FINAL PROJECT REPORT
WTFRC Project Number: #PR-04-431

Project Title: Biology and management of pear pests

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Budget History:

Item	**Year 1: 23,400**	**Year 2: 23,790**	**Year 3: 24,180**
Salaries	10,000	10,300	10,600
Benefits	3,000	3,090	3,180
Wages	10,400	10,400	10,400
Benefits			
Equipment			
Supplies			
Travel			
Miscellaneous			
Total	**23,400**	**23,790**	**24,180**

OBJECTIVES:

Advance our understanding about how cover crops affect biological control of pear psylla:

- Assess taxonomic composition of generalist predator community in cover crop vs tree, and make inferences about habitat preferences;
- Develop methods for marking predators in cover crop and tree canopy, to allow examination of predator movement;
- Develop methods (gut contents analysis) to assess predator feeding on pear psylla, allowing us to determine what species are likely important sources of biological control.

SIGNIFICANT FINDINGS:

- Developed methods (with V. Jones) for marking generalist predators in large sections of habitat (cover crop or tree canopy), and showed that these methods allow us to make inferences about the role of cover crop as a source of natural enemies moving into the tree;
- Developed methods (with T. Unruh) that allow us to determine whether field-collected generalist predators have been feeding on pear psylla, and showed that diet in field-collected predators tracks psylla densities;
- Showed that a complex of predator species occurs in cover crop and tree habitats; by determining densities of each species in either habitat, we were able to make inferences about whether a species is a cover crop specialist, tree specialist, or habitat generalist;
- Marking studies indicated that a percentage of predators do indeed move between habitats, and the movement occurs even in species we initially categorized as habitat specialists;
- In a comparison of cover crop vs grass understory plots, we showed: (1) higher densities of predators in both the understory and tree canopy of the cover crop plots (compared to grass plots); and (2) lower densities of psylla nymphs in the cover crop plots.

- **CONCLUSION:** Results suggested that generalist predators move between cover crop and tree in both directions, and that several of these species regularly contain psylla remains in their guts. We infer from our results that the cover crop acted as a source of predators moving into the tree, and that these predators contributed to statistically significant drops in densities of psylla nymphs.

- **UNKNOWNS (to be assessed in a new project):** We are not certain that predator specimens which colonized the tree canopy from the cover crop were also responsible for prompting the decrease in psylla numbers, because we did not look for both the marker and psylla remains simultaneously in our assayed specimens. Also, the cover crop was obviously highly attractive to generalist predators (based upon predator densities), whereas our marking results suggest that a comparatively low percentage of the predators collected in the tree canopy had originated in the cover crop. It would be useful to develop methods that allow us to prompt higher rates of movement from cover crop into tree.

RESULTS AND DISCUSSION

Plots. A legume cover crop composed of winter pea, hairy vetch, and crimson red clover was used in all studies. Control plots had a ryegrass/fescue understory. Two sets of plots were used (Figure 1). The three large plots were used to develop marker technology. Five smaller plots, each paired with a grass control plot, are the source of our sampling results (taxonomic questions, pest and predator densities). Specimens for gut contents analysis were collected from throughout the orchard.

Sampling studies. Table 1 shows abundance data for 3 taxa of generalist predators (ladybird beetles - Coccinellidae, green lacewings - Chrysopidae, true bugs - Heteroptera), with the 5 numerically most common species in each taxon. The data suggest that the predator community includes habitat specialists (e.g., *Chrysopa oculata* in the cover crop and *Chrysopa nigricornis* in the tree) and habitat generalists (e.g., *Chrysoperla plorabunda* and *Coccinella septempunctata*). From these data, one would hypothesize that a cover crop is unlikely to be a direct source of biological control of pear psylla by species such as *C. oculata* (cover crop specialist) or *C. nigricornis* (tree specialist), but that it could be useful with respect to control of psylla by a generalist such as *C. plorabunda*. However, our marking data (see below) suggest that the data in Table 1 may be somewhat misleading in providing insight into rates of movement between habitats. (PP+) in the Table indicates that Horton has reared the predator successfully on a diet of pear psylla; (PP-) indicates psylla is not an appropriate diet. Asterisks indicate that both adults and immatures were collected.

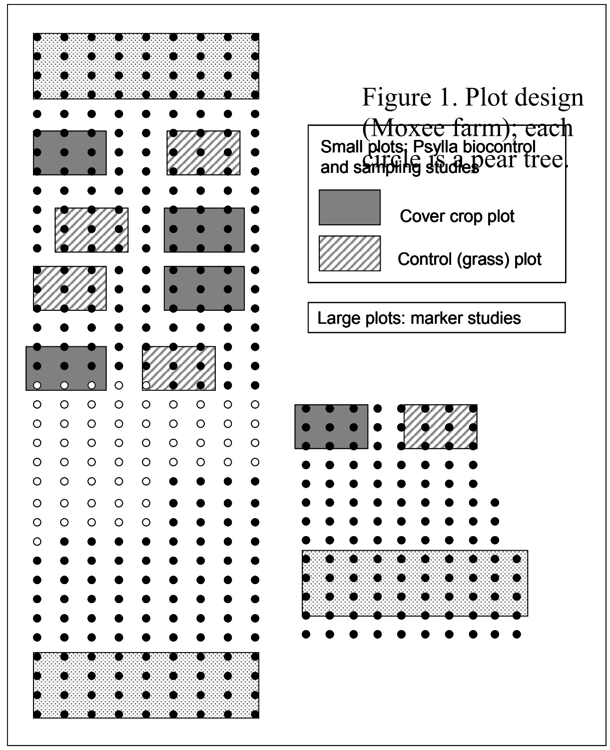


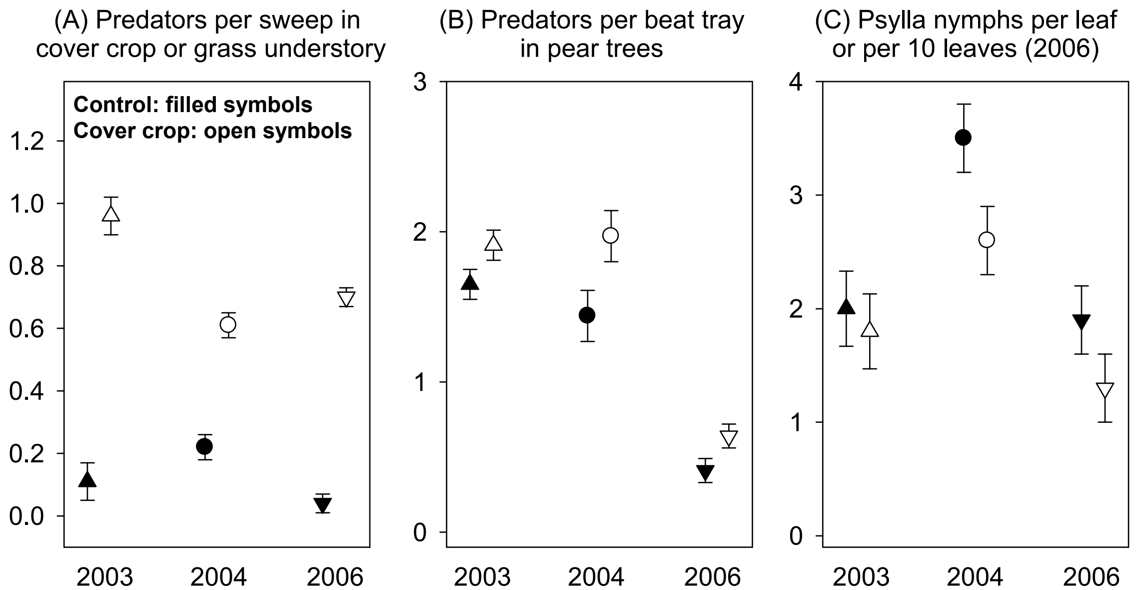
Figure 1. Plot design (Moxee farm); each circle is a pear tree.

Table 1. Numbers of generalist predators in 3 major taxa collected in 2003-06 from a legume cover crop (by sweep net) and pear trees (by beat tray).

Taxon	No. Collected[1] in		Apparent Habitat Preference		
	Cover crop	Tree	Cover crop	Tree	Habitat generalist
Heteroptera					
Orius tristicolor (PP+)	662*	24*	X		
Geocoris spp.	329*	0	X		
Nabis sp.	99*	8	X		
Deraeocoris brevis (PP+)	59*	1159*		X	
Anthocoris tomentosus (PP+)	10*	459*		X	
Chrysopidae					
Chrysopa oculata (PP+)	194*	3	X		
Chrysoperla plorabunda (PP+)	132*	155*			X
Eremochrysa sp.	33	111		X?	
Chrysopa coloradensis (PP+)	13	5*			X?
Chrysopa nigricornis (PP+)	4	47*		X	
Coccinellidae					
Hippodamia convergens (PP-)	382*	15*	X		
Coccinella septempunctata	127*	118*			X
Coccinella transversoguttata	116*	36	X?		
Hyperaspis lateralis	112	95			X
Harmonia axyridis (PP+)	11*	159*		X	

Predator densities were substantially higher in the cover crop than the grass understory (Fig. 2A). (Each mean in Fig. 2A-C is a seasonal mean, obtained by averaging over several sample dates per year). Densities of predators in the tree canopy were also higher in the cover crop plots than the control plots (Fig. 2B: by Anova, P = 0.036), suggesting that predators did indeed move from the orchard floor into the tree canopy. Densities of psylla nymphs in the cover crop plots were 90%, 74%, and 68% of the densities noted in the control plots for the three years, respectively, suggesting that the higher densities of predators in the cover crop plots led to biological control of psylla nymphs (Fig. 2C: by Anova, P = 0.028). Note that the data in Figure 2C are expressed as numbers of nymphs per leaf (first two years) or per 10 leaves (third year, in which psylla densities were low all season).

Fig. 2. Small plot (3 trees) trials showing effects of legume cover crop on predator densities in ground cover (A) or tree (B), and densities of psylla in tree (C).



Marking trials (Jones and Horton). Egg white, milk, and wheat flour were used to mark cover crop, tree canopy, and tree trunk, respectively (by Horton). Egg white was diluted to 20% (2004) or 10% (2005-2006) in water, and applied through a boom sprayer attached to an ATV. Milk was diluted to 20% and applied with a hand gun sprayer. Wheat flour was applied in bands to tree trunks by painting the product on. Predators were collected by dislodging them onto sections of cardboard treated with sticky trap, and removing them individually into microcentrifuge tubes for assaying. The specimens were collected by Horton, and shipped to Vince Jones to be processed for presence of markers.

In 2004, we began development of the egg white marker (for cover crop). Over 97% of arthropods collected from the cover crop carried the marker (Table 2), indicating that our application and assay methods were very good. More interestingly, 23% of arthropods from the tree also carried the marker, indicating that these specimens had visited or originated from the cover crop. The study was repeated in 2005 and 2006 to obtain better taxonomic resolution of the lacewings and ladybird beetles (Table 3). Tree specialists, cover crop specialists, and habitat generalists (from Table 1) all carried the marker.

Table 2. Percentage of specimens from tree or cover crop scoring positive for egg white (which was applied to the cover crop only) summer 2004.

Taxon	Tree[1]		Cover Crop[1]	
	N	% Positive	N	% Positive
Anthocoris tomentosus	248	21.4	61	93.4
Deraeocoris brevis	98	19.4	70	98.6
Nabis sp.	2	0	24	100
Orius tristicolor	3	33.3	318	97.5
Lygus spp. (pest)*	9	55.6	249	96.4
Chrysopidae*	12	58.3	20	100
Coccinellidae*	15	80.0	238	100
Spiders*	96	14.6	192	96.9
TOTALS	**483**	**23.0**	**1172**	**97.6**

[1]Tree collected insects were all adults; cover crop samples included some immature insects.
*Multiple species

Table 3. Presence of a cover crop marker on insects collected from cover crop or tree canopy; June-July 2005-2006. Adult insects only. ND – no data.

Specialization Group/Species	Tree collected		Cover crop collected	
	# Tested	% Positive	# Tested	% Positive
Tree specialists				
Anthocoris tomentosus	138	31.2	13	100
Deraeocoris brevis	267	15.7	46	97.8
Chrysopa nigricornis	3	33.3	ND	ND
Eremochrysa sp.	34	11.8	6	83.3
Harmonia axyridis	24	8.3	ND	ND
Cover crop specialists				
Orius tristicolor	10	20.0	830	97.5
Nabis sp.	2	50.0	52	100
Geocoris spp.	25	24.0	283	86.9
Chrysopa oculata	ND	ND	6	83.3
Hippodamia convergens	1	0	68	98.5
Coccinella transversoguttata	9	0	2	100
Habitat generalists				
Chrysoperla plorabunda	57	12.3	15	93.3
Chrysopa coloradensis	2	0	ND	ND
Coccinella septempunctata	22	4.5	13	100
Hyperaspis lateralis	3	0	5	100
Tree specialists	466	19.7	65	96.9
Cover crop specialists	47	19.1	1241	95.2
Habitat generalists	84	9.5	33	96.7
TOTALS	**597**	**18.3**	**1339**	**95.3**

The milk marker was less effective than egg white at marking predators (Table 4), either because of spray coverage or because insects less readily pick up a milk residue than an egg residue. The results suggest that there was some movement from the tree canopy into the cover crop by predators. Leafroller larvae and ladybird beetle larvae were allowed to walk across tree trunks brushed with wheat flour. All 7 leafrollers and all 12 beetle larvae that were assayed had picked up the marker. We believe that this method can be used to monitor movement between orchard floor and tree canopy by arthropods that use the tree trunk for these movements.

Table 4. Percentage of arthropods collected from tree or cover crop marked with milk; marker applied to tree canopy. 2006 study.

Sample 1		Sample 2	
TREE		TREE	
Anthocoris tomentosus	25.5	*Deraeocoris brevis*	28.6
Deraeocoris brevis	20.7	Spiders	23.2
Orius tristicolor	22.7	Pear psylla	45.3
Spiders	37.7		
Pear psylla	33.5		
COVER CROP		COVER CROP	
Orius tristicolor	8.6	*Orius tristicolor*	3.6
Geocoris spp.	4.8	*Geocoris* spp.	4.5
Spiders	22.7	Spiders	4.7

Gut contents analysis (Unruh and Horton). Two methods were developed by Tom Unruh to detect psylla remains in predator guts: (1) a method to detect psylla DNA in predator guts (by use of PCR), and (2) a method to detect psylla proteins (by use of ELISA). In 2004-2006, Horton collected predators from the tree canopy at 2-3 week intervals throughout the summer, and provided them to Unruh for analysis with ELISA; a portion of the 2005 sample was also analyzed using PCR. Also, in 2004-2005, we collected several predator species from the cover crop, to assess whether they might show evidence of having fed on psylla nymphs, which we would interpret as evidence that the predators moved between tree canopy and cover crop. Horton also collected leaf samples on each sample date to determine densities of psylla nymphs in the orchard.

The PCR method successfully identified psylla DNA in the guts of predators collected from the tree canopy or the cover crop (Table 5); the latter result suggests that predators collected from the cover crop had recently visited the tree canopy and fed upon pear psylla. ELISA showed that a small percentage of the cover crop specialist, *Orius tristicolor*, collected from the cover crop, did indeed have psylla remains in the gut (again suggesting some movement to the tree and feeding upon pear psylla). ELISA was used to analyze the seasonal tree collections, and results are shown for two predatory bugs: *Deraeocoris brevis* and *Anthocoris tomentosus* (Figure 3). Both species appear to feed readily on psylla when the pest is abundant, and to be much less likely to have psylla remains in the gut when psylla densities are low. Thus, proportion of specimens scoring positive for psylla were lower in 2006 than 2004-2005, which correlated with yearly differences in psylla densities. Within-season patterns in gut contents results also tracked within-season patterns in psylla densities; this is particularly clear in the 2004 and 2005 data (Figure 3).

Table 5. Incidence of psylla protein (ELISA) or psylla DNA (PCR) in guts of field-collected predators. Specimens collected from cover crop and tree.

	# Assayed	% positive for psylla
COVER CROP		
ELISA (2004)		
Orius tristicolor (June)	87	2.3
Orius tristicolor (July)	137	0.7
PCR (2005)		
Deraeocoris brevis	15	66.7
Hippodamia convergens	15	86.7
Coccinella septempunctata	14	53.3
Coccinella transversoguttata	10	10.0
PEAR TREE (PCR 2005)		
Deraeocoris brevis	15	66.7
Coccinella septempunctata	15	73.3
Harmonia axyridis	15	100.0

Fig. 3. Percentage of field-collected *Anthocoris* spp. or *Deraeocoris brevis* scoring positive for presence of pear psylla proteins (bottom panel); top panel shows psylla densities in orchard on dates predators were collected.