and predatory mites).

Apple rust mite populations ranged from 213-424 mites/leaf before any treatments were applied; however, because of the randomization, the differences among treatment means were not significant. Differences were apparent by the first post-treatment count (29 June, or 3 DAT1[1]). At that time, both lime-sulfur treatments (which at that time were identical in having only a single application on) and the 1X micronized sulfur treatment had significantly lower densities of rust mites than the check. By 3 July (7 DAT1) all treated plots had significantly lower rust mite densities than the check; however, populations in the check had dropped precipitously. This drop coincided with a period of high temperatures from the 92°F (24 June) to >100°F (26-27 June). Hot weather is known to adversely affect rust mites. Populations had recovered by 29 August, at which time there was likely no residual effect of the treatments.

Seasonal CMDs are not a very good indicator of treatment effect in this case because of the variability in the pre-treatment counts and the late season recovery. For this reason, a secondary analysis of CMDs was done, eliminating the pre-treatment count, and dropping all counts after 21 July (check had dropped to zero by the next date). In this analysis, all treatments suppressed rust mites in relation to the check (Fig. 2). The greatest degree of suppression was in the 3X application of lime-sulfur, followed by the 1X application of flowable sulfur, then the 1X application of lime-sulfur. One anomaly is that the 3X application of flowable sulfur had significantly higher seasonal rust mite populations than the 1X application. ATS (1X and 3X) caused a slight suppression of rust mites.

[1] DAT1; days after application #1; DAT2; days after application #2.

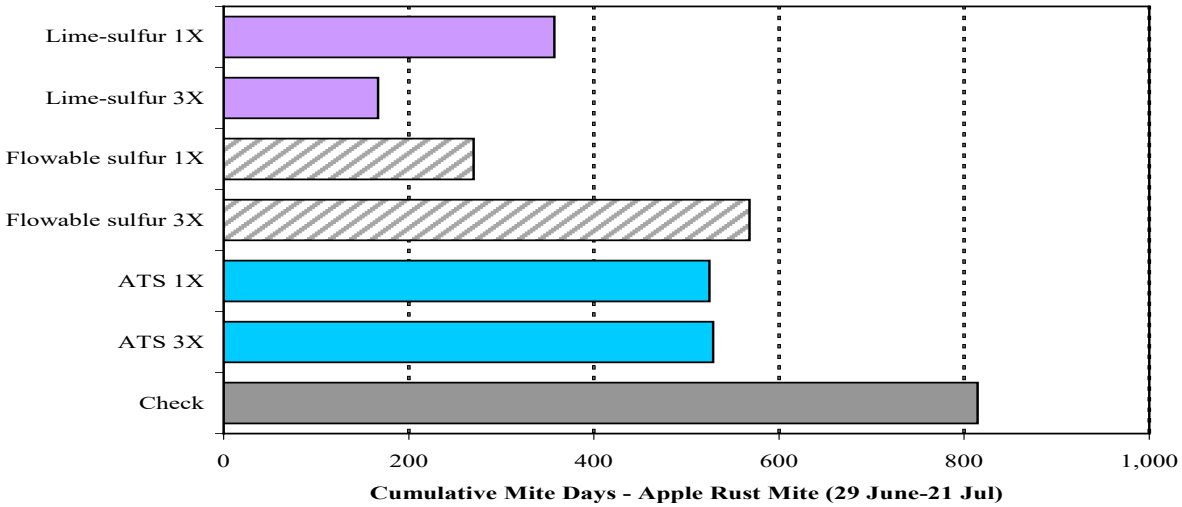

Fig. 2. Apple rust mite seasonal populations following treatments of sulfur-containing pesticides.

Predatory mite populations were moderate before treatment and peaked in the check on 3 July. The low seasonal levels likely reflected low prey densities during the summer. Few treatment differences were apparent during the critical post-treatment period except on 10-18 July, when most treatments had lower predatory mite densities than the check. This is reflected in the CMDs for predatory mites (Fig. 3). Unlike the rust mite CMDs, all treatments affected predatory mites to approximately the same degree.

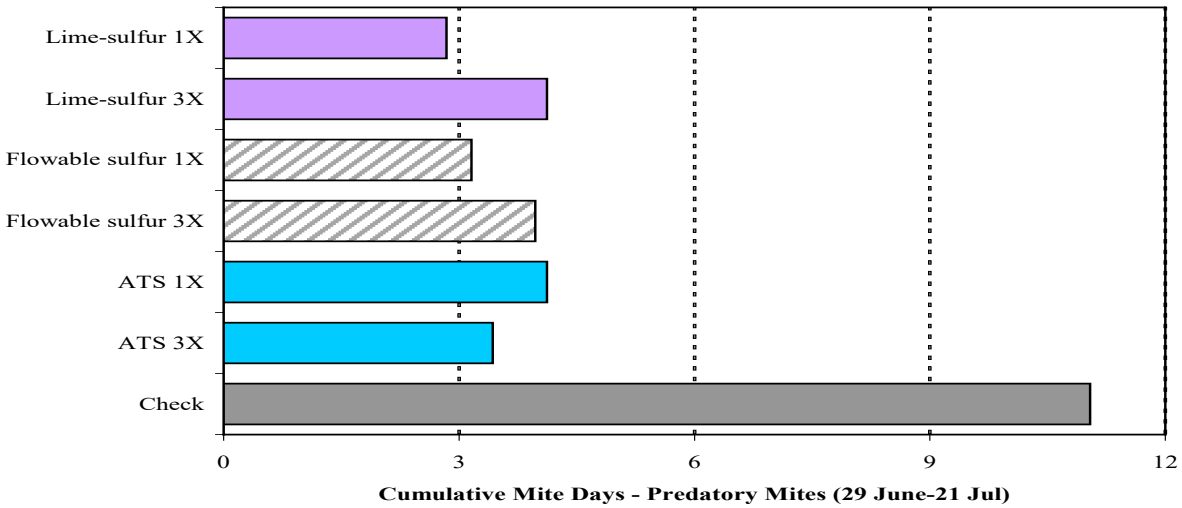

Fig. 3. Predatory mite seasonal populations following treatments of sulfur-containing pesticides.

Tetranychid mites were low during the entire course of the test, never exceeding 2 mites/leaf. There was a slight trend for the 3X flowable sulfur treatment to have a higher level of mites; however, this was based on only two count dates and overall populations were too low to be meaningful.

3. Small plot field trials (additive effects of codling moth and thinning materials)

The tetranychid mite species composition throughout the season in this block was 70% twospotted spider mite, 19% McDaniel spider mite, and 11% European red mite (data not shown). Mites were low during the spring and summer, with a peak in late August in several of the treatments. Peaks of any magnitude occurred only where all three groups of materials were combined (lime-sulfur, carbaryl, and the codling moth insecticide). The treatments lacking both thinners had no meaningful increase in mite populations, as did the treatments that combined lime-sulfur with the codling moth program. The common element with all treatments with elevated mite levels was the addition of two applications of carbaryl. The highest peaks occurred in the Assail and Rimon treatments, an intermediate peak in the Imidan treatment, and the lowest peak in the Calypso treatment. This is reflected in the cumulative tetranychid mite days for those treatments (Fig. 4).

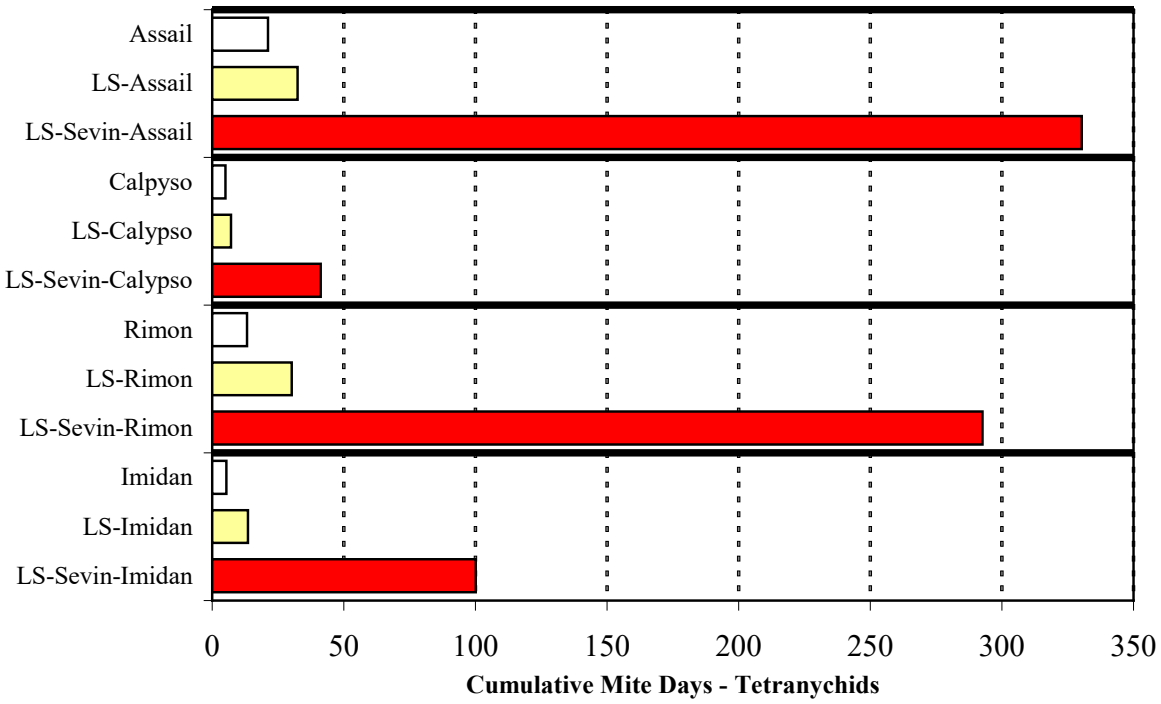

Fig. 4. Cumulative tetranychid mite days resulting from various combinations of codling moth materials and apple thinning materials.

Differences among treatments in predatory mite densities were not dramatic. There was a peak of predatory mite population in the LS-Sevin-Assail treatment, likely in response to the high tetranychid population. The seasonal densities indicated high numbers in both the Imidan and Assail treatments; the three Rimon treatments had some of the lowest predatory mite populations. Apple rust mite population densities followed the same trend as the predatory mite populations with the highest populations in the Assail and Imidan treatments.

CONTINUING PROJECT REPORT
WTFRC Project Number:

Project Title: CSI in the orchard: finding the killers of 4 key apple pests

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City: Wapato
State/Province/Zip WA 98951

Co-PI(2): D Horton
Organization: USDA-ARS
Telephone/email: 509-454-5639 horton@yarl.ars.usda.gov
Address: 5230 Konnowac Pass Rd
City: Wapato
State/Province/Zip WA 98951

Cooperators: Eugene Miliczky and Nina Barcenas

** This is an interim report for a 1 year project (see budget notes) and a second, expanded report will be provided to the commission in the next reporting period

Budget 1:
Organization Name: USDA-ARS
Contract Administrator: Carolyn Yager
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Item	Year 1: 2006
Salaries	13825
Benefits	7122
Wages	
Benefits	
Equipment	
Supplies	
Travel	
Miscellaneous	
Total	20947

Footnotes: Supports Eugene Miliczky

Budget 2:
Organization Name: WSU-Entomology
Contract Administrator: Barb Smith
Telephone: 509-335-5504
Email address: niehoff@wsu.edu

Item	Year 1
Salaries	21243
Benefits	2897
Wages	
Benefits	
Equipment	
Supplies	
Travel	
Miscellaneous	
Total	24140

Footnotes: Supports Pablo Palmandez

Understanding the causes of pest insect mortality is critical to discovery of the best bio-rational methods to control these pests. Unlike death from disease or parasitoids, unwitnessed predation cannot be measured with confidence - the predator eats the evidence. The most unbiased measure of predation is direct observations of predators eating the prey (pest) of concern or direct measurement of a predator's consumption history based on physical or biochemical gut content analysis. This study is designed to perfect methods to determine key predators of 4 key pests of apples by detection of prey remains in the form of PCR of prey DNA in the predator's guts. This information will form the basis of both more intensive and extensive studies of insect predation on codling moth, OBLR, woolly and rosy apple aphids.

This project was funded in late February of 2006, began in late May, and should be finished by mid-March 2007. Except for design of primers and pilot DNA analyses, we have not conducted the DNA analysis portion of the work which naturally follows the specimen collection and preparation phases. Hence we include here an interim report with the commitment to provide a final report with all data from the 2006 season collected analyzed and summarized the February 2008 reporting session.

Objectives:

1) **Design multiplex PCR methods to specifically detect DNA of codling moth, OBLR, woolly and rosy apple aphids in predator guts**
2) **Collect predators throughout the season and the day/night cycle and use the method to estimate predation frequency and to rank predator importance**
3) **Conduct laboratory feeding studies to help interpret data collected in the field**

Significant findings (by objective):

1. A new extraction protocol was developed, PCR primers were redesigned to work better with all predator species, and a multiplex PCR method was developed which allows simultaneous detection of codling moth and OBLR remains.
2. About 2,500 predators were collected from 6 orchard sites. Both the orchard types, pest and predator collection success are reviewed under the Results and Discussion and Table 1. Predators are currently being extracted and with gut content analysis to follow.
3. Feeding and digestion trials were conducted for a large, common carabid species *Pterostichus* sp., and for the aggressive house spider, but gut contents have not been conducted for these yet

Results and discussion (by objective):

Objective 1: Multiplex PCR methods were developed for codling moth and OBLR detection. This means both species can now be detected simultaneously in a single PCR amplification for a specimen, reducing by half the expense of predator gut analysis. The PCR primers were redesigned to be more sensitive in some problematic predator groups (ladybeetles and carabid beetles especially). The original primers, developed to mitochondrial CO1, were replaced with primers for the ITS2 spacer region of the nuclear genome, a much more species specific and globally variable non-coding region of animal genomes. We also developed a rapid salt alcohol DNA extraction protocol but we still must dissect the gut from very large predators (large carabid beetles and spiders)

Objective 2: Predators were collected from 6 orchards using 3 major collection methods: beat tray samples, 24 hr pitfall trapping, and by visual search and collect. Six orchards were sampled and over 2,500 predators were collected. DNA analysis has just begun and is not reported here. The

following narrative describes the orchard and provides a summary of predator abundance by predator type and by orchard Table 1).

Orchards:

1. Moxee fujis was a mixed variety block consisting primarily of Fujis in which various experimental insecticide treatments were applied in addition to herbicides. Our samples were taken in untreated parts of the orchard. Uncultivated land with mixed native and introduced plants surrounded this orchard on all sides. This block was heavily infested with Codling moth and we released OBLR larvae on sample trees on 2 dates.
2. Moxee small block consisted of 19 apple trees of various varieties bordered by pears and soft fruits on 3 sides and uncultivated land on the fourth. No insecticide treatments applied to the trees but early in the season the ground cover was sprayed with diazinon for ant control. and herbicides were used. This block was heavily infested with Codling moth in the second generation and we released OBLR larvae on 2 dates.
3. Mike Young's was a commercial 3 acre organic block of large golden delicious trees mostly bordered by other orchards with some exposure to uncultivated land. This orchard was very heavily infested with Rosy apple aphid but incidence of Codling moth was low due to mating disruption and repeated granulovirus applications.
4. Scott Leach's was a commercial organic block of red and golden delicious trees bordered by other orchard and an irrigation canal. The orchard had a moderate infestation of Rosy apple aphid and a low to moderate infestation of Wooly apple aphid, noticeable mostly on crown suckers. Codling moth incidence was low.
5. Wallace block was a nearly abandoned block of red and golden delicious trees bordered by other orchards and a highway. This orchard had received very little management for a number of years. The irrigation system was poorly maintained and the trees had not been pruned. Also, the orchard had not been mowed for more than a year and small trees of several species had established themselves in the understory. Codling moth and San Jose scale infestations were both ~100% on the fruit and there was a low to moderate infestation of Wooly apple aphid. Experimental trapping/monitoring of codling moth was being conducted in this orchard.
6. The Garza Office block is a former commercial organic orchard consisting of red and golden delicious trees now being used for experimental codling moth treatments. It was surrounded by other orchards and weedy, uncultivated land. We sampled in control plots where coding moth levels were as high as 50% fruit damage. There was also a low level of Wooly apple aphid, primarily around the bases of the trees.

Table 1. Summary of 2006 predator collection data for CSI project. Abbreviations for expected prey: CM = codling moth; LR = leafroller; RAA = Rosy apple aphid; WAA = Wooly apple aphid. Abbreviations for type of sample: PT = pitfall trap; BT = beat tray; HC = hand collection; CB = cardboard band/bundle; SN = sweep net. Relative abundance of predators indicated by: - absent; + low abundance; ++moderate abundance; +++ high .

Orchard Number						
Parameter	1	2	3	4	5	6
Expected Prey	CM, LR	CM, LR	RAA	RAA, WAA	CM, WAA	CM, WAA(?)
Types of Sample	PT, BT, HC,CB	PT, BT, HC, CB	PT, BT, HC, SN	BT, HC	PT, BT, HC, CB	PT, BT, HC
Sampling period	June - August	June - October	June – July	May - July	July - October	August – October
Predator abundance	Moderate	Moderate to high	High	High	High	Moderate
# predators taken	100 – 200	> 500	> 500	300 -400	> 500	300 – 400
	Relative abundance of predator taxa collected					
Lacewings	+	+	+++	+++	++	-
Predatory bugs	+	++	+++	+++	+++	-
Lady beetles	+	+	++	++	++	-
Syrphid flies	-	-	++	++	+	-
Earwigs	+	+++	++	-	+	-
Ground beetles	++	+++	+++	-	+++	++
Rove beetles	+	++	+	-	+	+
Ants	+	+	-	++	-	-
Spiders	++	+++	++	++	++	++
Daddy-longlegs	-	+	+	-	+	+

Note on revised scope of objective 2 . Collections of predators in trees infested with Rosy apple aphid showed a very strong bias toward three predator groups in this order of abundance: lacewings>ladybeetles>syrphid flies> all others (mostly predatory bugs). Observations make it clear that these species are in curled leaves eating the aphids arguing against expensive assays of these predators; bugs will be the focus of PCR studies for aphids. However, in those instances where Woolly aphid is at low densities, all predators encountered will be examined.

Objective 3. Evidence of predator feeding on our key pests must be interpreted based on knowledge of feeding frequency and digestion rates. We completed 2 laboratory feeding and digestion studies , a large carabid beetle species and a large spider species. We will conduct additional feeding studies of ladybeetle(s) and earwigs over the next few months.

Budget notes :
Originally funds were requested for 33% salary for 1 year for Nina Barcenas and Eugene Miliczky. With a verbal OK of the WTFRC, Nina's role was replaced by Pablo Palmandez at a lower salary and longer duration following Nina taking a teaching position. This portion of the grant went directly to WSU and the portion of the effort to be executed by Pablo in the Unruh lab is still ongoing and will be completed around mid-March. Gene's portion of the effort is substantially completed except for a modest amount of time to help in insect and especially spider identification.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AE-05-504 (WSU Project No. 13C-3643-3190)

Project Title: Management of leafrollers in apple orchards

PI: Jay F. Brunner
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Cooperators: Mike Doerr, Steve Garzinski, John Dunley

Budget 1:
Organization Name: WSU **Contract administrator:** Mary Lou Bricker & Sally Ray
Telephone: 509-335-7667**;** 509-663-8181x221 **Email address:** (mdesros@wsu.edu); saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries [1] (AR-0.25); (AP-0.083) [1]	7,048 (AR) 4,603 (AP)	7,401 (AR) 4,817 (AP)	**7,512 (AR) 4,898 (AP)**
Benefits (AR-46%); (AP-34%) [1]	3,242 (AR) 1,565 (AP)	3,626 (AR) 1,638 (AP)	**3,456 (AR) 1,665 (AP)**
Wages [2]	5,400	5,400	**6,000**
Benefits	540	540	**690**
Equipment	0	0	**0**
Supplies [3]	1,500	1,500	**1,500**
Travel [4]	2,000	2,000	**2,000**
Miscellaneous	0	0	**0**
Total	25,898	26,922	**27,721**

[1] **Kathleen Pierre (AR Associate in Research)** - rearing and maintenance of leafroller colonies, **Mike Doerr (AP Administrative Professional)** – management of project and bioassay efforts.
[2] Summer labor to assist with rearing of leafrollers.
[3] Leafroller diet components, plastic Petri dishes, glassware. Cell phone charges are allowed under this grant.
[4] Pays for a vehicle for six months used part-time on this project, plus fuel and maintenance costs.

NOTE: the Washington Commission on Pesticide Registration has funded a proposal in the amount of $27,710 per year, primarily to support a Ph.D. graduate student.

Objectives:

1. Develop a dose-mortality bioassay method for insect growth regulators (IGRs) and other new insecticides to establish baseline toxicity data for leafrollers (OBLR and PLR).
2. Develop discriminating concentrations for key insecticides.
3. Use molecular methods as a tool for early detection of resistance development in leafrollers and codling moth. **(NEW)**
4. Evaluate levels of resistance in leafroller populations from orchards suspected of having resistance issues with insecticides.
5. Characterize any cross-resistance in leafrollers between old and new insecticides.
6. Evaluate new insecticides for control of leafrollers in field tests.

Significant findings:

1. There was a good dose-response when OBLR larvae were fed pyriproxyfen. The response was expressed as increasingly abnormal larvae and pupae with increasing dose. Based on this test a discriminating dose of between 0.3 and 1.0 ppm pyriproxyfen could be used to evaluate field-collected leafroller larvae for signs of resistance.
2. A new diet incorporation bioassay was developed and used to evaluate two field populations of codling moth to a new insecticide, rynaxypyr (Altacor). The LC_{50} value for the susceptible laboratory colony was 0.13 (0.08-0.18). The LC_{50} value for the field populations was 0.08 and 0.17.
3. A diet-dip bioassay was evaluated as a method for replacing our leaf-dip method. There was a good dose-response noted for OBLR exposed to pyriproxyfen. No OBLR survived to emerge as adults when exposed to 300 ppm, and less than 30% emerged when exposed to 30 ppm.
4. In 2006, OBLR populations near Quincy and Pasco showed resistance to azinphosmethyl (35.6 and 11.3 times), spinosad (1.9 and 7.2 times) and methoxyfenozide (***Pasco only*** - 10.8 times).
5. OBLR populations collected from Pasco in 2005 and reared in the laboratory on artificial diet through eight generations showed significant declines in resistance to spinosad, to a level the same as the susceptible colony, while methoxyfenozide and azinphosmethyl reversions were not as dramatic, suggesting that their mechanisms of resistance are linked.
6. In field-aged bioassays, residues of novaluron (Rimon) were not as long as those of methoxyfenozide for PLR or OBLR. Rynaxypyr (Altacor) and spinetoram (Delegate) showed a long residual activity against OBLR larvae, similar to spinosad (Table 3). Against CM larvae rynaxypyr showed very good residual activity, lasting as long as azinphosmethyl and longer than spinosad. In an ovicidal bioassay, novaluron had slightly better residual activity than methoxyfenozide.
7. Field trials demonstrated that emamectin benzoate (Proclaim) was an excellent product for control (99-100%) of leafroller larvae in spring or summer. Rynaxypyr at 3 and 4 ounces per acre provided control of CM as good as azinphosmethyl, but rynaxypyr at 2 ounces did not provide adequate control. Spinetoram (Delegate) applied 3 times per generation provided excellent control of CM. Acetamiprid (Assail) and thiacloprid (Calypso) provided control of CM similar to azinphosmethyl, but in several trials acetamiprid caused outbreaks of spider mites.

Methods:

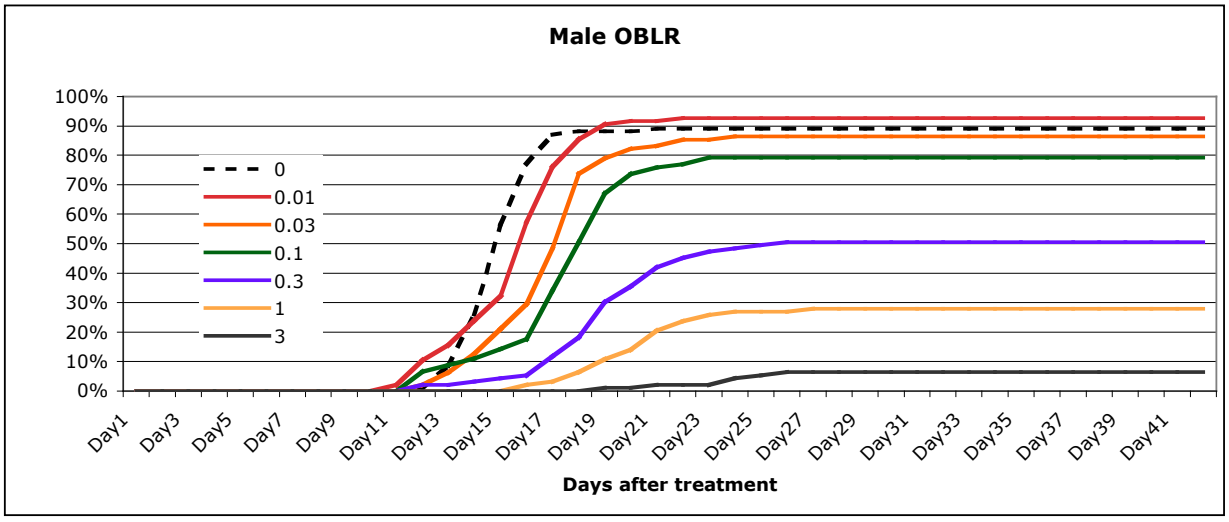
IGR dose-response bioassay – A known dose of pyriproxyfen (Esteem) was applied to a small leaf disk and allowed to dry. A last stage OBLR larva was placed in an arena with the treated disk for 24

h. All larvae that consumed the entire disk were then transferred to an arena with artificial diet, and their development was followed until adult emergence or death. Doses used were 0 (control), 0.01, 0.03, 0.1, 0.3, 1 and 3 ppm. Criteria used to assess the effect of pyriproxyfen dose were the expression of normal or abnormal larvae or pupae, dead larvae or pupae and emergence of adults.
Diet incorporation bioassay – A new bioassay method was used to assess the effect of a new insecticide, rynaxypyr (Altacor), against codling moth and we are working on the use of this method to evaluate several insecticides against leafrollers. The artificial diet used was a "stonefly diet" that is commercially available. Different concentrations of rynaxypyr were mixed into the stonefly diet and small amounts were placed into a small arena with a CM larva. Mortality of CM larvae was determined after 14 days.
Diet-dip bioassay – Another bioassay method was evaluated to determine its value as a replacement for the leaf-disk bioassay since leaves are not always readily available for such studies. Cubes of artificial diet were dipped in different concentrations of pyriproxyfen, allowed to air dry and then placed in an arena with fifth instar OBLR larvae. Larval development was then followed using criteria described in the IGR dose-response bioassay.
Survey of field-collected populations – In 2006 only two populations of OBLR larvae were collected. They were reared to the second generation (F2) in the laboratory, and neonate larvae were used in a leaf-disk bioassay to determine their susceptibility to azinphosmethyl (Guthion 50WP, Bayer CropScience) and spinosad (Success 2SC, Dow AgroSciences). Another population was collected from Mattawa where spinosad seemed to fail in summer, but sufficient larvae have not been reared to conduct bioassays.
Reversion of resistance – In 2005 a field-collected OBLR population showed resistance to azinphosmethyl, spinosad and methoxyfenozide. The population was reared through eight generations (F8) without selection pressure from insecticides and evaluated again for resistance levels to these three insecticides.
Field-aged bioassay – A field-aged bioassay was used to assess the residue longevity of new insecticides. Trees were treated with candidate insecticides and at regular intervals leaves or fruit were collected. Leafroller or codling moth larvae were placed on the leaves or fruit and mortality assessed after appropriate periods to determine the efficacy of aged residues.
Field trials – Numerous field tests (14) were conducted evaluating the efficacy of new insecticides against leafrollers and codling moth. Treatments were applied either by handgun or airblast sprayer in replicated deigns. Assessment of leafrollers was made by counting live and dead larvae following treatment and for codling moth the number of injured fruit after each generation.

Results and discussion:

IGR and leafroller development – Last year we demonstrated that mid-sized OBLR larvae (third/fourth instars) exposed to pyriproxyfen (Esteem) mixed into an artificial diet did not die but were delayed in their development until they were removed from the diet. This year's focus was on developing a better understanding of how pyriproxyfen impacted last instar OBLR larval survival when they were fed a specific dose, then reared on untreated diet. While several factors were measured, two that seem to capture the impact were the number and time required for normal emergence of adult moths exposed to different doses. The untreated OBLR males (and females) emerged as adults between 13-18 days after feeding on the leaf disk (untreated). Larvae exposed to the lowest doses of pyriproxyfen showed a slightly elongated duration of emergence (12-21days) and reduced levels of emergence as dose increased. Higher doses of pyriproxyfen significantly reduced successful adult emergence (larvae or pupae died) and emergence was significantly delayed relative to the control group (Fig. 1).

Fig. 1. Percent emergence of male OBLR following exposure to different doses of pyriproxyfen.



OBLR exposed to a dose of pyriproxyfen showed deformities as larvae (Fig. 2) or pupae (Fig. 3). These larvae or pupae lived for long periods (50 days) but eventually died. Possible discriminating doses for expression of OBLR larvae abnormalities or death have been determined by probit analysis.

Fig. 2. OBLR larvae showing abnormalities after consuming pyriproxyfen compared to a larva (normal) that did not receive a dose of pyriproxyfen.

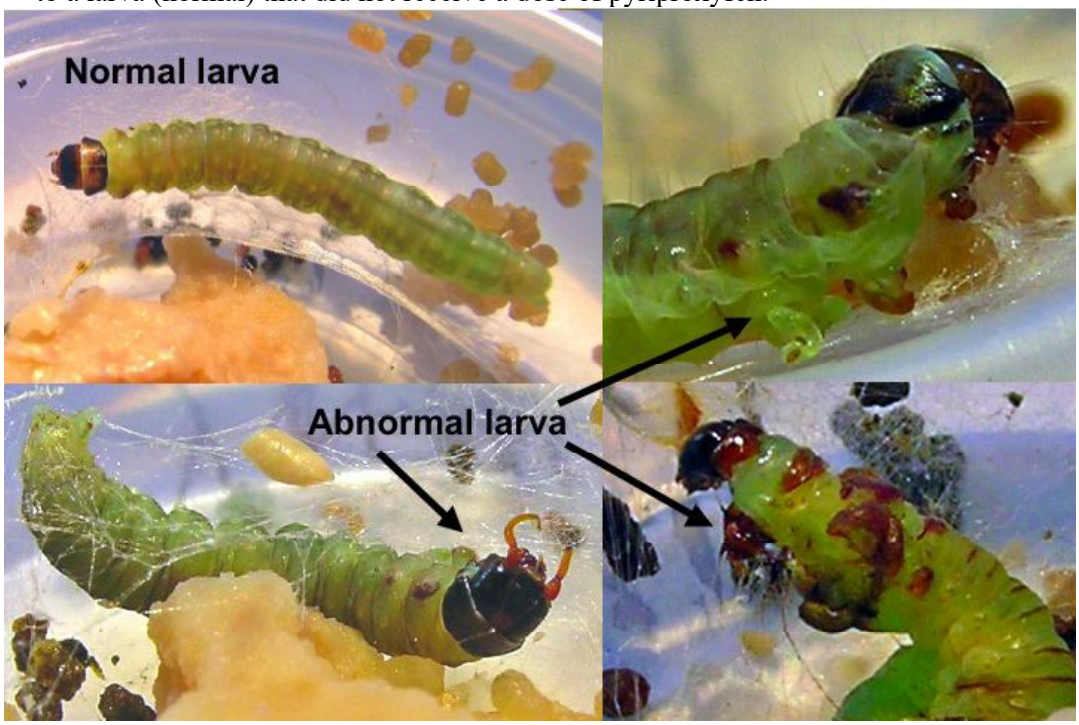
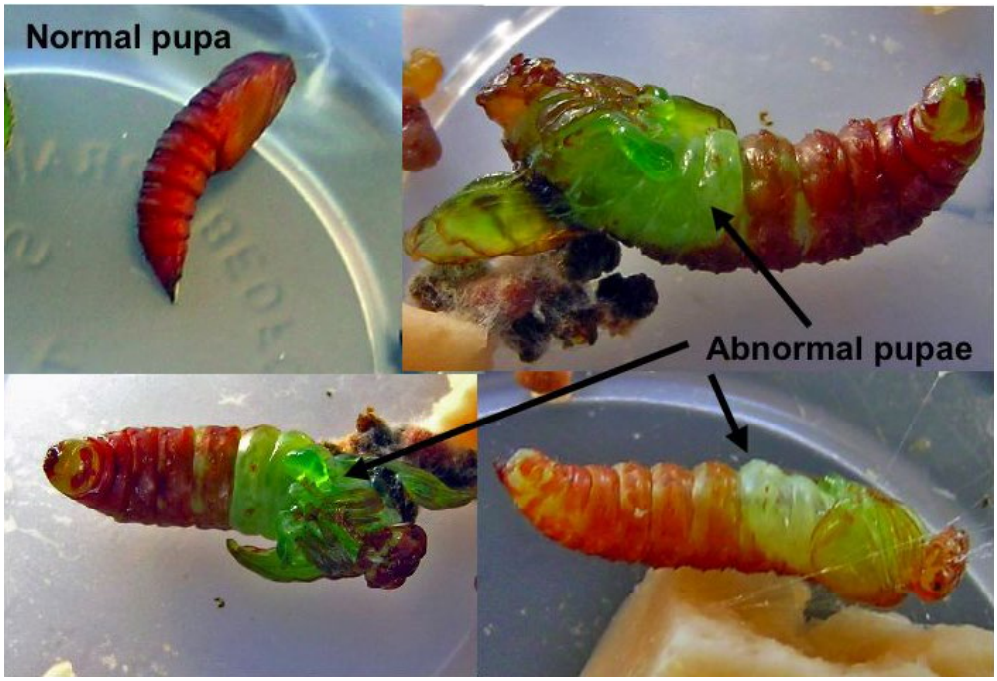


Fig. 3. OBLR pupae showing abnormalities after consuming pyriproxyfen compared to a larva (normal) that did not receive a dose of pyriproxyfen.



Diet incorporation bioassay – Rynaxypyr (Altacor, DuPont) is a new insecticide due to be registered for use on tree fruit in 2008. We have been working with the company to develop methods and baseline data on the susceptibility of codling moth and leafrollers to this product. Efforts to develop an adult bioassay were not successful (2005 studies). Therefore, a larval bioassay was the only alternative. Baseline data for a reference population (USDA-ARS, Wapato) were obtained and two field populations were evaluated. There was no difference in susceptibility between the two field populations (Table 1) and the USDA colony, but both field populations were not under commercial selection pressure. A population collected from CA did not provide enough larvae for testing, and an OR population is in diapause and will be tested once adults emerge and larvae are available. Plans are to expand testing of field populations in 2007 from the western and eastern U.S. and other locations worldwide.

Table 1. Insecticidal Dose-Response of *Cydia pomonella* neonate larvae.
Evaluations 96 hours after exposure of neonate larvae to E2Y45-20 SC or E2Y45-35WG incorporated in artificial diet.

			Probit Analysis Parameters				
Compound	**Population**	**N**	**Slope ± SE**	**LC_{50} ($CI_{95\%}$)**	**LC_{90} ($CI_{95\%}$)**	**χ^2**	**g**
ŹAltacor 35WG	LAB colony (USDA- Wapato, WA)						
	Rep 1	240	4.9 ± 1.2	0.16 (0.10-0.21)Ź	Ź0.29 (0.22-0.48)	Ź58.34	0.24Ź
	Rep 2	240	2.0 ± 0.5	0.04 (0.01-0.06)	0.18 (0.11-0.45)	Ź44.02	0.26Ź
	Rep 3	Ź240	5.3 ± 1.5	0.17 (0.10-0.22)	0.29 (0.22-0.48)	Ź49.35	Ź0.30
	Average	Ź3 reps	4.6 ± 1.3	Ź0.13 (0.08-0.18)	0.25 (0.18-0.51)	Ź28.39	Ź0.32
	TFREC (wild, OP susceptible)	240	1.5 ± 0.3	0.08 (0.03-0.15)	0.62 (0.33-1.8)	Ź76.62	Ź0.21
	Brogan (wild, OP susceptible)	200Ź	3.6 ± 1.9	0.17 (no limits)	0.39 (no limits)	Ź41.94	1.05Ź

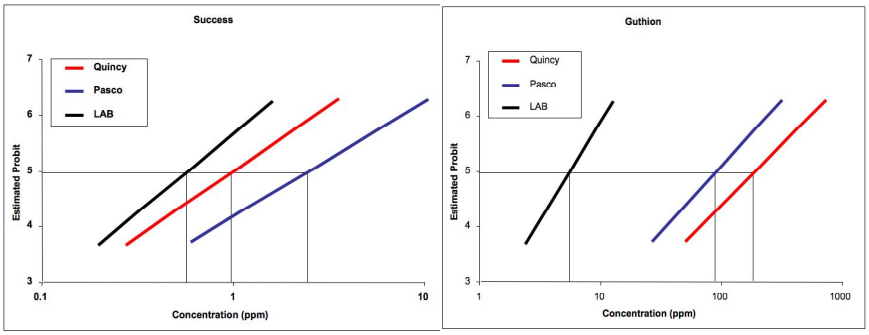
Diet-dip bioassay – Most OBLR larvae consumed the cube of diet dipped in pyriproxyfen over the 24-h period allowed. The percentage of larvae pupating or emerging as adults was high in the

control, 90% and 100%, respectively (Table 2). The percentage of larvae pupating at lowest dose of pyriproxyfen was similar to the control, but the percentage declined as dose increased for both males and females. The same was true for the percentage of pupae emerging as adult. There was less than 30% adult emergence at 30 ppm and no emergence at 300 ppm. The average number of days as a larva tended to increase with dose, although at high doses when few larvae survived the average time in the larval stage actually declined. The average number of days in the pupal stage again tended to increase with pyriproxyfen dose, especially at the highest doses.

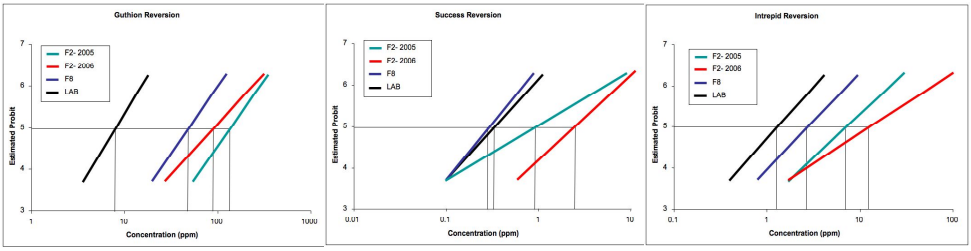
Table 2. The percent pupation and emergence and days in a life stage of OBLR exposed to different doses of pyriproxyfen.

Dose	Sex	% pupation	% emergence	Days as larvae	Days as pupae	Total
0	F	90.0%	100.0%	6.6	14.0	20.6
0.03	F	86.7%	84.6%	6.4	13.6	20.1
0.3	F	90.0%	59.3%	7.2	14.1	21.3
3	F	50.0%	40.0%	7.9	15.3	23.2
30	F	16.7%	20.0%	8.0	21.0	29.0
300	F	6.7%	0.0%	3.0		0.0
0	M	100.0%	96.7%	4.4	12.3	16.7
0.03	M	100.0%	86.7%	5.6	12.8	18.4
0.3	M	73.3%	63.6%	6.0	14.5	20.5
3	M	53.3%	81.3%	10.5	19.2	29.7
30	M	23.3%	28.6%	6.1	26.0	32.1
300	M	6.7%	0.0%	3.5		0.0

Survey of field-collected populations. In 2006 OBLR populations near Quincy and Pasco showed resistance to azinphosmethyl (35.6 and 11.3 times), spinosad (1.9 and 7.2 times) and methoxyfenozide (***Pasco only*** - 10.8 times). The spinosad resistance level is the highest we have detected in WA but our data suggest that this population should still be controlled if using the high field rate; however, methoxyfenozide would likely not provide sufficient control to be commercially acceptable.



Reversion of resistance. OBLR populations collected from Pasco in 2005 and reared in the laboratory on artificial diet through eight generations showed significant declines in resistance to spinosad, to a level the same as the susceptible colony, while methoxyfenozide and azinphosmethyl reversions were not as dramatic suggesting their mechanisms of resistance are linked.



Field-aged bioassay – In field-aged bioassays residues of novaluron (Rimon) were not as long as those of methoxyfenozide for PLR or OBLR. Rynaxypyr (Altacor) and spinetoram (Delegate) showed a long residual activity against OBLR larvae, similar to spinosad (Table 3). Against CM larvae rynaxypyr showed very good residual activity, lasting as long as azinphosmethyl and longer than spinosad. In an ovicidal bioassay novaluron had slightly better residual activity than methoxyfenozide.

Table 3. Corrected percent mortality of leafroller and codling moth exposed to residues of insecticides for different periods.

			Corrected percent mortality (DAT)				
Insecticide	Rate/a	Year	1	7	14	21	28
Pandemis leafroller (7-d evaluation)							
Rimon 0.83EC	32 fl oz	2006	90.3	88.6	50.0	72.7	
Intrepid 2F	16 fl oz	2006	100.0	100.0	100.0	100.0	
Obliquebanded leafroller (7-d evaluation)							
Rimon 0.83EC	32 fl oz	2006	91.7	86.1	64.4	62.0	
Intrepid 2F	16 fl oz	2006	100.0	100.0	100.0	100.0	
Altacor 35WG+HMO	4.0 oz	2006	98.1	100.0	100.0	100.0	100.0
Success 2SC	6.0 fl oz	2006	100.0	100.0	92.5	95.7	100.0
Delegate 25WG	6.4 oz	2006	100.0	100.0	100.0	100.0	100.0
Codling moth neonates (7-day evaluation)							
Altacor 35WG+HMO	4.0 oz	2006	100.0	72.2	93.8	91.1	86.2
Guthion 50WP	2.0 lb	2006	100.0	94.4	91.7	82.3	89.6
Success 2SC	6.0 fl oz	2006	85.0	72.2	33.3	67.7	41.3
Delegate 25WG	6.4 oz	2006	100.0	94.4	41.7	91.1	65.5
Codling moth ovicide (10-day evaluation)							
Rimon 0.83EC	32 fl oz	2006	74.4	87.2	82.3	87.4	
Intrepid 2F	16 fl oz	2006	78.4	76.1	72.2	79.5	

Field trials – Field trials demonstrated that emamectin benzoate (Proclaim) was an excellent product for control (99-100%) of leafroller larvae in spring or summer. Five insecticides (spinosad, novaluron, emamectin benzoate, methoxyfenozide, and pyriproxyfen) applied to control overwintering larvae in spring provided excellent carryover activity, essentially eliminating leafroller populations in the same blocks in summer. Novaluron applied three times per generation and timed at the egg-laying period was effective against CM. However, it was not as effective when applied only twice per generation indicating that the residual activity was not sufficient, especially in the second generation. Acetamiprid (Assail) and thiacloprid (Calypso) provided control of CM similar to azinphosmethyl, but in several trials acetamiprid caused outbreaks of spider mites. Rynaxypyr (Altacor) at 3 and 4 ounces per acre provided control of CM as good as azinphosmethyl, but rynaxypyr at 2 ounces did not provide adequate control. Spinetoram (Delegate) applied 3 times per generation provided excellent control of CM. In 2006 we gained experience with some numbered products under development. Some appear to have promise as controls for leafroller and/or CM.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AE-05-503 WSU Project No. 13C-3643-4190

Project Title: Codling moth management with pheromones: key unanswered questions

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Composite Budget:
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Telephone: 509-335-7667; 509-663-8181 x221 **Email address:** mdesros@wsu.edu; saray@wsu.edu

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3: 2007**
Salaries [1]	130205	112353	**108,305**
Benefits [2]	38081	39455	**37,073**
Wages [3]	15000	19500	***37,000****
Benefits	1800	1950	**4,075**
Equipment	3000	0	**0**
Supplies [4]	7012	11700	**10,700**
Travel [5]	8100	10500	**9,000**
WSU total	83586	81393	**91,629**
MSU total	48730	53756	**54,915**
USDA total	35082	39609	**40,689**
Ag Canada total	35800	20700	**21,420**
Miscellaneous	0	0	**0**
Total	203198	195458	**206,153**

[1] For WSU part only - salary (1 mo.) for Senior Scientific Assistant; salary (11 mo.) for Research Assoc.
[2] Benefits for Senior Scientific Assistant; 34% for Research Associate.
[3] Hourly help to assist with setting up experimental apparatus, collection and analysis of data.
**** Increase of $20,000 in WSU portion of grant is for Vince Hebert's program to collaborate in assessing release and purity of different pheromone products. Also reflects some reduced allocation to other PIs.***
[4] Supplies will include lures, traps, flagging materials, cell phone charges and fuel.
[5] Travel to experimental plots; pays for one car for 6 months.

Budget 1:
Organization Name: Washington State Univ. **Contract Administrator:** Mary Lou Bricker; Sally Ray
Telephone: 509-335-7667; 509-663-8181 x221 **Email address:** mdesros@wsu.edu; saray@wsu.edu

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	55,515	50,169	**43,846**
Benefits	14,471	17,366	**14,908**
Wages	5,000	7,500	**25,000**
Benefits	600	825	**2,875**
Equipment	3,000	0	**0**
Supplies	2,500	2,500	**2,500**
Travel	2,500	2,500	**2,500**
Total	83,586	80,860	**91,629**

[1] For WSU-TFREC part only – salary (1 mo.) for Senior Scientific Assistant; salary (11 mo.) for Research Associate.

[2] Benefits for Senior Scientific Assistant; 34% for Research Associate.

[3] Hourly help to assist with setting up experimental apparatus, collection and analysis of data. ***$20,000 wages plus $2,300 benefits will go to Vince Hebert's program to collaborate in assessing release and purity of different pheromone products. Also reflects some reduced allocation to other PIs.***

[4] Supplies will include lures, traps, flagging materials, cell phone charges and fuel.

[5] Travel to experimental plots, pays for one car for six months.

Budget 2:
Organization Name: Michigan State University **Contract Administrator:** On file
Telephone: **Email address:**

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	27,000	27,810	**28,644**
Benefits	10,530	10,846	**11,171**
Wages	5,000	6,000	**6,000**
Benefits	600	600	**600**
Supplies	2,000	3,000	**2,000**
Travel	3,600	5,500	**4,000**
Total	48,730	53,756	**52,415**

Budget 3:
Organization Name: USDA-ARS, Wapato **Contract Administrator:** On file
Telephone: **Email address:**

Item	Year 1: 2005	Year 2: 2006	Year 3: 2007
Salaries	18,690	19,438	**20,215**
Benefits	7,280	7,571	**7,874**
Wages	5,000	6,000	**6,000**
Benefits	600	600	**600**
Supplies	2,512	5,000	**5,000**
Travel	1,000	1,000	**1,000**
Total	35,082	39,609	**40,689**

Budget 4:
Organization Name: Agriculture Canada **Contract Administrator:** On file
Telephone: **Email address:**

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3: 2007**
Salaries	29,000	15,000	**15,600**
Benefits	5,800	3,000	**3,120**
Supplies	0	1,200	**1,200**
Travel	1,000	1,500	**1,500**
Total	35,800	20,700	**21,420**

Project goal: Increase the efficacy of pheromone-based technologies for codling moth management.

Project objectives:

1. Determine the active space of different pheromone sources (females, lures, dispensers, flakes, fibers, etc.) under MD and non-MD situations. (WSU, MSU)
2. Determine where in the tree CM females call. (WSU, USDA, CA)
3. Determine the aggregation of CM in MD and non-MD orchards. (WSU, CA)
4. Determine the impact of pheromone purity, addition of minor pheromone components, and plant volatiles on attraction of CM in MD orchards. (USDA, MSU, CA)
5. Determine the spatial arrangement of pheromone sources that maximizes MD. (WSU, MSU)
6. Define the effect of host plant volatiles on CM pheromone biology. (USDA, CA)
7. Characterize responses of CM from different geographical areas to pheromones and plant volatiles as baselines for future assessments of resistance. (USDA, WSU, MSU)
8. Utilize the information in objectives 1-6 to optimize pheromone delivery technologies for CM control and monitoring. (WSU, MSU)

Project highlights:

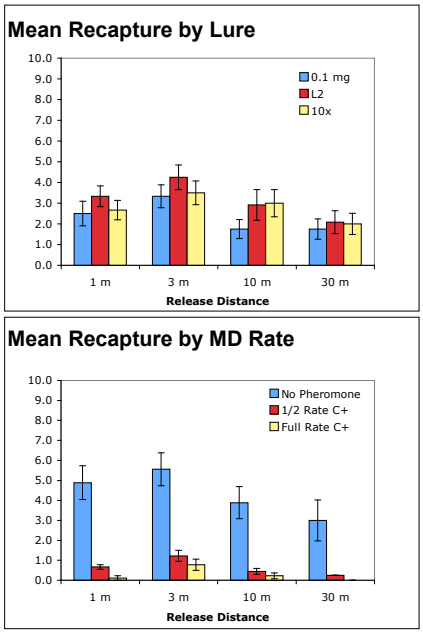
Significant findings.

- **1a)** 0.1 mg lures were shown to be a good mimic of virgin female CM in mark-release-recapture studies.
- **1b)** Active space of the female mimicking 0.1 mg lure appears to be less than 10 m and is further constrained at full rates of an Isomate treatment.
- **2a)** Both male and female CM were found in the top thirds of trees; significant differences in moth behavior in the field were observed between laboratory and wild moths. Female moths did not avoid pheromone dispensers.
- **2b)** Wild, diapause-destined CM are more likely to disperse from their tree of origin than CM not destined for diapause.
- **3)** A high suction vacuum system represents a good method for sampling CM, and likely many other insects, within trees. However, an alternative method using low volume pyrethrum sprays may provide quicker data collection of moth distributions over larger areas.
- **4a)** Male CM flown (wind tunnel) in a pheromone background were not able to orient to female sex pheromone extracts to a greater extent than to synthetic codlemone.
- **4b)** A plant volatile has been identified that, in combination with pear ester, appears more attractive than pear ester alone.
- **5a)** Placing even a small number of microdispensers (six per tree) above or around a trap provided significant MD during the first flight.
- **5b)** Very high densities of "mesodispensers" are capable of almost 100% mating suppression in CM, further supporting the hypothesis that higher numbers of pheromone point sources may be a key factor in improving MD.
- **6a)** Pear ester alone is not as attractive as codlemone, and combinations of pear ester and codlemone are equally attractive when presented separately. However, in choice tests combination lures are more attractive than codlemone-alone lures.
- **6b)** Male CM were less likely to locate pear ester lures after brief exposures to pear ester dispensers.
- **6c)** MD attributable to plant volatiles, either alone or in combination with codlemone, does not appear to be improved compared to MD due to codlemone alone.
- **7a)** Laboratory moths from two colonies were more responsive to codlemone and pear ester/codlemone combo lures than wild moths from Canada, Washington and Michigan.

- **8)** Application of between 30-100 Hercon flakes per tree provided adequate MD in small plot studies using a known number of marked laboratory moths. Therefore, the application efficiency of microdispensers needs to be greatly improved for the new technology to be effective.

Objective 1 – Active space.

Methods – Mark-release-recapture studies were performed in British Columbia (BC) and Wenatchee (WA-W). The objective of the BC experiments was to compare the active space of the 0.1 mg lure and caged virgin females. The objectives for the WA experiment were to compare the active space of three lures, including the 0.1 mg lure, in the absence of MD as well as to characterize the impact of MD on the active space of the female mimic lure.

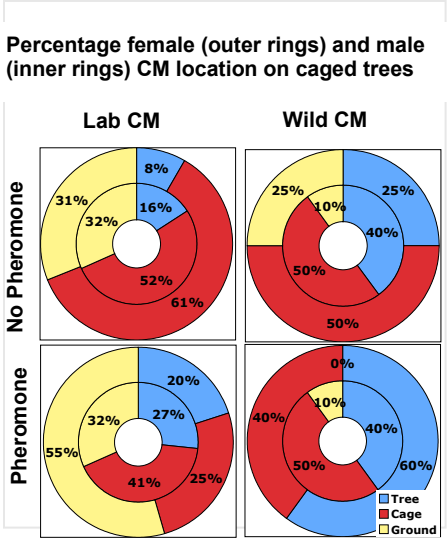


Results – In the BC experiment during the spring, virgin females recaptured about 5% of CM males from 5 m, 23% from 10 m, 10% from 20 m, 6% from 40 m, and 7% from 80 m. Slightly less CM were recaptured during the summer although a similar pattern was observed for percentage recapture by release distance. The lower level of recapture during the summer may have been due to increasing canopy complexity. The fact that the closest release point did not have the highest rate of recapture suggests that there is an optimal distance of detection. A comparison of traps baited with the 0.1 mg lure or virgin females showed a very similar level of recapture (31.7% and 30.1%, respectively). In the WA-W experiment, similar numbers of moths were recaptured with the three lure types; however, the 0.1 mg lure differed from the higher dosage lures in that it caught more moths from within 3 m than from 10 m (upper figure). Most of the moths captured from within 10 m of the trap were captured within three days, while moths released farther than 10 m, were typically recaptured after three days. Pheromone treatments (Isomate) greatly reduced trap capture, and almost all of the moths recaptured under a full rate were from less than 10 m and within three days. These results support the hypothesis that the female mimicking, 0.1-mg lure has an active space of less than 10 m while the higher dosage lures may have a larger active space. The addition of pheromone further constrained the attractive range of the female mimic to less than 10 m (lower figure).

Plans for 2007 – Further studies will be done to compare the recapture of laboratory moths vs. protein-marked wild moths; recapture distances will also be developed for orchard blocks with different types of mating disruption (i.e, flakes or fibers vs. Isomate or sprayable pheromone).

Objective 2 – CM location in the canopy.

Methods – Studies were performed at both Yakima (WA-Y) and WA-W. In WA-W, fluorescently marked laboratory and wild CM of both sexes were observed on caged trees in the presence and absence of MD in order to determine where moths were most active. In addition, a digital video observation system capable of nighttime observations of tethered individual CM or moths responding to a pheromone source was developed in WA-W. In WA-Y, synthetic trees and floor interception traps were used to determine where laboratory or wild larval CM on the tree or orchard floor were most likely to pupate.



Results – In observations made in WA-W, lab and wild males and females were found at similar heights (top third of trees) on both the tree and cage. However, compared to lab moths, wild moths of either sex were found at least twice as often on trees and somewhat less frequently on the ground. The addition of pheromone increased the proportion of both males and females on trees but also increased the proportion of lab females observed on the ground. Also, fluorescent powder and several lab males were observed on Isomate dispensers, indicating contact with dispensers by males. In preliminary video recordings performed in late July, the 0.1 mg lure attracted more moths than either flake or fiber dispensers. Male activity was recorded after dark, not during dusk, typically within a 1- to 2-hr window of time. In the WA-Y experiment, approximately 75% of both diapause-destined and non-diapause-destined lab CM, as well as non-diapause-destined wild CM, pupated on the trunk of the artificial tree, whereas only about 50% of diapause-destined wild CM were found on the trunk. The remaining diapause-destined wild CM were found in floor interception traps. This suggests that diapause-destined CM are more likely to disperse as larvae compared to non-diapause-destined CM, which might result in denser larval aggregations in the overwintering generation.

Plans for 2007 – Further studies will be performed to determine whether differences in calling location exist for laboratory females, overwintered wild females, and summer generation lab females in the presence and absence of MD. A camera system will be used to record the temporal pattern of female calling and male response throughout the growing season.

Objective 3 – Aggregation of CM in orchards.

Methods – In WA-W, trees were seeded with marked male and female lab CM and then either sampled with the vacuum system or air-blast sprayed with a low volume of natural pyrethrums. The ground around sprayed trees was covered using contractor plastic to facilitate recapture of moths. In Michigan (MI), separate trials using the vacuum system were performed, capturing both wild moths on uncaged trees, as well as recapturing marked lab moths from trees and orchard rows under screen tents. MI vacuum samples were collected during both daytime and early evening.

Results – In WA-W, both spraying and vacuuming returned similar numbers of moths (about 8% recapture) for the first two runs, but the spray method returned 5-fold (8% vs. 40%) more moths on the third run. Both methods captured wild larval CM but did not capture any wild adult moths. MI trials capturing wild moths demonstrated that both male and female CM could be captured throughout the trees with more CM captured during the evening than the daytime. Laboratory CM released in caged trees were recaptured from the cage, trees, and ground, although only about 50% of CM released were recaptured. The data collected in MI suggest that moths are active throughout the trees and may move between the trees and ground.

Plans for 2007 – Larger-scale trials utilizing the spray technique will be conducted in 2007 to assess the temporal and spatial pattern of wild CM in infested orchards. Additional trials using the vacuum sampler will be done to assess the effect of pheromone dispenser placement on the positioning of male and female CM within trees.

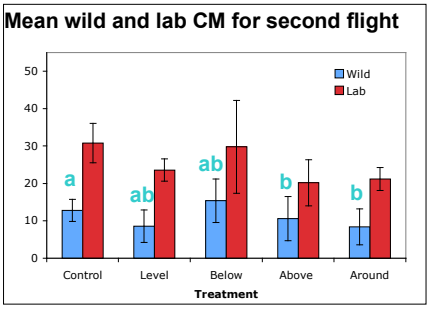
Objective 4 – Pheromone purity, components and plant volatiles.

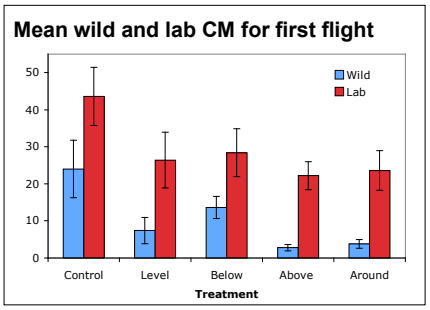
Methods – A laboratory experiment was carried out in BC, and field experiments were carried out in WA-Y. The BC experiment consisted of flying male CM to either synthetic codlemone or female CM pheromone gland extract in a wind tunnel. Experiments at WA-Y included three field trials, the first of which identified potential plant volatile blends for further testing. The second tested the most promising blend versus individual blend components, and the third tested the relative attractiveness of either green-uninfested, green-infested, and ripe apples or ripe pears to both male and female CM.

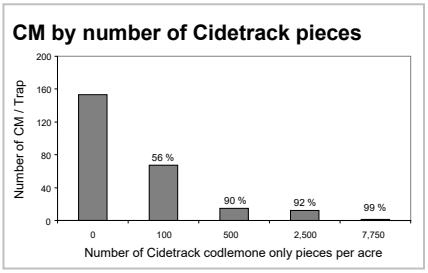
Results – Wind tunnel experiments conducted in BC indicated that the addition of a pheromone background greatly reduced the overall response of CM males compared to a clean background (33% and 63%, respectively). However, little difference in the number of source contacts was found for CM responding to artificial codlemone versus female gland extract in pheromone backgrounds, (45.5%, 55.5%). These results suggest that minor components of natural pheromone (vs. synthetic codlemone)

are not sufficiently attractive to interfere with MD. Field experiments conducted at WA-Y and in New Zealand successfully identified a two-component blend of pear ester and another plant volatile that proved 5- to 10-fold more attractive to both male and female CM than pear ester alone. In fruit attractiveness studies ripe pears proved the most attractive followed by ripe apples. Green apples were not more attractive than a control lacking fruit, regardless of infestation status.
Plans for 2007 – The new plant volatile blend identified at WA-Y will be further tested in the field and under laboratory conditions. Further experiments will be performed testing the attractiveness of pear ester in the presence and absence of codlemone and other plant volatiles.

Objective 5 – Spatial arrangement of competing pheromone sources.
Methods – An experiment to determine the effect of changing the placement of pheromone dispensers relative to a pheromone trap on MD was conducted in WA-W, and an experiment to determine the effect of dispenser density on MD was performed in MI. The WA-W experiment tested five arrangements of six Scentry No-Mate fibers relative to a pheromone trap (no fibers, and fibers in line with, above, below, or around the trap) and was performed using both wild moths as well as marked lab moths. The MI experiment examined the capture of wild moths in small plots containing 0, 100, 500, 2,500, or 7,750 mesodispensers per acre. Mesodispensers used in the Michigan trial consisted of small pieces of Trécé Cidetrack codlemone dispensers.
Results – In the WA-W experiment, wild moths were significantly disrupted by the six fibers placed above or around the trap during the first flight (upper figure). However, during the second flight wild moths were not disrupted and lab moths were never disrupted throughout the experiment (upper and middle figures). These data further suggest that the second flight of moths may be more difficult to disrupt compared to the first and that significant differences may exist between the behavior of wild and laboratory CM. A clear dose response was visible in the MI experiment with the three highest densities resulting in excess of 90% disruption relative to the control (lower figure).

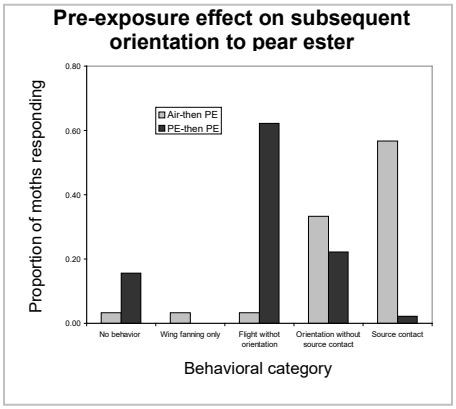






Plans for 2007 –Experiments conducted in 2007 will focus on examining the relationship between the density and strength of dispensers to successful mating disruption of both wild and laboratory CM. Experimental designs will account for overall dosage of pheromone and include the use of marked laboratory CM as well as wild CM.

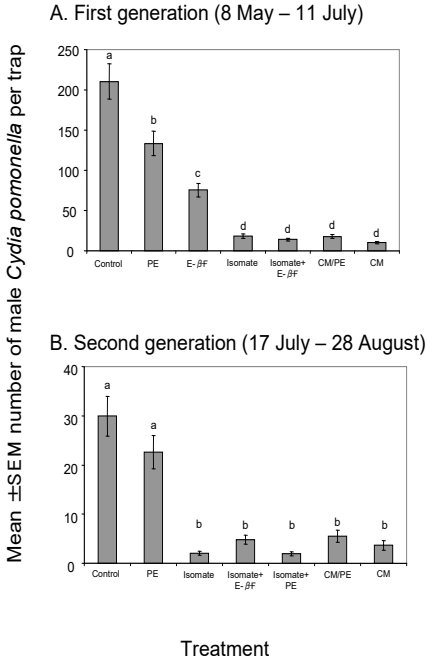
Objective 6 – Effect of host plant volatiles on CM pheromone biology.
Methods – Laboratory wind tunnel and field experiments exploring the importance of host plant volatiles were conducted in BC and in MI. Wind tunnel experiments in BC examined the relative attractiveness of codlemone, pear ester, or combination lures in clean air as well as in pheromone-laden air. The major objectives of the MI studies were to determine if laboratory pre-exposure to pear ester results in desensitization of male CM to either



codlemone or pear ester, to test a lure featuring a new plant volatile (M-Lure), to explore the possibility of lure/cultivar interactions, and to further test the potential for plant volatiles to contribute to better MD in the field.

Results – BC no-choice wind tunnel experiments examining the response of male CM to either codlemone or pear ester lures in the presence or absence of background pheromone indicated that with or without the presence of background pheromone, 0.1 mg codlemone lures were substantially more attractive than pear ester lures, with combination lures experiencing a similar level of attraction to codlemone-only lures. However, in choice experiments where both a codlemone and a combination codlemone-pear ester lure were tested, combination lures attracted significantly more moths than the codlemone-alone lures in both the presence (66% and 34%, respectively) and absence of background pheromone but only when lures contained considerably more (10-1000x) pear ester than codlemone. The results of the BC experiments suggest that while male CM are capable of detecting pear ester, additive response to the plant volatile is only apparent when CM are given a choice between codlemone or a combination of codlemone and pear ester. The MI wind tunnel experiment examining the effects of brief pre-exposures to pear ester showed that there was not a significant reduction in the number of male CM source contacts to codlemone. In contrast, exposures to codlemone alone, as well as a combination of codlemone and pear ester did reduce codlemone source contacts compared to pre-exposures to clean air (20%, 25% and 65%, respectively). Pre-exposures to pear ester did significantly reduce the number of source contacts by male CM to a pear ester lure, although pre-exposure also appeared to increase the chance of flight in males (upper figure). These results indicate that contact with concentrated plant volatiles has the potential of desensitizing male orientation to less concentrated sources. The field trial comparing the M-Lure to DA (pear ester) lures as well as an L2 (codlemone) lure showed that neither of the plant volatile lures was comparable to the codlemone lure. However, in the absence of pheromone the DA lure caught significantly more moths than the M-Lure during the first (8 and 0.1 moths/trap respectively) and second (10 and 2.5 moths/trap, respectively) flights. In addition, the DA lure capture had a higher female-male sex ratio compared to the M-Lure (8 and 26%, respectively). Cultivar type may have had an effect on capture by both codlemone and plant volatile lures in orchard blocks lacking mating disruption, although this may have been attributable to either differences in cultivar volatile profiles or CM population density. In the experiment addressing the potential of MD by plant volatiles, either alone or in combination with codlemone, only E-β-farnesene provided significant MD compared to an untreated control. However codlemone dispensers provided the best levels of disruption, and combination dispensers did not provide better disruption than codlemone alone (lower figure).

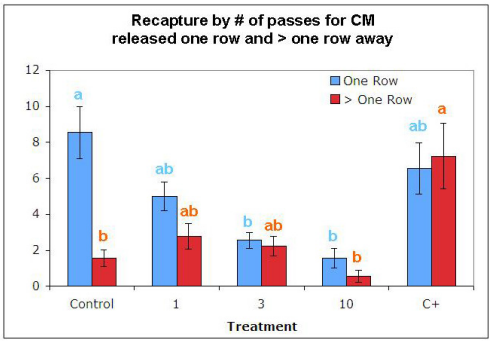
MD Using plant Volatiles, Codlemone, and Combination dispensers



Plans for 2007 – Future studies will explore the importance of cultivar and plant volatiles on MD as well as CM monitoring via pheromone traps.

Objective 7 – Baseline characterization of behavior and electrophysiology.
Methods – A series of laboratory wind tunnel experiments was performed in BC characterizing the response of wild moths from BC, MI, and WA-W as well as USDA (Yakima) and SIR (BC) lab moths to pear ester, synthetic codlemone, and combinations of codlemone and pear ester. Electroantennograms (EAG) were performed with CM male and female moths of the wild BC, wild WA-W, and SIR and USDA lab populations against codlemone, female sex gland extract, and pear ester. In addition, the quantity of sex pheromone present in several of the CM strains was determined.
Results – Male SIR moths were the most responsive to both the 0.1 mg codlemone lure and the combination lure (74.2% and 65.8%), followed by the wild BC moths (60% and 53.9%), wild WA-W moths (59.9% and 62.1%), and wild MI moths (42.1% and 27.1%). None of the CM populations tested made source contacts to lures loaded with pear ester, although upwind flight was detected for the SIR (59%), USDA (26%), and wild BC (8%) moths. EAGs to codlemone and pear ester revealed that both wild BC and WA populations had nearly identical responses to codlemone and were significantly less responsive to pear ester compared to codlemone. In contrast, male SIR and USDA CM had nearly identical responses to both codlemone and pear ester. Female EAGs revealed that none of the populations tested were very responsive to codlemone although all strains were responsive to pear ester with response increasing with dose. Furthermore, females of the wild BC population were significantly more responsive to pear ester than females from the wild WA-W, wild MI, or USDA, and SIR laboratory populations. The sex pheromone glands of SIR females were found to have slightly more sex pheromone than those of the wild BC strain (7.9 ng and 6.2 ng).
Plans for 2007 – Studies in 2007 will include further determination of the quantity of sex pheromone present in females of the various CM populations, as well as the response of males from the various populations to female sex pheromone extracts from different populations.

Objective 8 – Development and optimization of pheromone delivery technology.
Methods – The objective of this experiment was to directly compare the MD provided by three rates of Hercon CM MicroDisrupt flakes with an untreated control and Isomate in small orchard plots. Marked male CM were released one row downwind of single orchard rows treated with either 0, 1, 3, or 10 passes of a flake applicator (approximately 11 flakes/tree/pass), or the full rate of Isomate.



Results – Both the 3- and 10-pass treatments (ca. 33 and 110 flakes per tree) recaptured significantly fewer CM compared to the control for CM released one row downwind. Surprisingly, the Isomate treatment caught the highest number of CM released from more than one row away, significantly more than either the 10-pass treatment or the control. These results indicate that at least 30 flakes/tree are needed to provide mating disruption in small blocks. They also suggest that small blocks treated with the labeled rate of Isomate dispensers have the potential to attract CM males over relatively large distances but do not provide disruption-orientation to a trap.
Plans for 2007 – Similar experiments will be conducted in larger orchard blocks with hand-applied flakes, fibers, or other microdispensers. Dispenser release rate will be addressed, as well as the number of point sources, so that the effect of total amount of codlemone can be separated from the number of point sources. This will potentially allow us to suggest the optimal number and strength (release rate) of dispensers needed to achieve optimal MD of CM.

CONTINUING PROJECT REPORT **YEAR**: 1 of 4
WTFRC Project Number: AE-06-603

Project Title: Sprayable foam for trapping and killing codling moth larvae.

PI:	Peter Landolt	**Co-PI(2):**	Greg Glenn
Organization:	USDA, ARS	**Organization:**	USDA, ARS
Telephone/email:	509-454-6570	**Telephone/email:**	510-559-5677
	landolt@yarl.ars.usda.gov		gmg@pw.usda.gov
Address:	5230 Konnowac Pass Rd,	**Address:**	800 Buchanan St.
City:	Wapato	**City:**	Albany
State/Province/Zip	WA 98951	**State/Province/Zip:**	CA 94710

Cooperators: Lerry Lacey and Gary Judd

Budget 1:
Organization Name: USDA, ARS **Contract Administrator:** Carolyn Yager
Telephone: (509) 454-6575 **Email address:** cyager@yarl.ars.usda.gov

Item	**Year 1:** 2006	**Year 2:** 2007	**Year 3:** 2008	**Year 4:** 2009
Salaries	21,000	0	21,700	22,500
Benefits	6,400	0	6,600	6,800
Wages	0	0	0	0
Benefits	0	0	0	0
Equipment	0	0	0	0
Supplies	3,000	0	3,000	2,000
Travel	600	0	600	0
Miscellaneous				
Total	31,000	0	31,900	31,300

Project Objectives:
1. Develop, test, and select a biodegradable replacement, to be applied as a liquid or semi-solid to a tree trunk.

2. Evaluate pesticides and pathogenic nematodes in a candidate foam material to determine both larval recruitment, mortality and duration of effectiveness.

3. Compare cardboard banding and a biodegradable foam in apple orchards for efficacy and cost assessments.

Significant Findings:
1. Comparisons of polyurethane foam and cardboard banding showed a superiority of the foam in recruiting greater numbers of larvae that are seeking spin up sites.

2. Laboratory evaluations of several additional materials showed a clear connection between foam cell (bubble) size and efficacy, and superiority of a starch based material over others.

3. An industrial foamer with an application wand has been located that appears to be suitable for both mixing of experimental materials and application of foam to tree trunks in an orchard.

Methods:

Polyurethane foam (commercial Great Stuff ®) was applied to tree trunks in a two inch wide band. Other trees were banded with 2 inch wide cardboard banding. The materials were removed in late September and numbers of codling moth cocoons counted.

A series of materials were manufactured in the Albany laboratory to provide candidates for testing. These materials were both natural and synthetic, but were all designed to be light weight, porous, and inexpensive. Base materials were cardboard, wood fibers, starches, straw, polystyrene, polyurethane, and concretes. A range of densities of materials were also made for testing.

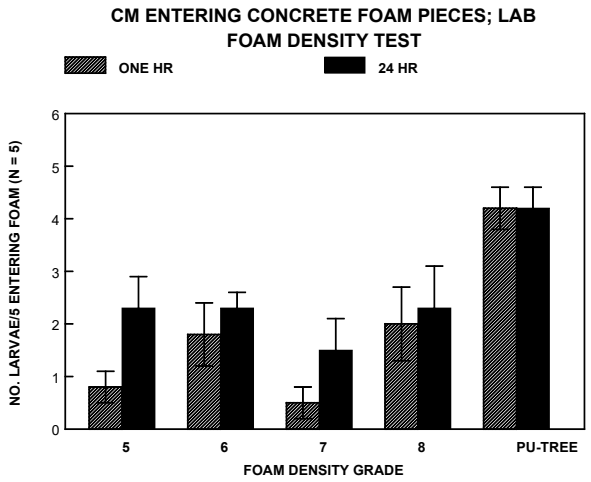
Candidate foam materials were tested in the laboratory to rank types of materials and material consistencies for their acceptance by mature codling moth larvae. Larvae from the USDA laboratory colony were removed from cocoons and placed individually in 16 oz plastic cups with pieces of test materials. Materials were scored at 1 and 24 hours for larval contact with and entry into the material. Great Stuff polyurethane foam and cardboard bands were used for comparison, as positive controls. A series of materials were tested using this arena assay. These comparisons are intended to provide candidate types of materials for use in more advance testing and formulations.

Results and Discussion:

Best results in laboratory assays were with polyurethane foams, a fiber reinforced foam, a fiber roll, a straw/starch formulation, and cardboard. These results supported the hypotheses that efficacious materials facilitated codling moth entry by chewing through the material and by the presence and size of air pockets or cells. All materials that strongly recruited larvae were “chewable” and open in consistency.

Table 1. Percent of mature codling moth larvae entering piece of test material held in 16 oz plastic cup in laboratory. N = 10 to 20.

Material



ring Test Material

	30 min	24 hours
Rice Straw/Starch Foam	20	100
Concrete Foam	0	60
Fiber Reinforced Concrete Foam	20	90
Mearl 10 Cement Foam	0	20
Mearl 5 Cement Foam	0	0
Pressed Cork	0	25
Starch Fiber Foam	0	15
Pressed Board	0	10
Fiber Foam	20	70
Foam Freeze Starch	0	0
Polyethylene foam	0	50
Polyurethane Foam	10	90
Polystyrene Foam	10	65
Large Pore Starch Foam	90	90
Fiber Roll	100	100
Great Stuff®	50	100
Card Board	85	85

Comparisons of densities of ultra-light concrete did not show an improvement in efficacy with decreasing density and all densities were inferior to cardboard banding. The lack of acceptance by some larvae may have been due to the toughness of the concrete, despite the presence of numerous small air pockets.

It was surprising nonetheless to see codling moth larvae bore into concrete and spin up cocoons within the concrete.

Plans and Time Line for 2007.

January to April. Additional foams will be laboratory-tested for acceptability to codling moth larvae, in Wapato. These assays will evaluate starch based materials, potentially with other materials and adjuvants, in part to provide consistency and strength, and later to add some water resistance. The goal of these assays will be to select a formulation for first field trials in June 2007.

April/May. One or more candidate materials will be evaluated in the Albany laboratory, using the commercial foamer applicator, to determine the feasibility of applying the material to tree trunks. Alterations will be made if necessary to the materials, most likely to alter the consistency of the resultant foam.

June. A field trial will compare a foam formulation to cardboard banding for efficacy in recruiting codling moth larvae to spin up. Applications to apple tree trunks will be made in early June, and counts made of cocoons in early July.

May to August. Formulation alterations will be made in Albany to provide water resistance to the foam. A second generation foam will then be tested in the laboratory in Wapato using the arena assay design to determine if changes in the formulation impacted acceptability to larvae. In addition, preliminary attempts will be made in the laboratory to test a pesticide and nematodes in the foam formulation. These materials will be evaluated in the laboratory, using the arena bioassay, in comparison to foam without pesticide or nematodes. Data will be obtained on recruitment of larvae into the foam (to test the hypothesis of no repellency of the treatments) and on mortality and survival of larvae within the foam.

August /September. Field trials will evaluate the second generation foam, in comparison to cardboard banding , to evaluate efficacy in recruiting larvae in the field, but also to durability when exposed to irrigation sprinklers.

September 2007 into January 2008. It is anticipated that a series of laboratory assays will need to be done to evaluate and compare several pesticides at different dosages, and different dosages of nematodes, to select dosages that provide optimum results in anticipation for field testing in 2008. In addition, information obtained from the two field trials may indicate the need for additional fine tuning of the foam formulation to provide durability and rain fastness. Any changes to the formulation would necessitate additional laboratory testing before the field season.

Budget Notes: The funding for the 2006 season was not available until October 2007, due in part to difficulties in working out an agreement between WTFRC and ARS regarding proposed changes in overhead charges. For this reason, we request that the WTFRC 2006 funding be used by us in 2007, and that additional funding be postponed one year. This requested change is reflected below in the budget table.

Budget:

Project Duration: 4 years (2006-2010)			Current year: 2007	
Project Total (4 years):			Current Year request: 0	
Year:	Year 1	Year 2	Year 3	Year 4
Total:	$31,000	0	$31,900	$31,300
Item:	Year 1	Year 2	Year 3	Year 4
Salaries	$21,000	0	$21,700	$22,500
Benefits	6,400	0	6,600	6,800
Wages	0	0	0	0
Benefits	0	0	0	0
Equipment	0	0	0	0
Supplies	3,000	0	3,000	2,000
Travel	600	0	600	0
	-------	-------	-------	-------
Total Request	$31,000	0	$31,900	$31,300

Salary requests are for partial support of a biological technician at Wapato (½ time), and partial support for a chemistry technician at Albany, CA (½ time). Travel request is to cover Dr. Glenn's travel from California to participate in WTFRC meetings.

Supplies include materials for preparation of foams, a foamer applicator, olfactometer and arena assay supplies, banding, pesticides and other chemicals, codling moth rearing materials, and nematodes.

CONTINUING PROJECT REPORT

Project Title: Collaborative WTFRC research programs

PI: Jim McFerson
Organization: WTFRC
Telephone/email: 1 509 665 8271
mcferson@treefruitresearch.com
Address: 1719 Springwater Ave
City: Wenatchee
State/Province/Zip WA, 98801

Cooperators: Tom Auvil, Felipe Castillo, Tory Schmidt, WTFRC, Wenatchee, WA
Ines Hanrahan, WTFRC, Yakima, WA

OBJECTIVES:

1. Conduct field trials on crop load management, use of reflective covers, sunburn suppression, russet management, rootstock evaluation, and lenticel-based skin disorders in grower cooperator orchards.

2. Assist WTFRC funded research programs with trial setup, maintenance, and sampling.

3. Manage soil sample collection regarding Penbotec and Scholar registration.

RESULTS AND DISCUSSION

WTFRC field trials

In 2006, the Washington Tree Fruit Research Commission (WTFRC) conducted 65 trials on apple, pear, cherry, peach, and nectarine within its internal research program, covering topics such as crop load management, reflective groundcovers, sunburn suppression, fruit finish, rootstock evaluation, and lenticel-based skin disorders (Table 1). All trials were conducted in grower-cooperator orchards. Funding was used to hire seasonal labor (10 people/year), to repair and maintain equipment, to purchase supplies, and to cover crop loss. Most products evaluated were donated by industry suppliers (Table 2). A number of trails were conducted with financial support from private companies (Table 3). Detailed project reports are included elsewhere in this document.

We have developed strong ties with various organizations specializing in international student exchange (i.e. Experience International, Ohio State University International Agriculture Exchange Program). In recent years, we have hosted interns from Germany, Mexico, and Austria. We encourage students to actively participate in industry events and to educate WA growers about practices in their respective home countries.

Table 1: WTFRC internal program field trials in 2006.

	Apple	Pear	Cherry	Peach	Nectarine
Crop load management	22	3	3	1	1
Reflective fabric*	7	2	5	1	
Sunburn suppression*	1				
Fruit finish*	6				
Lenticel breakdown	4				
Rootstock	9				
				Total:	**65**

* Products donated by industry suppliers

Table 2: Companies and Institutions that contributed materials and services to the WTFRC internal program in 2006.

Contribution	Company
Chemicals	Amvac
	BASF
	Cascade Distributing Co.
	D & M Chemical
	Fine Agrochemicals
	GS Long
	JMS Flower Farms
	Nufarm
	Orcal Inc.
	Pace Intl.
	RainGard
	Rohm and Haas
	Valent
Other supplies	Extenday
	Willow Drive Nursery
	Wilbur-Ellis, Wenatchee
Labor	Crane and Crane
	Fleming's Valley View Orchards
	Stormy Mountain Ranch
	Valley Fruit
	Willow Drive Nursery
Lab space/equipment	USDA-ARS TFRL, Wenatchee
	WSU-TFREC, Wenatchee
Packing line time	Valley Fruit
	McDougall & Sons
Fruit donation	Auvil Fruit Company
	Crane and Crane
	Ron Wilcox

Collaborative Projects

The WTFRC internal program provided technical support with trial set-up, maintenance, and sampling for several WFTRC-funded research programs (Table 3). A growing number of scientists have taken advantage of the opportunity to utilize the internal program's extensive network of industry cooperators when conducting field trials. By using in-state locations in commercial orchards, increasingly relevant data has been generated for Washington growers by research programs around the world.

Betsy Beers: The focus of this project was to evaluate collateral effects of chemical thinners (namely lime sulfur and carbaryl) on populations of phytophagous and predatory mites at WTFRC trial sites. Leaf samples were collected every other week from 5 treatments each at three trials and delivered to the Beers lab for evaluation. (sample timing: late April to late July)

Curt Rom: WTFRC performed field evaluations of novel organic chemical bloom and postbloom thinners developed by Rom and his graduate student, Jason McAfee. Materials included essential plant oils, organic acids, and organic bases with potential to kill or damage pollen. Trial location, setup, spray applications, harvest, and quality analyses were performed by the WTFRC.

Don Elfving: The internal program continued its ongoing support of data collection for Elfving's PGR work, including whole tree bloom counts in April for three sites and harvest yields in the fall.

Peter Hirst: *a*) Understanding apple flower bud development: *WTFRC* selected 10 trees from both a Gala and a Fuji block, attached tags to 100 buds per tree, and divided buds into 3 categories. Two samples of each type were collected every 10 days until August, when we switched to every 20 days until trial completion in mid October. The samples were fixed in FAA solution and shipped to Purdue for analyses.

b) Mechanisms of apple fruit growth: WTFRC selected 10 trees from both a mature Red Delicious and mature Gala block and hand thinned all trees at full bloom to reduce crop load. Samples were collected weekly starting in May, switching to bi-weekly in July, until trial completion at harvest. Samples consisted of 2 fruit from each tree, which were labeled and measured before being shipped to Purdue.

Steve van Nocker: WTFRC established a simple replicated thinning trial in Gala. Chemical thinners were applied with the Proptec sprayer, followed by 3 intensive sampling events of fruitlet parts. Hundreds of fruitlets were dissected and frozen in the field at each sampling, and eventually shipped to MSU for molecular analyses.

Gennaro Fazio: The main focus of the rootstock plantings is to evaluate new Geneva rootstocks in soils with replant problems (see Fazio report). WTFRC conducts trial layout, planting establishment, data collection, and some horticultural management on an ongoing basis.

Karen Lewis/Tom Auvil: WTFRC moves mobile platforms between locations for industry demonstration and testing, occacionally providing training in platform operation.

FruitGard: WTFRC located rain-vulnerable cherry sites, set up trials, and applied formulations designed to reduce rain cracking. Internal staff also conducted field evaluations, and collected harvest fruit samples.

Ciba-Geigy: Trial location and setup were performed by the WTFRC for 2 trials (Ambrosia and Pink Lady). Apples free of defects were selected and subsequently bagged with two component color enhancement bags about two months before harvest. Roughly a month before harvest the outer bag was removed, ten days later the inner liner was removed and the stencil was applied. At harvest, fruit was transported to the WTFRC lab for stencil removal, followed by treatment with SmartFresh, tray packed, and shipped to the company.

Whiting/Elfving: WTFRC staff helped design the trial, located an appropriate site, laid out the trial, collected field data, harvested sample fruit, and delivered it to the Whiting lab for quality analyses.

Whiting: WTFRC worked jointly with Extenday and Whiting to develop reflective groundcover trials in cherry. Internal staff maintained the trial, collected harvest fruit samples, and delivered them to the Whiting lab for quality analyses.

Fallahi: WTFRC conducted all aspects of trial design, setup, application, data collection, and analysis for peach and nectarine chemical thinning trials. Fallahi advised internal staff regarding treatments and provided Tergitol for use in soft fruit and apple.

Table 3: WTFRC internal program: collaborative support for WTFRC-funded projects.

			WTFRC technical support			
Researcher	**Topic**	**Number of trials**	Site selection	Trial set-up	Data collection	Misc.
Betsy Beers WSU - Wenatchee, WA	Chemical thinner effects on arthropod populations	3	x	x	x	
Curt Rom UA, Fayetteville, AR	Novel chemistries and pollenicides for chemical thinning	2	x	x	x	x
Don Elfving WSU - Wenatchee, WA	Use of gibberellic acid to inhibit flowering in apple	3			x	
Peter Hirst Purdue, West Lafayette, IN	Molecular basis for fruit cell division and expansion	4	x	x	x	x
Steve van Nocker MSU, East Lansing, MI	Molecular control of fruitlet abscission	1	x	x	x	x
Gennaro Fazio Cornell, Geneva, NY	Next generation rootstocks for apple	9	x	x	x	x
Lewis/Auvil WSU - Ephrata, WA / WTFRC	Mechanized assistance of orchard labor	variable	x			x
FruitGard LLC* Wenatchee, WA	Cherry cracking prevention	3	x	x	x	
Ciba-Geigy* Basel, Switzerland	Logo imprinting on apple skin	2	x	x		x
Whiting/Elfving WSU - Prosser/Wenatchee, WA	Use of gibberellic acid to inhibit flowering in cherry	1	x	x	x	
Whiting WSU - Prosser, WA	Reflective fabric to improve cherry quality	4	x	x	x	x
Fallahi UI - Parma, ID	Cropload management in softfuit	1	x	x	x	
	Total:	**33**				

* received financial support from company

Soil sample collection

In collaboration with the Northwest Horticultural Council and EPA, the internal program is managing soil sample collection supporting the registration of two new postharvest fungicides (Penbotec and Scholar). We are currently maintaining 24 sampling sites (Table 4). Soil samples are taken prior to application, directly after application, and post-season. WTFRC is responsible for collecting, shipping, and correct documentation of all soil samples. We anticipate to complete soil sampling in spring of 2007.

Table 4: Wastewater sampling: cost-effective data collection to support registration of postharvest fungicides needed by industry.

	Number of sites in WA	North central Washington	Yakima Valley
Sites established in 2005	11	5	6
New sites added in 2006	13	2	11
Total in 2006	**24**	**7**	**17**

CONTINUING PROJECT REPORT **YEAR**: 1 of 3
WTFRC Project Number: AH-05-508

Project Title: Employing biological elements of orchard ecosystems

PI: Mark Mazzola
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Telephone/email: 509-664-2280 ext. 207/mazzola@tfrl.ars.usda.gov
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Address 2: 1104 N. Western Ave.
City: Wenatchee
State/Province/Zip WA 98801

Cooperators: Ray Fuller, Chelan, WA

Budget 1: (*Required information – please complete all information***)**
Organization Name: USDA-ARS **Contract Administrator:** Chuck Myers
Telephone: 510-559-6019 **Email address:** cwmyers@PW.USDA.GOV

Item	**Year 1: 2005**	**Year 2:** 2006	**Year 3:** 2007
[1]**Salaries**	22,800	23,940	25,137
Benefits	6,840	7,182	7,541
[2]**Wages**	12,000	12,000	12,000
Benefits	3,600	3,600	3,600
Equipment	0	0	0
[3]**Supplies**	8,000	8,000	8,000
[4]**Travel**	800	800	800
Miscellaneous			
Total	54,040	55,522	57,078

Footnotes: [1]Postdoctoral Research Associate, 0.4 FTE; 0.6 FTE of this position is funded through other external funds and will contribute to this program; [2]Research Assistant, 0.5 FTE; [3] Enzymes, media, plasticware, chemicals, gases, greenhouse supplies, rootstocks; [4]Travel to field sites to collect soil, manage field trials, collect growth and yield data.

Project Overview:

For sites lacking significant lesion nematode populations, pre-plant *Brassica napus* seed meal amendment used in conjunction with a post-plant mefenoxam (RidomilGold EC) soil drench has provided levels of replant disease control, growth and yield equivalent to pre-plant soil fumigation (Mazzola & Mullinix, 2005). Preliminary studies with alternative seed meals suggest that disease control can be improved upon and may circumvent need for the post-plant mefenoxam application. Realization of this outcome would allow for the implementation of this disease control strategy in organic production systems.

The overall objective of this program is to develop an integrated management method compatible to conventional and organic apple production systems that provides the shortest time frame to initial commercial harvest when re-establishing orchards on sites previously planted to apple. As similar biological entities appear to have a role in replant problems encountered in pear, peach and cherry (Mazzola, unpublished data; Browne et al., 2002), it is plausible that such a system would have utility across tree fruit production systems.

Specific objectives:

1.) Examine the capacity of Brassicaceae seed meals to suppress the biological complex inciting replant disease and enhance tree growth in replant orchard soils.

2.) Determine the mechanism(s) by which these soil amendments provide control of the various plant parasites and pathogens that incite replant disease development, with emphasis on *Rhizoctonia solani*.

3.) Assess the influence of rootstock genotype on composition of resident *Streptomyces* populations and the efficacy of RSM-induced disease suppression

Goals and Activities 2007:

- Studies will continue to monitor growth and yield of the Gala/M26 block established at the CV orchard in May 2005, which seeks to determine the efficacy of different brassicaceae seed meal amendments for the control of replant diseases.
- Studies will be initiated to determine the value of a brassicaceae seed meal-based approach for use in single-tree replacement practices in orchard systems.
- A new planting will be established in a commercial orchard to evaluate the utility and efficacy of a composite seed meal amendment for control of replant disease in organic management systems.

Significant Findings 2006:

- Irrespective of seed meal type, 18 month post-plant growth increment of Gala/M26 apple in soils receiving a pre-plant amendment in conjunction with a post-plant mefenoxam soil drench was as great or greater than trees grown in soils receiving pre-plant soil fumigation
- In the absence of post-plant Ridomil drench, only seed meal from the mustard *Sinapis alba* cv. IdaGold improved tree growth to a level equivalent to soil fumigation.

- Ridomil (mefenoxam) soil drench alone did not improve tree growth.

- When applied in a commercial organic orchard, a composite *Brassica napus+Brassica juncea* seed meal amendment suppressed amplification of *Pythium* populations which is consistently observed in response to *B. napus* seed meal alone.

- Composite *Brassica napus*+*Brassica juncea* amendment significantly improved tree growth, at times to a level superior to pre-plant fumigation, but the response was rootstock dependent.

- All seed meals were demonstrated to provide control of *Rhizoctonia* by modifying the orchard soil microbial community, but *Brassica juncea* was shown to have an additional chemical mechanism, production of allyl-isothiocyanate.

Orchard Gala/M26 Field Trial

A field trial was established at the Columbia View Research and Demonstration Orchard, and was initiated with tree removal (Red Delicious/Seedling) from the site in October, 2004. In addition to *B. napus* cv. Dwarf Essex (canola), seed meal of *Sinapis. alba* cv IdaGold (yellow mustard) and *B. juncea* cv Pacific Gold (oriental mustard) were employed with or without a post-plant mefenoxam (Ridomil) soil drench. These seed meals were chosen based upon our data obtained in controlled environment studies, and data from the literature, suggesting the relative activity of these materials toward the pathogens and parasites causing replant disease. In a preliminary greenhouse trial, *B. juncea* was superior to other seed meals for control of lesion nematode (Table 1).

Table 1. Impact of seed meal on lesion nematode populations in soil and gala seedling roots

Treatment[z]	*Pratylenchus*/g soil	*Pratylenchus*/g root
Control	217b	370c
DE	19a	79ab
Athena	5a	111b
IG	7a	105b
PG	1a	2a

[z]DE and Athena=*Brassica. napus*; IG=*Sinapis alba*; PG=*Brassica. juncea.*

B. juncea also seemed a suitable choice for organic systems as in greenhouse trials it did not stimulate *Pythium* populations resident to orchard soils (Cohen and Mazzola, 2006). In the field, *B. napus* and *S. alba* amendments increased *Pythium* populations by greater than an order of magnitude (Table 2), and caused significant infection of Gala/M26 roots. All seed meal amendments, but not pre-plant Telone-C17 fumigation, provided significant control of *Rhizoctonia* root infection (Table 2), however control of *Rhizoctonia* in response to *B. juncea* seed meal at times was less effective than *B. napus* seed meal (Mazzola et al., 2007).

Table 2. Impact of soil treatments on soil populations and Gala/M26 root infection by fungal pathogens.

Treatment	*Pythium spp.* propagules per g soil	*Rhizoctonia* root infection (%)
Control	265b	14.0a
Telone C17	65a	11.7ab
Brassica juncea PG	75a	5.0b
Brassica napus DE	5320c	6.6b
Sinapis alba IG	4515c	2.8b

[z]Values followed by the same letter are not significantly different according to the Tukey test (P=0.05)

B. juncea, B. napus or *S. alba* seed meal with post-plant mefenoxam significantly improved tree growth over the first two growing seasons at a level equivalent to pre-plant soil fumigation (Table 3). The comparative results obtained with *B. napus* with or without post-plant Ridomil drench were identical to that obtained in a previous field trial (Mazzola and Mullinix, 2005). *Sinapis alba* seed

meal alone resulted in tree growth that was equivalent to Telone-C17. The basis for enhanced growth of apple in response to post-plant application of mefenoxam was seed meal dependent; control of *Pythium* in *B. napus* treated soils and control of *Phytophthora* in *B. juncea* amended soils.

Table 3. Impact of pre-plant seed meal amendment and post-plant mefenoxam soil drench on 18-month increase in trunk diameter (mm) of Gala/M26 apple planted at CV orchard, Orondo, May 2005

Pre-plant soil treatment	No post-plant treatment	Post-plant mefenoxam
Control (no treatment)	9.d8[z]	11.3cd
Telone-C17	15.2ab	-
Brassica juncea PG	11.1cd	16.8a
Brassica napus DE	11.4cd	13.1bc
Sinapis alba IG	13.4bc	16.1a

[z]Values followed by the same letter are not significantly different according to the Tukey test (P=0.05)

The initial fruit harvest from this experimental block was conducted in August 2006. No statistical differences (P=0.234) in yield were observed among treatments (Table 4). However, in all instances, the trend in yields were higher for trees grown in seed meal/mefenoxam treated soils than non-treated or fumigated soils.

Table 4. Impact of pre-plant seed meal amendment and post-plant mefenoxam soil drench on 2006 yields from Gala/M26 apple (kg/tree) established at the CV orchard, Orondo, WA in May 2005

Pre-plant soil treatment	No post-plant treatment	Post-plant mefenoxam
Control (no treatment)	0.00	0.17
Telone-C17	0.20	-
Brassica juncea PG	0.95	1.49
Brassica napus DE	0.78	0.94
Sinapis alba IG	0.45	1.55

Significance to industry: In concert with our previous studies (Mazzola and Mullinix, 2005), the data to date demonstrate that consistent control of replant disease can be attained using the *B. napus*/mefenoxam treatment on sites lacking significant lesion nematode populations. Likewise, superior pathogen control attained with *B. juncea*/mefenoxam treatment relative to soil fumigation may provide enhanced tree performance, which has been realized to date. While the initial finding from the *B. juncea* alone treatment was unexpected and disappointing, these data provided further information for developing effective and **consistent** control of replant disease in organic systems.

Orchard Rootstock Trial:

A field trial was established at a commercial organic orchard to evaluate the efficacy of a *Brassica napus/Brassica juncea* composite seed meal amendment for control of replant disease, and to assess whether the response was rootstock dependent. Composite seed meal or *B. napus* seed meal alone was applied on May 3 at 3.08 lbs per linear foot of tree row and rotovated into the soil profile. The site was planted with G11, G16, G30, M7, M9, M26 and seedling rootstocks. Data for G11 rootstock are not reported as all plants died irrespective of soil treatment.

B. juncea seed meal suppressed the repeatedly observed *B. napus*-induced proliferation of *Pythium* spp. (Table 5). In assessments completed to date, the *B. napus/B. juncea* significantly reduced lesion nematode populations recovered from roots of M26 and M7 rootstocks and to a level comparable to

or better than that attained through pre-plant soil fumigation. However, the level of nematode control attained was not comparable to what has been achieved with *B. juncea* alone in greenhouse trials (Table 6). Optimization of nematode control with the combination seed meal may require modification of application method to enhance soil retention of the *B. juncea*-derived allyl-isothiocyanate (AITC).

Table 5. Effect of treatments on *Pythium* soil populations.

	Pythium soil populations (cfu/g soil)	
Treatment	At planting (5.29.06)	At harvest (10.26.06)
Control	550b	604b
Telone-C17 fumigation	135a	350a
B. napus Athena	3890c	3917c
B. napus Athena+*B. juncea* PG	120a	300a

Table 6. Impact of soil treatments on recovery of lesion nematode from roots of apple rootstocks planted at Stormy Mountain Ranch, Chelan, WA

	Pratylenchus penetrans populations (#/g root)	
Treatment	M26	M7
Control	114b	189b
Telone-C17 fumigation	59a	104ab
B. napus Athena	109b	85a
B. napus Athena+*B. juncea* PG	42a	92a

As was observed in previous greenhouse studies, the growth response attained for any given treatment in the field was rootstock-dependent. In several instances, all treatments (including soil fumigation) failed to improve plant growth relative to that attained in non-treated orchard soils. Root growth of G30 planted in composite seed meal amended soil was superior to all other treatments, and root biomass of M9 was greatest in fumigated soil. Significant increases in shoot biomass were only observed in two instances; G16 in response to the composite seed meal amendment, and M7 in response to Telone-C17 soil fumigation. These outcomes were likely influenced by several factors including differential rootstock vigor and significant wildlife browsing.

Greenhouse rootstock/seed meal studies:

In concert with field trials, greenhouse studies were conducted to determine whether rootstocks contributed as a factor in disease control attained in response to brassicaceae seed meal amendment. Studies were conducted in the greenhouse using soils from the GC orchard, Manson, WA. Lesion nematode contributes significantly to disease development.

Control of specific pathogens was dependent upon seed meal type, rootstock and time. As observed previously, initial lesion nematode soil populations were significantly suppressed by all amendments, but control was not maintained for all seed meal treatments. Regardless of rootstock, *Brassica juncea* cv. Pacific Gold repeatedly and consistently suppressed root populations of this nematode over the 6 month study period (Table 7). Under very high nematode pressure, the *B. napus* and *Sinapis alba* treatments also provided extended nematode control, but this response was variable and often rootstock specific. In general, Malling and Malling-Merton rootstocks supported higher lesion nematode populations than did Geneva series rootstocks. Bagging *B. juncea* amended soils (to simulate tarping in the field) immediately after seed meal application provided a numerically superior level of nematode control, though statistically there was no difference from the non-bagged *B. juncea* treatment. The bagging of *B. juncea* amended soils was conducted as a means to retain the volatile AITC, which based upon our laboratory studies, is produced within 30 minutes of *B. juncea* seed

meal amendment. Yield of AITC from treated soil was complete within 24 hours of seed meal amendment.

Pythium populations recovered from soil and apple roots. As in studies described above, *B. napus* and *S. alba* amendments stimulated *Pythium* populations, increasing from 150 propagules/g in the non-treated control soil to as high as 12000 ppg (*B. napus* Athena; Table 8). Although *B. juncea* amendment depressed *Pythium* spp. soil populations, bagging *B. juncea* amended soils for 48 h prior to dispensing soil into pots virtually eliminated recovery of this pathogen from soil and apple roots.

Table 7. Impact of brassicaceae seed meal amendments on lesion nematode root populations

Experiment 1	Lesion nematode populations (# per g root tissue)										
Treatment	G11	G16	G30	M7	M9	Nic29	M26	MM106	MM111	Seedling	B9
Control	-	196	-	710	254	284	480	1758	850	371	142
Pasteurized	-	8	-	8	12	11	2	6	3	3	4
B. napus DE	-	110	-	189	98	205	136	78	321	28	179
B. napus Ath	-	214	-	178	127	230	142	177	189	11	105
S. alba IG	-	91	-	212	162	242	153	246	163	85	174
B. juncea PG	-	0	-	11	16	14	5	2	9	2	9
Experiment 2											
Control	434	1269	472	930	672	1556	393	499	1085	956	578
Pasteurized	3	3	6	2	0	2	0	0	1	20	1
B. napus DE	101	202	139	884	891	612	1588	456	168	514	891
B. napus Ath	30	46	52	752	619	235	954	236	251	270	300
S. alba IG	109	236	153	638	310	176	429	1642	1053	335	476
B. juncea PG	6	6	2	5	0	2	11	32	2	192	1
B. juncea-bag	0	0	0	1	0	0	0	1	0	17	2

Interestingly, ultimate composition of the *Pythium* population recovered from apple roots was dependent upon the type of seed meal applied to any given soil. The no treatment control population did not possess a clearly dominant species. In contrast, *P. irregulare*, *P. debaryanum,* and *P. heterothallicum/P. attrantheridium* dominated the populations recovered from roots of apple grown in *B. juncea, S. alba* and *B. napus* treated soil, respectively (Table 9).

Table 8. Effect of seed meal amendments on recovery or *Pythium* from GC soil and apple roots

Treatment	*Pythium* soil population (propagules/g soil)	*Pythium* root infection (%)
Control	145	7.3
Pasteurization	61	3.8
B. napus DE	4486	35.2
B. napus Athena	12818	44.6
S. alba IG	2563	43.7
B. juncea PG	154	3.4
B. juncea PG-bagged	0	1.2

This effect on species composition is extremely relevant as studies from this lab have previously demonstrated that this is great variation in virulence towards apple among *Pythium* spp. (Mazzola et

al. 2002). In general, among those dominating in the current study, isolates of *P. heterothallicum* and *P. attrantheridium* are less virulent than *P. irregulare* and *P. sylvaticum*.

Table 9. Effect of seed meal amendment on species composition of *Pythium* population

Pythium species	Control	*B. napus* DE	*B. napus* Ath	*S. alba* IG	*B. juncea* PG
P. attrantheridium	31.1	38.6	39.6	19.5	7.7
P. debaryanum	22.5	6.9	10.8	41.3	15.7
P. heterothallicum	14.5	49.0	47.6	20.7	4.8
P. irregulare	9.4	4.4	0.0	12.0	66.7
P. sylvaticum	22.4	1.0	2.9	6.4	5.2
P value	0.468	0.001	<0.001	0.049	<0.001

Significance to industry: The documented suppression of both lesion nematode and *Pythium* spp. in the field by composite *B. napus/B. juncea* seed meal amendment suggests its potential value for use in both conventional and organic production systems. Preliminary findings indicate that designer seed meal formulations may be developed for specific orchard replant sites based upon knowledge of the pathogen complex (e.g. presence or absence of lesion nematode) and the desired rootstock to be planted. These trials and associated laboratory studies, also point to a potential need for tarping soil immediately after *B. juncea* application to contain allyl-isothiocyante produced to yield optimum disease control performance, specifically the control of lesion nematode, a procedure that was omitted from the 2006 field trial.

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CONTINUING PROJECT REPORT **YEAR 2/3**

WTFRC Project # AE-05-502 Organization Project # 5853525758

Project title: Improving codling moth granulovirus (CpGV) transmission and activity

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Cooperators: Alan Knight, USDA-ARS, Wapato, WA
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Budget

Organization:USDA, ARS	**Contract Administrator:**Carolyn Yager
Telephone:509-454-6575	**Email:**cyager@yarl.ars.usda.gov

	Year 1 (2005)	Year 2 (2006)	**Year 3 (2007)**
Salaries and wages (includes benefits)			
Technician, partial support for GS5	20,000	20,000	20,000
Summer help, GS3, 1 FTE (3 months)	5,000	5,000	5,000
Chemicals, plastics, misc. materials	1,500	1,500	3,500
Total	$26,500	$26,500	**$28,500**

Objectives

- Continue study of attractants (pear ester) and feeding stimulants to improve uptake of virus
- Substitute continued work on autodissemination with additional work on UV protection of CpGV

Significant findings (2006)

- Pear ester added to CpGV in orchard studies caused (1) moderate reduction of fruit injury in apple during the second, but not first, generation and in 'Bartlett' pear at harvest and (2) a moderate increase in larval mortality and the percentage of shallow stings at 'Bartlett' harvest
- The mixing of CpGV, either in kaolin clay particle film (Surround®WP) or a water soluble lignin, reduced overall fruit injury, increased larval mortality and reduced deep entries in fruit (irradiated with harmful sunlight) with compared with CpGV alone.
- Our findings indicate that adjuvants and different spray formulations may improve the effectiveness and environmental persistence of CpGV, apparently through enhanced larval susceptibility and protective effects against solar degradation

Methods

Virus source. A Commercial preparation of CpGV 'Cyd-X®' (Certis USA, Columbia, MD) containing 3×10^{13} granules/liter was used as the virus source in all tests.

Orchard studies evaluating pear ester as a formulation additive (Objective 1) Orchard studies were conducted in apple and pear to evaluate the larval kairomone (*E,Z*)-2,4-decadienoate (pear ester) as a formulation additive to improve the efficacy of CpGV. Pear ester was a 5% a.i. microencapsulated formulation (PE-MEC, Trécé Inc. Salinas CA). All treatments were applied by airblast as dilute sprays (80-100 gal./A) and timed according to male Biofix and a degree-day (DD) phenology model for codling moth (Brunner et al. 1987). Buffer trees and under-tree sprinklers were used and no additional insecticides were applied to test plots during tests. In 2006, studies were conducted in a mixed apple block and 'Bartlett' pear. In apple, each replicate block consisted of 'Golden Delicious', 'Fuji', 'Gala' and 'Delicious'. Treatments comprised Cyd-X alone (3 fl./oz/A; 2.66×10^{12} virus granules/A), Cyd-X plus PE-MEC (1.9 g a.i./A), PE-MEC alone (same rate) and untreated controls. Five applications were made against the first generation, starting 1 June (288 DD). The second larval generation was not treated. In Bartlett pear, there were 10-20 trees per replicate. Treatments were Cyd-X alone (3 fl./oz/A), Cyd-X plus PE-MEC (1.5 g a.i./A), PE-MEC alone and untreated controls. Initial applications were made 18 May (309 DD) and treatments generally reapplied at 14 d intervals (range 6-14) for a total of seven applications until harvest. In both tests there were 5 replicates per spray treatment arranged within a randomized complete block design. No spreader/stickers were used. For assessments, fruit per tree fruit were sampled *in situ* mid season after the first larval generation and (in pear) again near harvest. 100 fruit were examined per replicate. Samples of infested fruit were picked and dissected in the laboratory to quantify larval mortality and shallow entries (≥ 5 mm). Weather conditions were generally dry and sunny.

Evaluate particle films and lignin-encapsulated formulations for improved solar stability of CpGV (Objective 2)

Laboratory tests were conducted [using previously described procedures (Lacey and Arthurs, 2005)], to evaluate the particle film Surround®WP (Engelhard Corp.) as a solar protectant for Cyd-X. In short, 'Fuji' apples were surface sterilized, sectioned, heat-treated and the cut section immediately sealed with wax and foil. Prepared fruit were sprayed with experimental treatments in a DeVries spray cabinet calibrated for 100 gal./A. Cyd-X was always tested at 3 fl.oz./A and Surround was tested at two rates (25 and 50 lb/A). After samples had dried, half from each treatment (UV-controls) were placed in plastic food containers and infested with 4 neonate codling moth. The remaining half

were placed in a reflective cabinet and exposed to UV (300-400 nm) and other wavelengths (visible, 400-800 nm and infrared (≥800 nm) with an Atlas Suntest CPS + solar simulator. Samples were irradiated with a discriminating dosage of accumulated radiant energy (9.36×10^6 joules/m^2). Infested apples were dissected after 10 days to determine stings, deep entries (≤ 6mm) and larval mortality. There were 5 replicate apples per treatment per test and the test was conducted 6 times.

Field studies were conducted in 8 year-old 'Delicious' at the USDA field station near Moxee, WA to evaluate lignin-based formulations of CpGV. Test formulations consisted of (1) Cyd-X, (2) 'lignin-encapsulated CpGV' manufactured by incorporating purified virus into a lignin-based wettable powder formulation by spray drying [detailed description of protocols presented in Arthurs et al. 2006] and (3) a lignin-based adjuvant containing a separate cross-linking agent consisting of $CaCl_2$ (33%) and sucrose (67%) that can be mixed together directly with a commercial virus product in the field. Rates were Cyd-X (3 fl.oz./A), CpGV/lignin formulation (77 oz./A) and CpGV/lignin adjuvant (128 oz./A); all treatments applied 2.66×10^{12} virus granules/A. Sprays were applied with motorized mistblower (100 gal./A) with NuFilm spreader/sticker (6fl.oz./A) to single tree plots (6 replicates) on two occasions (10 July and 1 August). Each time, sub-samples of fruit were removed for bioassay (to determine residual activity) up to 21 days post application.

Results and discussion

Pear ester as a formulation additive for CpGV (Objective 1). The benefits of adding pear ester to CpGV in our 2006 orchard trials in pome fruit were inconsistent. In 2006 apple tests, PE-MEC (1.9 g a.i./A) + CpGV (2.66×10^{12} granules/A) did not reduce fruit injury or increase larval mortality of first generation codling moth in mixed blocks compared with CpGV alone (Table 1). Data shown are pooled among all cultivars within each four-tree block for one-way analysis because the interaction of spray treatment and cultivar was not significant ($F_{9,59} = 0.5$, $P = 0.87$). However, data for larval mortality and shallow stings were significantly higher for PE-MEC alone versus the untreated control. In Bartlett pear, fruit injury was not reduced in the CpGV + PE-MEC versus CpGV alone, at mid season or harvest (Table 2). However in this case, both larval mortality and the percentage of larval stings that were shallow were significantly higher in the CpGV + PE-MEC over CpGV alone. As in apple, higher mortality and shallow stings were also observed in the PE-MEC alone versus untreated controls. Injury was also reduced in PE-MEC alone at mid-season.

Additional studies conducted in 2005 (data provided in 2005 report) and by other ARS scientist (notably in walnut) indicate several factors could determine the effectiveness of pear ester (PE-MEC) seen among crops and seasons. Factors affecting the responses of adult codling moth to pear ester (monitoring traps) have been fairly well studied. For example, the relative attractiveness of pear ester for adult codling moth varies between walnut and pome fruits and among apple and pear cultivars. In addition, dose and seasonal timing (crop phenology) influences the attractiveness of pear ester to adult codling moths. Unfortunately, the behavior of codling moth neonates to pear ester has not been as well studied. The importance of using different rates of pear ester in different crops is unknown, but could be a significant factor influencing the activity of sprays combining pear ester with insecticides. Comparative of the influences of pear ester on codling moth neonate host searching behaviors in walnut and pome fruits are also needed.

Table 1. Orchard evaluation in mixed apple (data pooled) of CpGV (Cyd-X formulation) applied with and without pear ester (PE-MEC) against codling moth, Moxee, WA, 2006

Treatment	% fruit injury[1]	% shallow stings	% mortality
Untreated control	37.3 ± 4.2	22.6 ± 1.8a	48.9 ± 4.3c
PE-MEC (1.9 g a.i./A)	32.8 ± 3.2	47.1 ± 4.8b	65.0 ± 4.3b
CpGV (3 fl.oz./A)	33.9 ± 6.6	67.7 ± 5.0c	88.9 ± 4.0a
CpGV + PE-MEC	34.3 ± 4.0	66.0 ± 2.9c	87.4 ± 3.4a

Table 2. Orchard evaluation in 'Bartlett' pear of CpGV (Cyd-X formulation) applied with and without pear ester (PE-MEC) against codling moth, Medford, OR, 2006

Treatment	Mid-season	Harvest		
	% fruit injury[1]	% fruit injury	% shallow stings	% larval mortality
Untreated	13.0 ± 2.6a	50.6 ± 2.9a	32.7 ± 3.2a	48.9 ± 3.2d
PE-MEC (1.5g a.i./A)	5.6 ± 1.7b	44.2 ± 3.3a	52.8 ± 5.2b	68.6 ± 2.7c
Cyd-X (3 fl. oz./A)	6.2 ± 1.5b	30.0 ± 5.2b	72.6 ± 3.0c	88.8 ± 2.8b
Cyd-X + PE-MEC	6.0 ± 1.2b	27.0 ± 3.6b	89.2 ± 1.6d	94.6 ± 0.9a

[1]Data show fruit injury (on tree), shallow stings (≤ 5mm) and larval mortality evaluated from infested fruit. Column means ± SEM (5 replicate plots) followed by different letters were significantly different, Fishers LSD, $P < 0.05$.

Evaluate particle films and lignin-encapsulated formulations for improved solar stability of CpGV (Objective 2)

The mixing of CpGV, either kaolin clay particle film (Table 3) or water soluble lignin (Table 4), reduced overall fruit injury, increased larval mortality and reduced deep entries in fruit compared with the commercial preparation alone. Our findings indicate CpGV protective effects against solar degradation through formulation in inert carriers. A second set of field tests showed similar results (data not shown). Unfortunately, differences were not always large and optimal rates, coverage and application strategies need to be confirmed in 2007 and in orchard tests to maximize formulation benefits. Further pH stabilization is also needed for the slightly alkaline lignin/adjuvant formulation.

Table 3. Laboratory evaluation of a particle film adjuvant (Surround®WP). Data based on irradiated fruit (9.36×10^6 joules/m^2) infested with four neonate codling moth.

Formulation[1]	Fruit sprayed with 7×10^9 granules/Liter (3 fl. oz./A)		
	% CM fruit penetration	% deep CM entries	% CM mortality
Untreated	92.3a	88.5a	14.4b
Surround (25lb/A)	80.8b	82.9a	22.5b
Surround (50lb/A)	55.8c	64.8b	49.2a
Cyd-X (3 fl.oz/A)	85.8ab	68.1b	43.3a
Cyd-X +Surround (25lb/A)	73.3bc	55.9b	50.8a
Cyd-X +Surround (50lb/A)	69.2bc	55.5b	59.2a

Data based on 30 irradiated fruit each infested with 4 neonate CM (6 replicates of 5 fruit/treatment)
[1]Mortality of larvae in non-irradiated fruit treated with virus was always >80%.

Table 4. Field evaluation of lignin formulations of CpGV (see methods) compared with a commercial product (Cyd-X®).

a) Fruit injury (shallow and deep entries combined)

Treatment	Days post spraying					
	0*	3*	7	10*	14	21
Control	4.4ab	4.1a	4.8	4.6a	4.7	4.1
Cyd-X (3 fl.oz./A)	4.5a	4.2a	4.8	4.5a	4.8	4.0
CpGV/lignin formulation	4.0b	3.0c	4.4	3.7b	4.4	3.9
CpGV/lignin adjuvant	4.3ab	3.6b	4.5	4.0b	4.5	3.6

b) Proportion of deep entries (≤ 6mm)

Treatment	Days post spraying					
	0*	3*	7*	10	14	21
Control	0.93a	0.88a	1.00a	0.93	0.93	0.97
Cyd-X (3 fl.oz./A)	0.40b	0.80b	0.90b	0.92	0.95	0.88
CpGV/lignin formulation	0.17c	0.45c	0.92b	0.83	0.97	0.88
CpGV/lignin adjuvant	0.27bc	0.47c	0.73c	0.87	0.93	0.85

c) Proportion larval mortality (total)

Treatment	Days post spraying					
	0*	3*	7*	10*	14	21
Control	0.45c	0.35c	0.30b	0.32b	0.32	0.42
Cyd-X (3 fl.oz./A)	0.85b	0.53b	0.40ab	0.40b	0.27	0.47
CpGV/lignin formulation	0.95a	0.80a	0.45a	0.58a	0.30	0.52
CpGV/lignin adjuvant	0.88ab	0.75a	0.47a	0.43b	0.35	0.48

[1]Data show mean ± SEM of 6 single tree plots (each tree was averaged for 5 fruit). Letters in columns indicate differences following significant 1-way ANOVA for that date*; $P<0.05$, Fisher's LSD. Proportion data were arcsine transformed prior to analysis

Proposed schedule of 2007 accomplishments

There is considerable evidence that a number of adjuvants and formulating materials may improve the efficacy of CpGV through enhanced environmental persistence and larval uptake. Although we have made some progress, important questions regarding formulation procedures and field application remain. Studies in 2007 will focus on the most effective strategies for the use of lignin-formulations and Surround, initially in the laboratory and then in the orchard using materials and methods described above. The effect of rainfastness, with and without stickers will also be evaluated using a rain simulator (DeVries spray cabinet).

References

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CONTINUING PROJECT REPORT **YEAR 1/2**

WTFRC Project # AE-06-602 Organization Project # 5853527380

Project title: *Heterorhabditis* nematodes for codling moth and oriental fruit moth control

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Cooperators: David Granatstein, WSU-TFREC , Wenatchee, WA

Budget

Organization: USDA, ARS	**Contract Administrator:** Carolyn Yager
Telephone:509-454-6575	**Email:**cyager@yarl.ars.usda.gov

	Year 1 (2006)	**Year 2 (2007)**
Salaries and wages (includes benefits): Technician, partial support for GS5 Summer help, GS3, 1 FTE (3 months)	$18,500	$18,500
Chemicals, plastics, misc. materials	1,500	1,500
Total	$20,000	$20,000

Objectives (modified per WTFRC instructions):

1. Screen several commercially available *Heterorhabditis* species in the laboratory using protocols developed at YARL to quickly determine the most efficacious species against CM and OFM larvae.
2. Conduct field trials in experimental orchards to determine efficacy of two or more *Heterorhabditis* species for CM and OFM control .
3. Isolate and evaluate *Heterorhabditis* species that are native to Eastern Washington orchards.

Significant findings in 2006

1. Laboratory screenings revealed moderate activity of *Heterorhabditis* species against cocooned codling moth.
2. Field trials in Wenatchee and Moxee showed that *Steinernema* species out performed *Heterorhabditis* species using 3 methods of assessment. Although OFM was less susceptible than codling moth to *S. feltiae* in the field, moderate control was obtained against this species in cardboard strips on bare ground.
3. Surveys for native insect specific nematodes yielded positive locations in Vantage, Euphrata, and East Wenatchee. The isolate from Vantage grew well in waxmoth and was identified as *H. bacteriophora*. However, small plots field trials did not produce significant mortality in sentinel cocooned codling moth larvae.

Methods, Results and Discussion

Laboratory bioassays were conducted on *Heterorhabditis* species against cocooned codling moth larvae in cardboard strips using the methods described by Lacey and Unruh (1998). All nematodes were grown in wax moth larvae in the laboratory using procedures prescribed by Kaya and Stock (1997). 100 larvae per replicate test were used for each nematode species, concentration and control. The larvae were exposed to 5, 10, or 20 infective stages of the 3 *Heterorhabditis* species. The tests were repeated on 3 dates. The results of these assays are reported in Table 1. The highest activity was observed with the *H. bacteriophora* and *H. marelatus* at the highest concentrations. Previous bioassays of *Steinernema feltiae* (a species used in previous field tests, Lacey et al. 2006a, 2006b) yielded significantly higher mortality (80-90%) at 10 infective stages per cm^2.

Moxee field trials. Individual single tree replicates and mulched plots and were marked within a block of Golden Delicious apples at the USDA experimental farm near Moxee. Half of the treatments (5 plots per treatment and control) in June were made in mulched plots and half (5 plots per treatment and control) on bare ground. Within each plot a cardboard strip containing 20 cocooned codling moth larvae and one containing 20 cocooned Oriental fruit moth (OFM) larvae were placed within each plots. In mulched plots the strips were placed 2-3 cm below the mulch. In bare plots they were placed on the surface, but covered with netting after applications to protect them from birds. Experimentally Table 1. Laboratory bioassay of experimentally producted infective stages of *Heterorhabditis* species against cocooned moth larvae.[1]

Nematode species	% mortality (conc. per cm2)		
	5	10	20
Heterorhabditis bacteriophora	35	53	74
H. marelatus	45	62	75
H.megidis	18	33	49

[1] control mortality was 0% for all test dates; tests were replicated on 3 different dates

produced nematodes of 4 species (*S. feltiae, H. bacteriophora, H. megidis* and *H. marelatus*) were applied at 0.4 billion per acre in the equivalent of 400 gal. per acre with a back pack sprayer. Plots were pre wetting using a light mist of water prior to applications and for 2 hours following application. The results of these trials are reported in Tables 2 and 3. Overal *S. feltiae* produced better control of both species. Mulch provided some advantage for the codling moth control but less activity was observed in OFM that were placed under mulch. None of the Heterorhabditis species that were tested provided adequate control.

Table 2. Spring field trial of experimentally produced insect-specific nematodes applied at 0.4 billion infective stages per acre for control of overwintering codling moth larvae in an experimental apple orchard near Moxee, WA.

Nematode species	% mortality -- location of larvae	
	In cardboard on bare ground	in cardboard under mulch
Control	0	0
Steinernema feltiae	61.22	85.27
Heterorhabditis bacteriophora	0	1.33
H. marelatus	1.43	3.82
H.megidis	0	5.56

[1]. Mean, minimum and maximum temperature during the 48 hours following treatment on June 12, 2006 were, 48.3, 60.4, 75.9°F, respectively. Relative humidity ranged from 40 to 100%.

Wenatchee field trial. Eighteen individual plots (13 ft. by 65 ft; 845 ft^2) (from center row to center row) were marked within rows of Gala var. apples. The ground beneath each plot was covered with 2-3 cm of wood chip mulch. Each treatment and control plot was be replicated 3 times and all treatments and controls were randomly assigned to plots in each of 3 rows. Treatment effects were determined with cocooned sentinel larvae in cardboard strips placed under the mulch (3 per plot), in logs lashed to tree trunks (3 per plot), and in cardboard bands secured to tree trunks (3 per plot). Sentinel larvae were placed in the orchard the day of application. The treatments consisted of three nematode species (*Steinernema carpocapsae, S. feltiae, and Heterorhabditis megidis*) produced by

Table 3. Spring field trial of experimentally produced insect-specific nematodes applied at 0.4 billion infective stages per acre for control of overwintering Oriental fruit moth larvae in an experimental apple orchard near Moxee[1].

Nematode species	% mortality -- location of larvae	
	In cardboard on bare ground	in cardboard under mulch
Control	0	0
Steinernema feltiae	70.30	55.49
Heterorhabditis bacteriophora	8.00	4.33
H. marelatus	15.24	10.87
H.megidis	2.86	6.47

[1]. see footnot table 2.

Becker Underwood. Two application rates (0.4 and 1.0 billion IJs/acre) were used for *S. feltiae and H. megidis* and one (0.4 billion IJs/acre) for *S. carpocapsae.* Applications were made using an airblast sprayer outfitted with D10 nozzles, with screens and swirl plates removed. Applications were made in 400 gal/ac (7.76 gal/plot). Irrigation was run 1 hour prior to and for up to 4 hours following application of IJs. Two days after application of infective juveniles (IJs), sentinel larvae were collected and incubated at 25°C for 5 days until mortality was determined. The results of these trials are reported in Table 4. The two *Steinernema* species provided excellent control in mulched plots. Only *S. feltiae* produced moderate mortality in tree bands at both rates and in logs at the 1 billion per acre rate. Only moderate levels of control were obtained *H. megidis* at the 1 billion per acre rate in mulch.

Table 4. Early fall field trial of commercially produced insect-specific nematodes applied at 0.4 and 1 billion infective stages per acre for control of overwintering codling moth larvae in an experimental apple orchard in East Wenatchee, WA.

Nematode species	% mortality -- location of larvae		
applicate rate/billion acre			
	Under mulch	within logs on trees	cardboard bands on trees
Control	0	1.3	0
Steinernema feltiae			
0.4	98.3	13.51	40.84
1	98.98	46.55	42.35
S. carpocapsae			
1	93.60	16.80	6.10
Heterorhabditis megidis			
0.4	23.53	11.69	3.59
1	42.13	19.01	3.56

[1] Mean, minimum and maximum temperature during the 48 hours following treatment on September 26, 2006 were, 46.5, 65.1, 90.6 °F, respectively. Relative humidity ranged from 30 to 100%.

Field trials were repeated at the Moxee site in late September using the same procedures outline earlier, except that OFM was not included in the fall tests and some of the species were from a commercial source and some were experimentally produced (*S. feltiae* and *H. megidis* were produced by Becker Underwood and *H. marelatus* & *H. bacteriophora* (Vantage) were produced in the laboratory.

Table 5. Early fall field trial of experimentally and commercially produced insect-specific nematodes applied at 0.4 billion infective stages per acre for control of overwintering codling moth larvae in mulched and unmulched plots in an experimental apple orchard near Moxee, WA.[1]

Nematode species	% mortality -- location of larvae	
	In cardboard on bare ground	in cardboard under mulch
Controls	0	0
Steinernema feltiae[2]	30.82	65.68
H.b. Vantage[3]	1.88	3.02
H. marelatus[3]	2.76	17.79
H.megidis[2]	13.48	22.11

[1] Mean, minimum and maximum temperature during the 48 hours following treatment on September 27, 2006 were, 46.1, 60.3, 78.7°F, respectively. . Relative humidity ranged from 30 to 100%. Very windy. [2] *S. feltiae* & *H. megidis* were produced by Becker Underwood [3] *H. marelatus* & *Hb*-Vantage were produced in the laboratory

A second trial was conducted in the fall of 2006 at the Moxee experimental farm using single tree plots. Sentinel larvae in logs and cardboard strips were placed in each of 5 trees for each treatment and control. Trees were treated with 1 million infective stages per tree in one liter of water using a back pack sprayer. Prior to treatment and for 2 hours following applications, the trees were lightly misted with water. Only the *S. feltiae* treatment resulted in good control in cardboard bands. Ostensibly the moisture remained high in the bands despite the breezy and cool conditions.

Table 6. Early fall field trial of experimentally and commercially produced insect-specific nematodes insect-specific nematodes applied at 0.4 billion infective stages per acre for control of overwintering codling moth larvae in logs and tree bands in single tree plots in an experimental apple orchard near Moxee.

Nematode species	% mortality -- location of larvae	
	In logs in trees	in cardboard tree bands
controls	0	0
Steinernema feltiae[2]	38.40	97.85
H.b. Vantage[3]	0	13.63
H. marelatus[3]	4.70	25.74
H.megidis[2]	15.09	28.96

[1]. Mean, minimum and maximum temperature during the 48 hours following treatment on October 6, 2006 were, 42.5, 52.4, 74.5°F, respectively. . Relative humidity ranged from 25 to 100%.
[2] *S. feltiae* & *H. megidis* were produced by Becker Underwood
[3] *H. marelatus* & *Hb*-Vantage were produced in the laboratory

Surveys for native insect specific nematodes using the wax moth baiting technique described by Kaya and Stock (1997) yielded positive locations in Vantage, Euphrata, and East Wenatchee. The isolate from Vantage was readily produced in wax moth larvae. It was identified as *H. bacteriophora*. Small plots field trials did not produce significant mortality in sentinel cocooned codling moth larvae.

Proposed schedule of 2007 accomplishments

We will continue survey for native insect specific nematodes, including in orchards where previous applications of *Steinernema* nematodes have been applied. The rationale being that nematodes that have survived years and moths after application may be better suited for conditions in our area. Application strategies that build upon the work conducted in 2006 and in previous studies at the Wapato lab will be investigated in 2007 in order to optimize the potential of candidate nematode species. We will also endeavor to work with other commercial producers of nematodes to be able to report to Washington growers which products are best suited for codling moth control under conditions in the Pacific Northwest.

References cited

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Lacey, L. A., D. Granatstein, S. P. Arthurs, H. Headrick and R. Fritts, Jr. 2006b. J. Entomol. Sci. 41: 107-119.

CONTINUING PROJECT REPORT **YEAR**: 2 of 3
WTFRC Project Number: AH-05-509

Project Title: Post-Plant Management of Lesion Nematodes in Apple Orchards in WA

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1) R. Fuller, Apple Grower, Stormy Mountain Ranch, Chelan, WA
2) D. Anyan, G. S. Long Co., INC, Yakima, WA
3) B. Hiromoto, Technology Officer, ABR LLC, Puunene, Hawaii
4) C. Ishida, Field R&D Scientist, Valent Biosciences Co.
5) Mr. Dale Gies, Precision Seeds, Moses Lake, WA.
6) Dr. Tom Forge, AAFC, Agazziz, BC, Canada

Budget 1:
Organization Name: WSU **Contract Administrator:** ML. Bricker / S. Brock
Telephone: 509-335-7667/509-786-9224 **Email address:** mdesros@wsu.edu / sabrook@wsu.edu

Item	**Year 1:** 2005	**Year 2:** 2006	**Year 3: 2007**
Salaries			
Benefits			
Wages[1]	10,811	10,811	**10,762**
Benefits[2]	1,189	1,189	**1,238**
Equipment			
Supplies[3]	1,500	1,500	**1,500**
Travel[4]	1,500	1,500	**1,500**
Miscellaneous			
Total	15,000	15,000	**15,000**

Footnotes:
[1]Wages are for time slip help to collect soil samples and assist with nematicide application.
[2]Time slip benefits are at 11.5%.
[3]Supplies include pipette tips, gloves for precise nematicide applications.
[4]Travel expenses include gas, mileage, meals and overnight accommodation to travel to the farms.
Note: Additional support will be sought from CSANR, WSU, for 12,000. All nematicides and mustard meal are donated by the manufacturers.

Objectives: The objective of this proposal is to study the effect of the following novel nematicides, (DiTera, SLS Enhanced Nematicide/Liquid Compost factor and NatureCur) on plant parasitic nematodes, especially on lesion nematodes under both conventional and organic apple orchards. DiTera is registered for Certified Organic Apples, while the registration of the other bio-nematicides is pending. Presently, Organic Apple Growers do not have effective bio-nematicides to control plant parasitic nematodes while Conventional Growers have limited choice of synthetic nematicides. In addition, we will evaluate the potential of the above nematicides to enhancing beneficial free-living nematodes in the soil. The second field season has been completed and we are requesting funds for the 3nd field season. This is a 3 year project and in the end of the project we will provide apple growers with new tools to control plant parasitic nematodes. The schedule of activities for 2007 will be the same as 2006. So far, we have obtained data that shows trends i.e. there is a reduction in plant parasitic nematodes but the reduction is not significantly different than the controls. However, for 2007 we expect to obtain statistically significant data – in comparison to the untreated control trees. In addition to the above bio-nematicides, we are planning to incorporate a mustard meal (defatted meal pellets from *Brassica carinata*) in one of the two apple orchards to evaluate plant parasitic nematode response to this novel mustard meal. We have obtained *B. carinata* in October 2006 and will incorporate it in the spring of 2007.

Application rates of nematicides

Treatment	Rates
LCF + SLS/CA[1]	2 quart/ acre at 1:400 dilution (LCF) and 1 quart / acre at 1% solution (SLS)
DiTera®[2]	15 pounds / acre
NatureCur[3]	5000 ppm
Brassica carinata meal[4]	1T/acre

[1]SLS/CA Enhanced Nematicide / LCF will be applied early spring (when soil temperature at 45^0 - 50^0F) and then monthly till October – watered in via micro-sprinklers.
[2] DiTera® ES will be applied early Spring (when soil temperature at 45^0 - 50^0F) and then monthly till October - watered in via micro-sprinklers.
[3]NetureCur will be applied early Spring (when soil temperature at 45^0 - 50^0F) and then monthly till October – watered in via micro-sprinklers.
[4]*Brassica carinata* meal will be applied, banded, early Spring (prior to root flash) – and watered in via micro-sprinklers.

Results from 2006 field season:

Rene Garcia Farm – NatureCur Treatment:

NatureCur increased the diameter of the treated trees in comparison to the untreated controls but the increase is not significantly different (Fig. 1).

There was a significant increase in yield from NatureCur treated trees in comparison to the untreated controls (Fig. 2).

NatureCur did not cause a significant reduction to the beneficial free living nematodes but it reduced the lesion nematodes in comparison to the untreated controls (Fig. 3).

Ray Fuller Farm – DiTera and SLS+LCF Treatment:

There was no effect on the trunk diameter of the bionematicide treated trees in comparison to the controls (Fig. 4). However, several trees were damaged by gophers and new ones were replanted.

The bionematicides did not cause a significant reduction to the beneficial free living nematodes in comparison to the controls (Fig. 5). In addition, the bionematicides did not cause a significant reduction to the lesion nematode in comparison to the controls except of the reduction caused to the lesion nematode by the Ditera treated M-26 rootstock trees (Fig. 6).

Fig. 1.

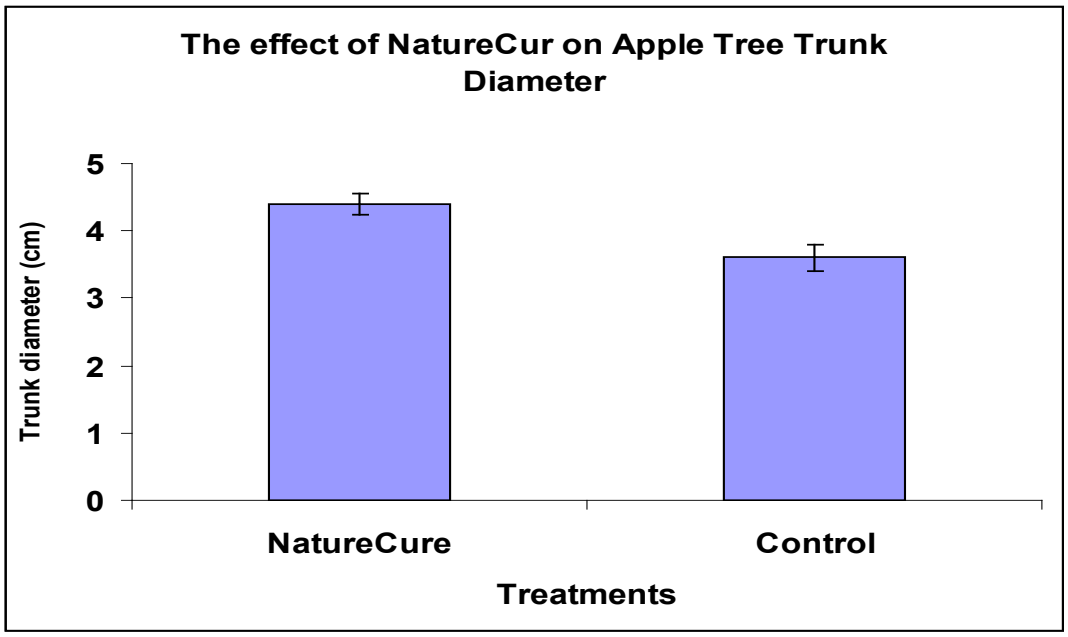


Fig. 2.

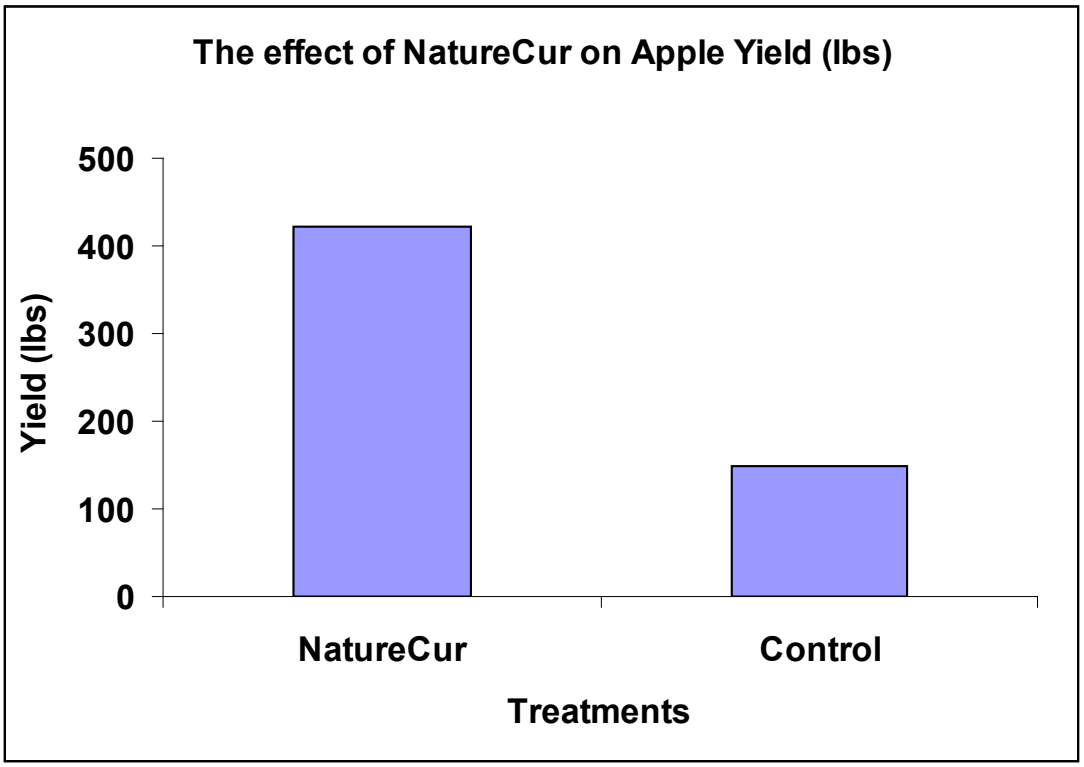

The effect of NatureCur on Apple Yield (lbs)
Yield (lbs)
500
400
300
200
100
0
NatureCur
Control
Treatments

Fig. 3.

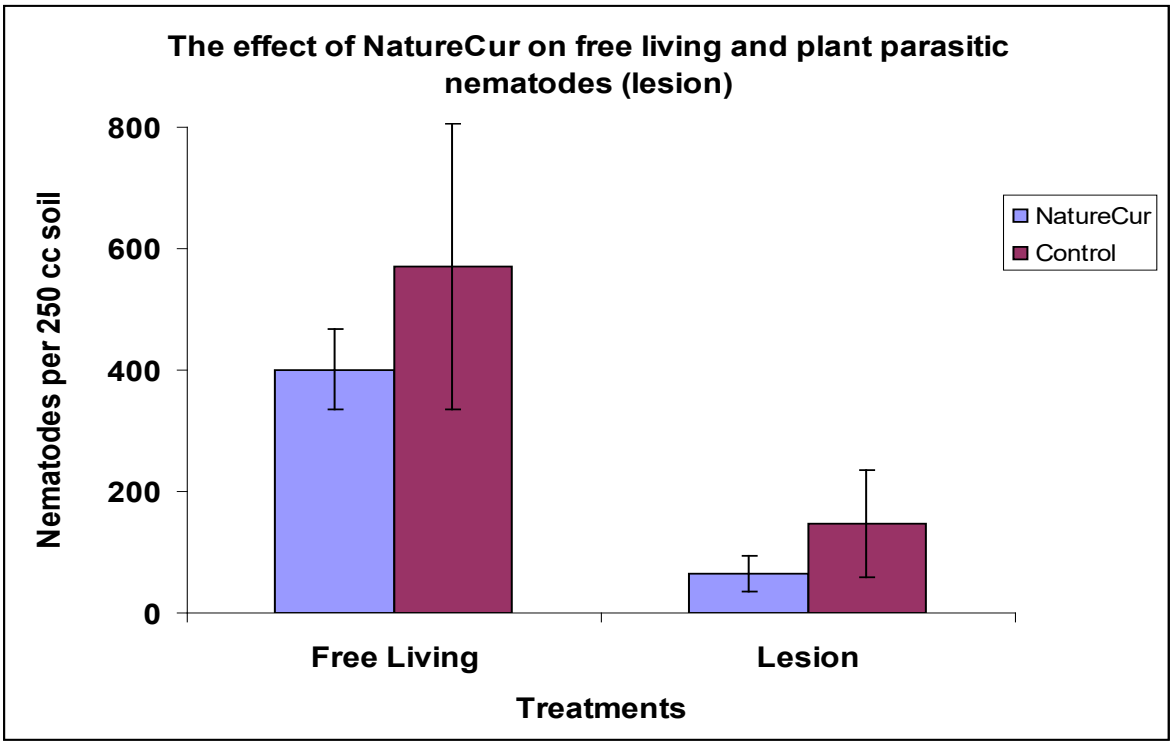

The effect of NatureCur on free living and plant parasitic nematodes (lesion)
Nematodes per 250 cc soil
800
600
400
200
0
NatureCur
Control
Free Living
Lesion
Treatments

Fig. 4.

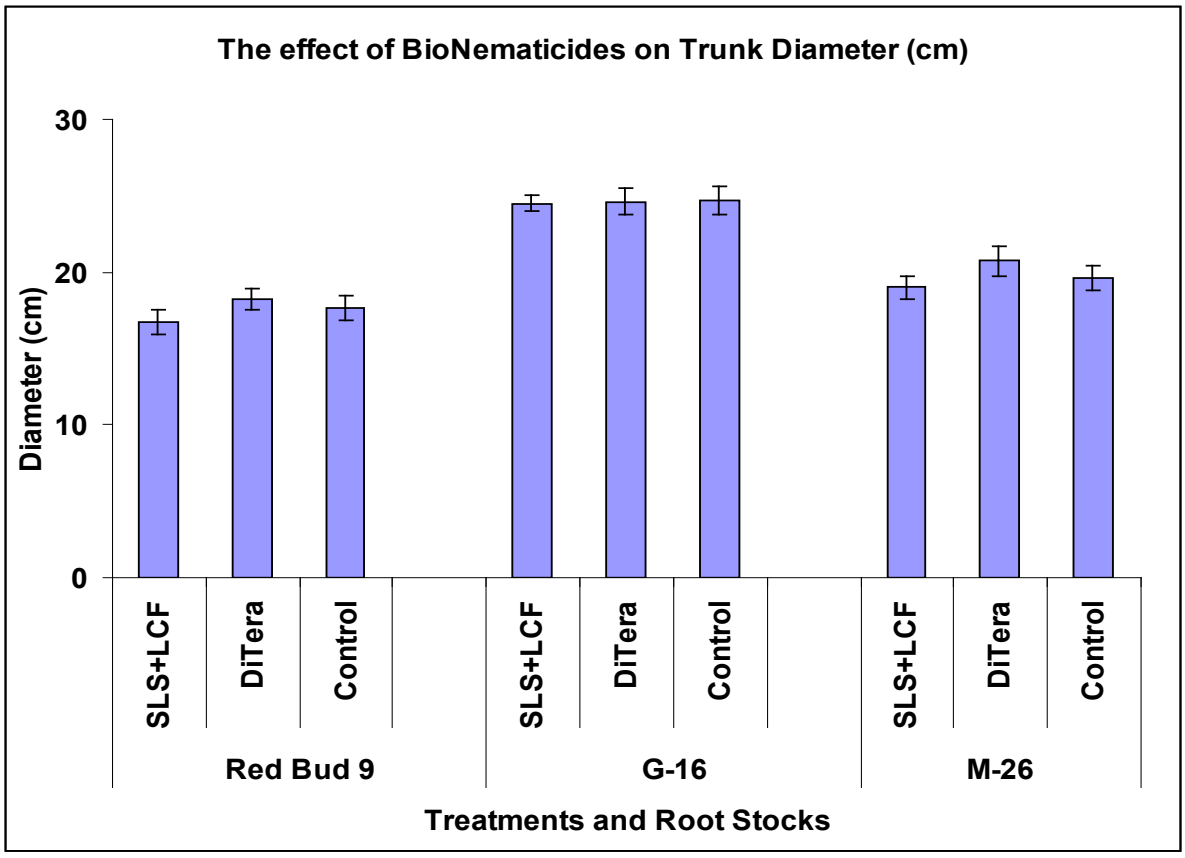

The effect of BioNematicides on Trunk Diameter (cm)
Diameter (cm)
30
20
10
0
SLS+LCF
DiTera
Control
SLS+LCF
DiTera
Control
SLS+LCF
DiTera
Control
Red Bud 9
G-16
M-26
Treatments and Root Stocks

Fig. 5

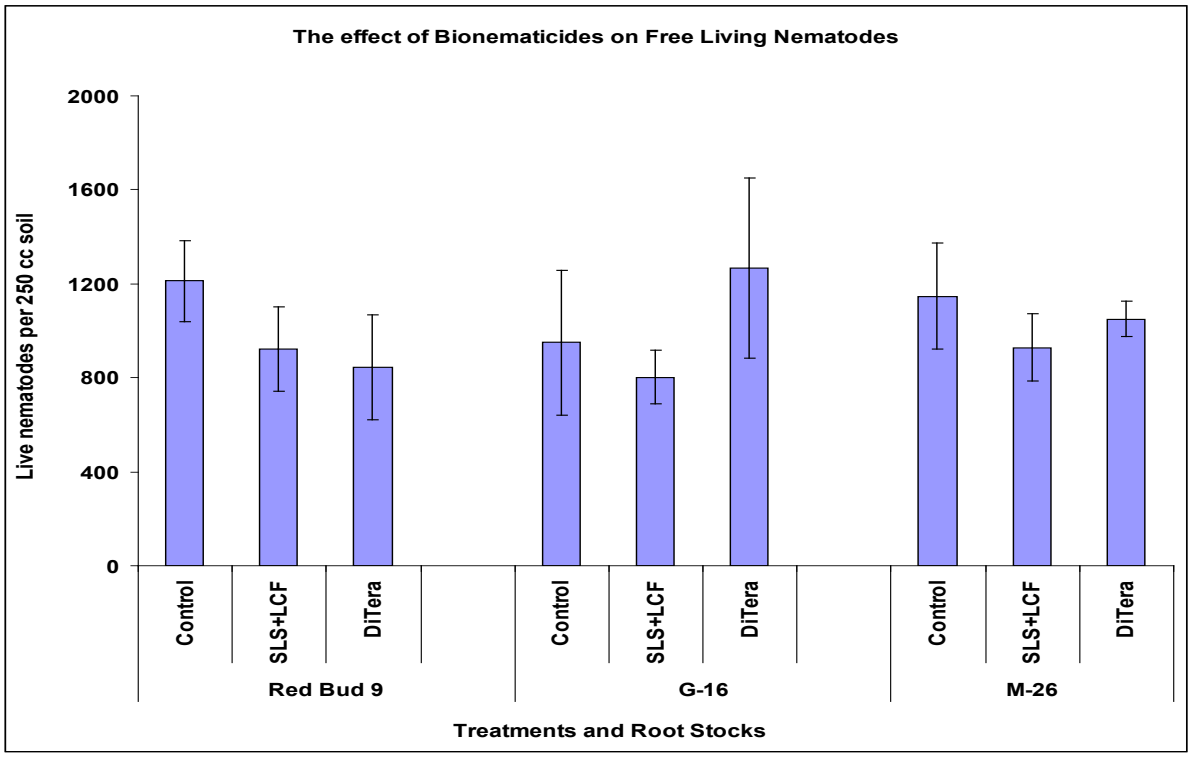
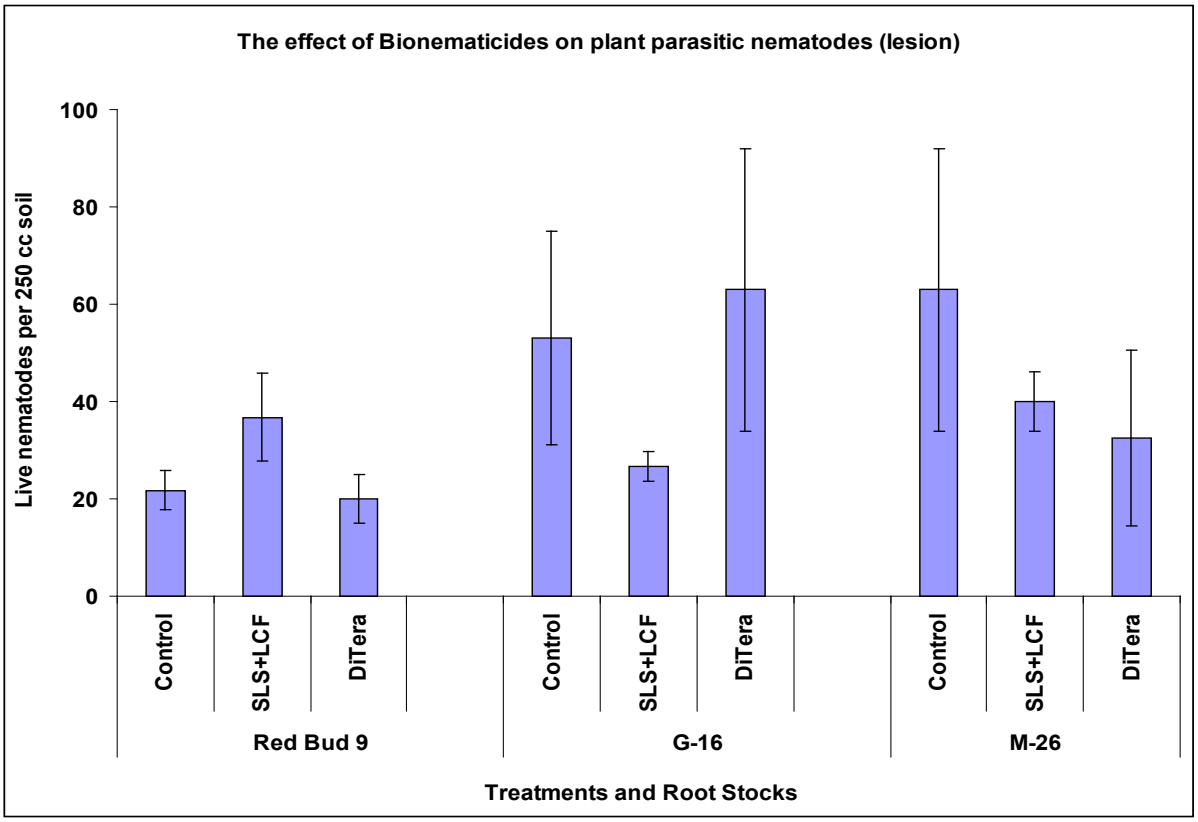

The effect of Bionematicides on Free Living Nematodes
Live nematodes per 250 cc soil
2000
1600
1200
800
400
0
Control
SLS+LCF
DiTera
Control
SLS+LCF
DiTera
Control
SLS+LCF
DiTera
Red Bud 9
G-16
M-26
Treatments and Root Stocks

Fig. 6.



CONCLUSIONS

NatureCur application if Garcia's Orchard significantly decreased lesion nematodes without reducing the free living beneficial nematodes.

The Bionematicide application in Fuller's Orchard was did significantly reduced the lesion nematodes but several trees were damaged by gophers – this part of the study needs to be repeated

It will take at least 1 more years for the trees to respond to the treatments and to obtain significant lesion nematode control.