FINAL PROJECT REPORT
WTFRC Project #AE-05-507 **WSU Project #13C-3643-6387**

Project title: Biology and Management of Secondary Pests of Apple
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Objectives:

1. Rosy apple aphid

1a. Identify the summer host or hosts of rosy apple aphid in Washington.
1b. Determine obligate status of the herbaceous host.

2. Woolly apple aphid

2a. Test chemical control tactics for aerial colonies.
2b. Test chemical control tactics for root colonies.
2c. Determine timing of peak migration root and aerial colonies.
2d. Conduct a world literature review of woolly apple aphid and its natural enemies.

3. Mites

3a. Examine the effects of newer codling moth control materials on integrated mite control.

Significant findings:

1. Rosy apple aphid (RAA)

1a. RAA colonies were found on both narrowleaf plantain, *Plantago lanceolata*, and broadleaf plantain, *P. major*, in orchards throughout central Washington. In a greenhouse experiment, aphids on these two weed species returned to apple trees in September and October. Rosy apple aphid was not found on other common weed species.

1b. Colonies on apple died out in July in a greenhouse, just as they did in the field; however, aphids kept under spring conditions (temperature and photoperiod) have continued to survive on apple alone.

2. Woolly apple aphid (WAA)

2a. Safe-T-Side oil had promising results for control of aerial colonies.

2b. Two systemic products applied as a soil drench, Admire 2F and Vydate 2L, and one applied as a foliar spray, NNP-316, showed activity against both root and aerial colonies on potted trees.

2c. First instars migrated from roots primarily from May through July, with a peak in early June (one site, Okanogan County). Very little migration was seen August through October.

3. Mites

3a. Of five commercial orchards, only one had seriously elevated tetranychid mite populations. The population was highest in the Rimon treatments, followed by Assail and Calypso, with the OP treatment having the lowest mite population. In this orchard, the three non-OP treatments had lower overall levels of predatory mites.

Methods:

1. Rosy apple aphid

1a. Survey of weed species for RAA. A survey for rosy apple aphid on various weed species was conducted in the following Washington counties (no. sites): Okanogan (3), Chelan (4); Douglas (6), Grant (1), Yakima (11), Benton (7), Klickitat (1), Franklin (1), Skamania (1), and in Umatilla, Oregon (2). Sampling concentrated on plantain (*P. lanceolata* and *P. major*), but other species were also sampled periodically. These species included dandelion (*Taraxacum officinale* Weber in Wiggers), dock (*Rumex* spp.), lady's thumb (*Polygonum persicaria* L.), lamb's-quarter (*Chenopodium album* L.), common mallow (*Malva neglecta* Wallr.), white clover (*Trifolium repens*), and yarrow (*Achillea* spp.). Both plantain species were present at some sites, but most had one species predominant. The majority of sites were orchards, conventional or organic, with a current population or history of rosy apple aphid.

Samples of plantain and other common weeds were taken from inside the orchards or in areas directly adjacent. Samples were collected biweekly from late June through October. Plants were sampled destructively by cutting them off at ground level in sufficient quantity to fill a 1-gal plastic bag. Sample locations were identified by GPS coordinates. Aphids were extracted from plant material using Berlese funnels (2 h, ca. 100°F). A duplicate sample was taken at each site to rear aphid parasitoids; these plants were placed into plastic tubs and stored for a month at room temperature next to a window. In addition, confirmatory samples were taken from rosy apple aphid colonies in trees. All aphids were stored in 70% ethanol and sent to WSU-IAREC, Prosser, for identification.

1b. Life cycle of RAA. The life cycle was investigated with a series of greenhouse experiments. The following three questions were addressed by these experiments:

Will RAA on apple colonize plantain species? In April, bare root 'Delicious' and 'Golden Delicious' apple trees were planted in 5-gal pots, then infested with RAA from a TFREC orchard. Four herbaceous weed species, narrowleaf plantain, broadleaf plantain, woolly plantain, and common lamb's-quarter was collected from orchards and waste areas on 15 May. Narrowleaf and broadleaf plantain are perennials introduced from Europe, while woolly plantain is a native annual (Whitson et al. 1992). All three plantain species were of interest as possible summer hosts in central Washington. Lamb's-quarter, not known to be a host of RAA, was used as a check. Three replications of each apple-herbaceous plant combination were placed in cages on 23 May. All plants were maintained in a greenhouse without modified lighting.

Can RAA reproduce continuously on apple? In April, potted 'Delicious' and 'Golden Delicious' apple trees were infested with RAA from the same TFREC orchard. Trees were divided among three cages. When trees were severely damaged by RAA they were replaced (every 2-4 wk) with uninfested trees of the same cultivar. Aphids were transferred from the old to the new trees. Trees were kept in a greenhouse with ambient photoperiod.

A second group of apple trees was removed from cold storage in June, potted, and placed in a growth room in July. The growth room was kept at 62-68°F with a light:dark photoperiod of 14:10 h. Three times in July these trees were infested with aphids from wild colonies, without success. The final attempt, from a colony on 'Granny Smith' apple in Smith Tract Orchard, Orondo, was made on 22 July, and this colony became established. The colony was provided with new potted apple trees every month.

Will RAA return to apple from plantain species? The original apple trees were removed from the apple-plantain treatment cages on 11 July and colonies were left to develop on plantain. On 1 September, uninfested potted apple trees (seedling) were placed in the cages. These trees had not been previously exposed to RAA in the greenhouse.

Plants were inspected for aphids at least weekly, and specimens were collected for identification on 20 June and 4 and 29 September. Plantain and apple leaves containing aphid colonies were placed in 70% ethanol and shaken to dislodge specimens, while the entire *C. album* plants were placed in Berlese funnels for 2 h to extract aphids. All aphids were stored in 70% ethanol and sent to WSU-IAREC for identification.

2. Woolly apple aphid

2a. Chemical control of aerial colonies. This test was conducted in a commercial apple orchard in Bridgeport, Washington. Twenty-eight apple trees were selected along a road between two blocks. Three WAA shoot colonies per tree were tagged. On 18 July, live and mummified aphids were counted in the tagged colonies. Developing larvae of *Aphelinus mali* Haldemann, a parasitoid of WAA, cause later instars to turn black, instead of the usual pale purple-gray, before the adult emerges (Beers et al. 1993). Trees were assigned to one of four blocks based on the initial population density, then randomly assigned one of seven treatments within blocks. Treatments were applied with a handgun sprayer to the point of run-off on 19 July. Live aphids and aphid mummies were counted the following day, then at 3-4 d intervals throughout the rest of the test.

Data 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.

2b. Chemical control tactics for root colonies. This test was conducted on potted apple trees in a greenhouse. A bulk soil sample was collected adjacent to a commercial apple block near Orondo, Washington. The soil was primarily sand (Quincy series [Mixed, mesic Xeric Torripsamments]). Fifty ¾ inch 'Golden Delicious'/EMLA 7 rootstock were planted in 18 liters of soil in 14-in plastic pots on 4 April. On 4 May, after trees had grown shoots approximately 15 cm long, the trees were infested by placing WAA-infested leaves on the branches. By the middle of May, aphids were well established and had formed shoot colonies. A new generation of aphids began by early June. On 13 June, half the soil in the pots was removed and replaced with bark mulch to expose part of the roots to the new mobile aphids. Root colonies developed naturally by aphids moving from shoot colonies. Shoots were heavily infested by the middle of July, and trees were under extreme stress.

On 21 July, 24 trees with visible root colonies were selected. On each tree, three shoot colonies were randomly selected and all other aphids removed to reduce the pest pressure. After bark mulch was removed, all exposed WAA on the roots were counted, and the missing soil was replaced with additional field soil. Trees were distributed into four replicate blocks based on the population of WAA on the roots, and treatments were randomly assigned within each block. Insecticides were applied on 24 July and again two weeks later. One product, NNP-316, was applied to the foliage to run-off with a 1-gal sprayer. Water mixed with NNP-316 was first acidified to a pH of 6-7 with a few drops/liter of 1N HCl. All other products were applied as soil drenches. Trees were fully watered 3 d before application, and then 1 liter of insecticide solution was poured onto the soil. This volume completely saturated the soil in the pots without losing any solution. Starting 3 d after treatment, trees were watered regularly, but minimal water was lost through the drainage holes of the pots. Shoot colonies were assessed periodically for 6 wk after the first application, and then all trees were lifted, the soil gently washed from the roots, and root colonies assessed.

Data 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.

2c. Timing of peak migration between root and aerial colonies. Three apple trees in a commercial orchard in Bridgeport were selected for monitoring. The trees were 8-yr-old Cameo scions grafted onto 15-yr-old rootstocks. Trees were selected for smooth bark that would accommodate sticky bands, history of a significant WAA population, and the soft insecticide program of the ranch. The trees had a circumference of 50-60 cm at 10 cm above the soil surface.

The sticky band trapping method was modified from that of Hoyt and Madsen (1960). Bands were made of a 3-cm wide strip of heavy-duty aluminum foil wrapped around the trunk and held in place with a thin layer of Tree Tanglefoot. A thin bead of Tree Tanglefoot was placed in the center of each band. One band was placed 15 cm above the soil surface to trap first instar nymphs moving up the trunk from the root colonies. A second band was placed 1 cm above the first to trap nymphs moving down from shoot colonies. Traps were set out 7 April and replaced weekly until frost, 27 October.

2d. Literature review: A collection of literature covering woolly apple aphid and its natural enemies was begun in the fall; an initial draft is written and will be finalized by 30 March, 2006.

3. Mites

3a. Effect of codling moth controls on mites: This test was conducted in five commercial orchards from Bridgeport to the Royal Slope. Cooperators had discretion over block size, with a minimum of one acre per treatment at each orchard. All treatments were applied by the orchard's personnel using their own equipment. Several growers elected to have 4-acre blocks so that an entire 400 gal sprayer tank could be used (at 100 gpa) to apply each treatment. Four of the orchards were primarily 'Delicious,' and the fifth one was 'Cameo.'

Treatments consisted of four different seasonal codling moth programs, applied at standard rates and timings for the given materials. Two applications of the products were made against first- and second-generation codling moth, with the key material paired with a "mite-neutral" material (Intrepid) in the second generation. The exception was Rimon, which was applied three times per generation. The treatments were Assail-Intrepid, Calypso-Intrepid, Rimon-Intrepid, and OP-OP.

Mites were sampled every 2-3 wk from late May through mid-September. One hundred leaves/plot were collected from the center portion of the plot and kept cool during transportation and storage. Mites were brushed from the leaves with a mite brushing machine and collected on a revolving sticky glass plate. The composite sample on the plate was counted using a stereoscopic microscope. All stages and species of phytophagous and predatory mites were recorded, including the eggs and motile stages of European red mite (ERM), *Panonychus ulmi* (Koch); twospotted spider mite (TSM), *Tetranychus* urticae Koch; McDaniel spider mite (MCD), *Tetranychus mcdanieli* McGregor [the eggs of TSM and MCD could not be distinguished and were recorded as a group]; western predatory mite, *Typhlodromus* (=*Galendromus*) *occidentalis* (Nesbitt); a stigmaeid predatory mite, *Zetzellia mali* Ewing; and motile stages of apple rust mite (ARM), *Aculus schlechtendali* (Nalepa).

Cumulative mite days (CMD) were calculated for tetranychid, predatory, and rust mites, giving an estimate of population densities integrated over the course of the test. CMD are the sums of the average density of mites on two dates multiplied by the number of intervening days.

Results and discussion:

1. Rosy apple aphid

1a. Survey of weed species for RAA. Both narrowleaf and broadleaf plantain support the summer generations of RAA in central Washington. Aphid identification is still in progress at the time of writing. In samples collected through September, 10 of 26 sites (38%) with broadleaf plantain had confirmed presence of RAA on that weed species. Sixteen of the 26 sites with narrowleaf plantain (62%) had confirmed presence of the aphid on that species. The presence of RAA on plantain appeared to be more related to the degree of RAA infestation in the orchard than the plantain species. The seven other weed species sampled were not a host for RAA, with the exception of one white clover sample. This was taken from an orchard with narrowleaf plantain, and two RAA were extracted from the foliage. These aphids may have crawled from a nearby narrowleaf plantain, and thus the clover was only a transient host on which the aphids do not reproduce or form colonies. Some parasitoid and hyperparasitoid specimens have been collected from apple and will be identified. A few parasitoid species have also been found on broadleaf and narrowleaf plantain.

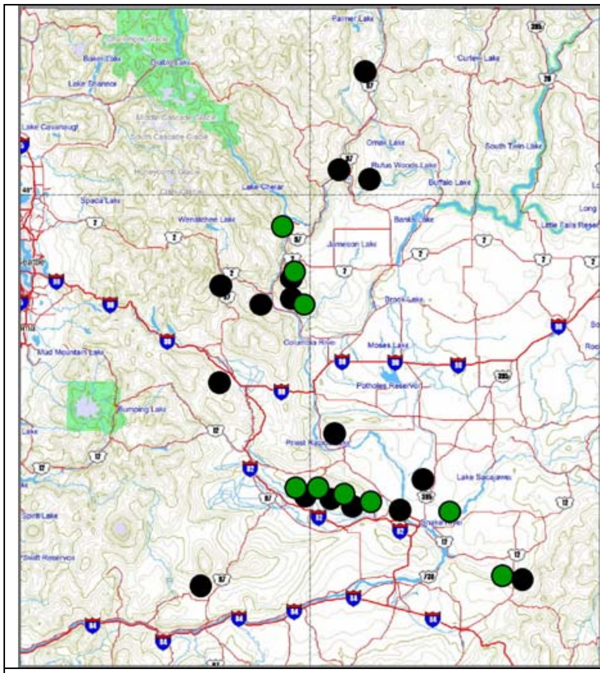	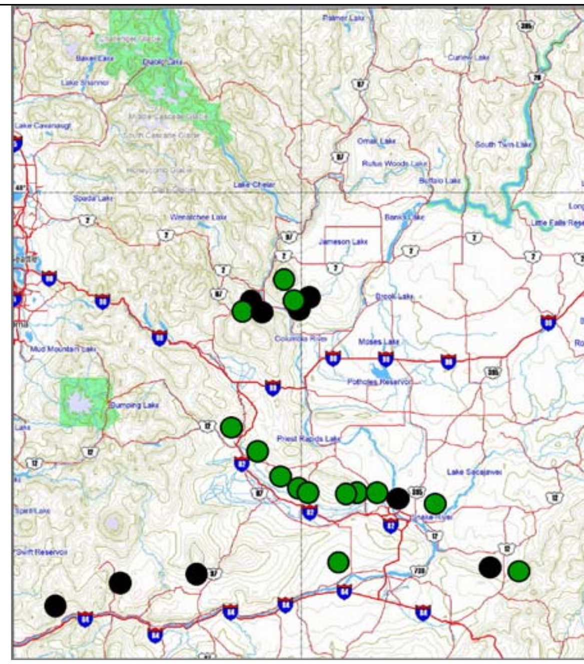
Fig 1a. Location of sites where rosy apple aphid was found (green circles) or not found (black circles) on broadleaf plantain, *Plantago major*.	Fig. 1b. Location of sites where rosy apple aphid was found (green circles) or not found (black circles) on narrowleaf plantain, *Plantago lanceolata*.

1b. Life cycle of RAA

Will RAA colonize plantain species? On 15 May, winged aphids were present on the apple trees; then by 10 June, winged aphids were present on all of the plantain species. No winged aphids were observed on lamb's-quarter. Colonies of wingless aphids were observed on all plantain species by 20 June. On 12 July, these were confirmed to be RAA on all replications. Wingless aphids on plantain were pale yellow, and those on apple were pink or purple (Matheson 1919). No RAA specimens were ever identified on lamb's-quarter.

Can RAA reproduce continuously on apple? Although new apple trees were made available to RAA colonies grown exclusively on apple, the colonies produced more and more winged forms and had disappeared by 11 July. By this time, greenhouse temperatures reached 95-105°F each day. A detailed account of field observations in New York determined that there were only four generations on apple in the spring and summer, with most colonies producing winged forms in the second and third generation (Matheson 1919). An aphid colony in the growth chamber collected from the field on 22 July was still composed of wingless forms by the first week in December. What remains to be determined is if by chance an unusual strain was collected that is capable of continuous reproduction on apple or if all of the normally heteroecious RAA in central Washington are capable of this given the correct environmental conditions.

Will RAA return to apple from plantain species? Black, winged aphids were present on narrowleaf and broadleaf plantain by 22 August. Colonies of yellow RAA could still be located and positively identified on 2 of 3 replicates of narrowleaf plantain and 2 of 3 replicates of broadleaf plantain by 3 September. Woolly plantain plants flowered and then slowly dried during the summer, in spite of regular watering. All three plants, along with the aphids, died by 1 August. In cages with successful plantain colonies, black, winged aphids were seen landing on seedling apple trees in early September. Colonies of yellow, wingless aphids were observed on 4 of the 6 apple trees within a week. On 29 September, 2 of the 3 apple trees caged with narrowleaf plantain, and 2 of the 3 caged with broadleaf plantain were confirmed to be infested with wingless RAA, which were produced by the migrating winged females.

2. Woolly apple aphid

2a. Chemical control tactics for aerial colonies. Thiodan and Diazinon reduced WAA populations by one day after treatment (DAT - Table 1), and populations in these treatments remained low for the rest of the test. Raynox (10% v/v) provided some suppression, but this occurred more slowly than with the two standard insecticides. The lower rate (5%) and Aza-Direct treatment means were never different from the check. Although Safe-T-Side oil appeared to cause a noticeable reduction in populations due to the variability, differences were not significant. Variance in the data increased throughout the experiment, as some colonies decreased while others increased. The gradual decrease in populations on unsprayed trees, as well as initial decreases on sprayed trees, could be partly explained by an increase in parasitized aphids (Table 2).

Table 1. Woolly apple aphid densities on apple before (18 July) and after treatment with various insecticides on 19 July, 2005.

		Live woolly apple aphids/colony					
Treatment	Rate/100 gal or conc v/v	18-Jul	20-Jul[x]	23-Jul[x]	26-Jul	31-Jul[x]	2-Aug
Raynox[y]	10% v/v	21.25 a	11.75 a	5.42 ab	5.50 a	7.92 ab	7.33 a
Raynox[y]	5% v/v	23.17 a	23.50 a	22.50 a	23.00 a	19.17 ab	23.83 a
Thiodan 50W	4 lb	20.75 a	0.17 b	0.58 b	0.17 a	0.00 b	0.00 a
Diazinon 50W	4 lb	12.42 a	0.58 b	0.33 b	0.17 a	0.00 b	0.00 a
Aza-Direct 0.0987L	32 fl oz	16.08 a	14.42 a	9.50 a	9.75 a	17.92 a	14.17 a
Safe-T-Side oil	1.5% v/v	16.25 a	4.58 a	2.33 ab	0.83 a	0.83 ab	0.00 a
Check	-----	15.58 a	18.83 a	13.75 a	13.50 a	10.17 ab	7.17 a

Means within columns followed by the same letter are not significantly different, Waller-Duncan *k*-ratio *t*-test, *k*-ratio=100.

[x]Data transformed ln(y+0.5) prior to analysis due to non-homogeneity of variances.

[y]Water treated with 14 oz/100 gal EDTA.

Table 2. WAA mummies parasitized by *A. mali* on apple trees treated with various insecticides on 19 July 2005.

Treatment	Rate/100 gal or conc v/v	Parasitized (mummies) woolly apple aphids/colony					
		18-Jul	20-Jul[x]	23-Jul	26-Jul	31-Jul	2-Aug
Raynox[y]	10% v/v	5.00 a	9.67 a	11.08 a	10.08 a	10.83 a	11.50 a
Raynox[y]	5% v/v	5.42 a	6.25 ab	7.50 a	7.75 a	7.83 a	8.83 a
Thiodan 50W	4 lb	4.67 a	5.50 b	7.17 a	7.17 a	7.17 a	7.17 a
Diazinon 50W	4 lb	4.83 a	7.00 ab	7.17 a	7.17 a	7.17 a	7.17 a
Aza-Direct 0.0987L	32 fl oz	2.50 a	5.42 ab	7.08 a	6.33 a	7.67 a	9.25 a
Safe-T-Side oil	1.5% v/v	3.75 a	5.50 ab	7.58 a	7.67 a	7.92 a	8.17 a
Check	-----	6.58 a	10.50 a	10.83 a	11.92 a	11.08 a	12.00 a

Means within columns followed by the same letter are not significantly different, Waller-Duncan *k*-ratio *t*-test, *k*-ratio=100.
[x]Data transformed ln(y+0.5) prior to analysis due to non-homogeneity of variances.
[y]Water treated with 14 oz/100 gal EDTA.

2b. Chemical control tactics for shoot and root colonies: Aphid pressure was high in this test, with 138-191 WAA per shoot colony before treatments were applied (Table 3). Vydate provided the best knockdown of aphid colonies on shoots; however, both NNP-316 and Admire also significantly reduced populations by 7 DAT (31 July). Excessive heat in the greenhouse on 5-6 August, just before the second application, caused a substantial reduction in shoot populations on all trees. Although WAA began to recover on untreated shoots, populations remained extremely low on all treated trees. Heat, combined with heavy pest pressure, resulted in death of 5 of the 24 trees (treated as missing values in the analysis).

Table 3. WAA populations on shoots and roots of potted apple trees before (20 July) and after treatment with various insecticides

Treatment	Rate/vol	Shoot colonies/tree				Root colonies/tree	
		20-Jul	31-Jul	7-Aug[x]	27-Aug[x]	20-Jul	27-Aug
NNP-316[y]	150 g/liter	148.33 a	97.50 ab	0.00 b	0.00 b	27.75 a	9.25 b
Admire 2F	32 fl oz/100 gal	151.67 a	100.17 ab	0.00 b	0.00 b	28.25 a	0.00 b
Vydate 2L	2.5 ml/gal	137.50 a	1.67 d	0.00 b	0.00 b	23.25 a	0.00 b
Vydate 2L	5 ml/gal	239.17 a	31.67 bc	0.00 b	0.00 b	26.00 a	0.00 b
Vydate 2L	10 ml/gal	190.83 a	38.58 cd	0.17 b	0.00 b	42.50 a	0.00 b
Check	------	184.17 a	253.33 a	4.58 a	70.00 a	30.00 a	155.00 a

Means within columns not followed by the same letter are not significantly different, Waller-Duncan k-ratio t-test, k-ratio=100.
Treatments applied 24 July and 7 August.
[x]Data transformed ln(y+0.5) prior to analysis due to unequal variances.
[y]Tank mixed with 0.25% vol:vol Destiny methylated seed oil.

Root colonies on the trees were not extensive (Table 3), only becoming established on roots growing in the bark chips. However, large galls were produced around feeding sites. Five weeks after the treatments began, all products had severely reduced root colonies. The low rate of Vydate, as well as Admire, provided excellent control. NNP-316 appeared to be transported throughout the apple tree, reaching the root colonies by a foliar application alone.

2c. Timing of peak migration between root and aerial colonies. Very few first instars were caught in April. Aphids moving from root colonies started increasing in May and peaked in early June (Fig. 2). Migration declined slowly until reaching near zero in mid-July. Aphids moving down from shoot colonies increased in May and stayed constant until mid-July, after which they, too, decreased to near zero. During peak activity, movement up from root colonies surpassed movement down. After July, very few first instars were trapped for the remainder of the year.

In West Virginia, aerial colonies increased in May, peaked in June, and declined in July and August. In some years aerial colonies increased again in September or stayed low (Brown and Schmitt 1994). Observations were different in California (Hoyt and Madsen 1960), where first instars moving on the trunk were trapped continuously throughout the summer and fall. Activity seemed to decrease during the rainy season, February through May; however, no repeat observations were made in different years.

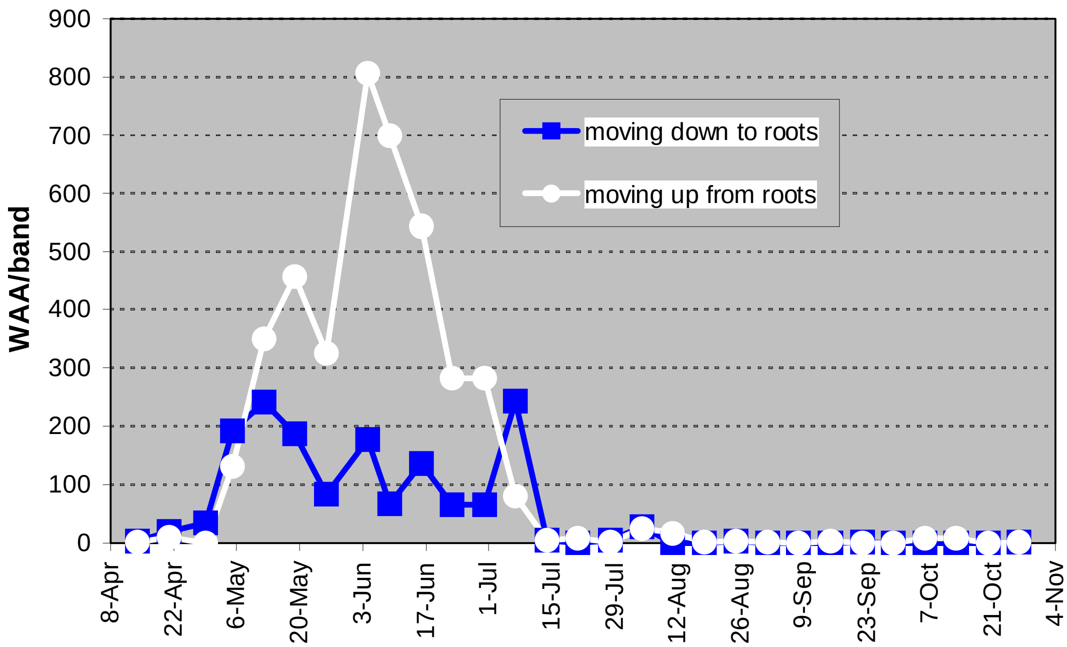

Fig. 2. Mean number of first instar woolly apple aphids trapped in sticky bands, Bridgeport, WA.

2d. Literature review. At the time of writing, the literature review is about half completed. The final version will be completed by February 2006.

3. Mites.

3. Effect of codling moth program on mites. Mites remained at relatively low densities (<2 mites/leaf) in four of the five orchards. One orchard, QLR, peaked at >50 mites/leaf in the Rimon treatment, although this orchard had slightly elevated levels in all the treatments (Fig. 3a). Because the treatments were not replicated within orchards, statistical analysis is not possible; however, at QLR the cumulative mite days were highest in the Rimon plot, followed by Assail and Calypso (very similar), with a lower level in the OP plot. Cumulative mite days did not differ greatly among treatments (within orchards) in the orchards in which mite populations stayed low. Predatory mites peaked in midsummer in three of the five orchards, with ARR and SLH having the highest seasonal populations in late August-early September. There was little variation among the treatment CMDs for predatory mites in ARR and SLH, but some of the treatments had lower predatory mite seasonal densities in relation to the OP standard in QLR and BAN (Fig. 3b). The RYL orchard had lower predatory CMDs in the Rimon treatment but a high level in the Calypso treatment.

No codling moth damage was found in any orchard or treatment after either first or second generation (data not shown).

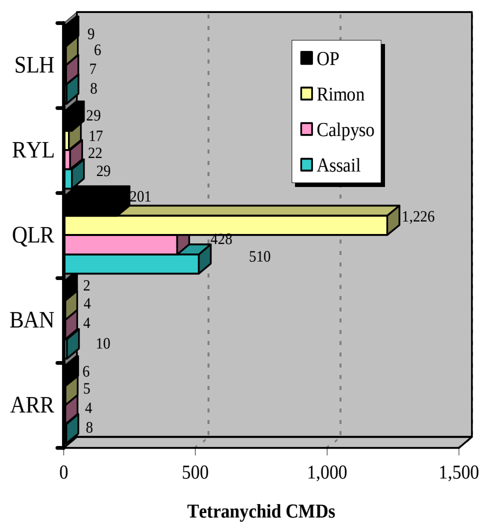

Fig. 3a. Tetranychid CMDs resulting from different codling moth programs.

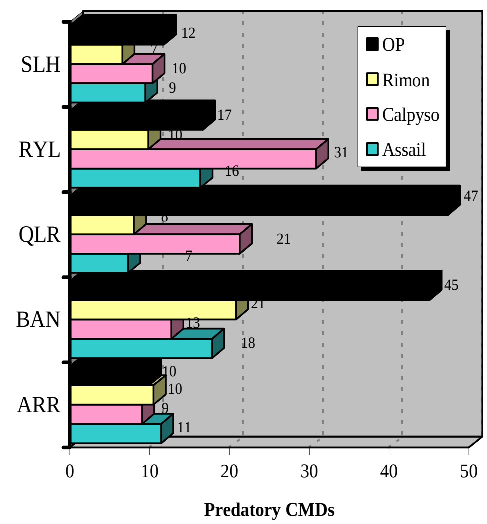

Fig. 3b. Predatory mite CMDs resulting from different codling moth programs.

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Budget:

Project title: Biology and Management of Secondary Pests of Apple
PI: Elizabeth H. Beers
Project duration: One year
Current year: 2005
Project total (1 year): $38,344

Item	Year 1 (2005)
Salaries[1]	$17,453
Benefits[2]	6,412
Wages[3]	9,360
Benefits (11%)	1,092
Equipment	-
Supplies[4]	1,000
Travel[5]	3,000
Miscellaneous	-
Total	**$38,344**

[1]Salaries: S.D. Cockfield (0.4 FTE); R. Talley (0.0625 FTE).
[2]Benefits: 36% for Cockfield, 41% for Talley.
[3]Time-slip wages (600 hours).
[4]Cell phone charges are allowed under this grant.
[5]Travel to research plots.