

FINAL PROJECT REPORT**WTFRC Project Number:** AE-04-428 (WSU Project No. 13L-3643-6367)**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	6,112
Benefits	6,146	6,392	2,246
Wages	11,000	11,000	4,142
Benefits	1,760	1,760	455
Equipment	0	0	0
Supplies	3,200	3,200	1,939
Travel	3,200	3,200	1,501
Miscellaneous			
Total^a	45,793	46,858	16,395*

*Pear portion of expenses only. An additional \$33,288 was received from the apple committee.

Significant progress this year:

- We developed a new marking protein (wheat flour) that can be brushed on the tree trunk and used to measure movement of immature insects and spiders moving up and down the trunk.
- We developed a method to correct for the differences in residual marking of our egg and milk marking proteins. This should reduce the likelihood that we bias our estimates of movement between the tree canopy and the ground cover.
- Our studies this year confirm that the ground cover is important to some of the predators and that they move freely between the two habitats.
- Foliage samples this year showed that we rarely detected drift in the canopy originating from the marker applied to the cover crop.
- Foliage samples collected from the cover crop did detect markers that were applied to the canopy of the tree, even when tarps were spread over the cover crop. In one set of experiments (Objective 1B) the drift was minor, but in another (Objective 1D) it was severe enough to invalidate the marker data for insects moving from the canopy to the ground cover.
- Our marker studies demonstrated that we could determine movement patterns that would not be detectable using normal sampling methods; specifically, if the insect has a daily movement pattern that happens at night, normal sampling would not detect that. The markers easily detect that sort of movement and allow us to quantify what proportion of the population moves in that fashion.

Objective 1. Determine the contribution of the orchard ground cover to natural enemy populations and biological control that occur in pear trees.

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This year a number of marking techniques were attempted, beyond what we tried in the previous years. These new techniques were aimed at expanding our previous studies and attempting to make sure that there were no problems with drift. There were no rain events during any of the experiments reported below, and under-tree irrigation (low pressure emitters) was applied only on the weekends after the week's samples had already been collected.

1A. Using a wheat flour marker to measure movement up and down a tree trunk.

This technique involved brushing dry wheat flour on the a tree trunk in a band 6 inches wide and then having leafroller larvae and ladybird beetles walk over the residue and determining if they acquired the mark. The wheat flour marker is a newly developed marker. Although the sample size was relatively small, we found that all the insects that walked over the band acquired the mark at extremely high levels. This treatment will be highly effective at measuring the importance of insects moving up and down the tree trunk (*i.e.*, immature insects or spiders) as opposed to flying between the ground cover and tree canopy.

1B. Treating the canopy with milk marker, no ground treatment.

This set of experiments was to determine how efficient the milk marker was at marking insects in the tree canopy and how extensive the drift problem under the tree would be. These experiments were tested over three periods: June 14-19, June 26-30, and July 10-14. Trees were sprayed with 20% whole milk and Sylgard® 309 spreader at 80 ppm. Before applying the milk marker, a tarp was placed below each tree to be treated. After each tree was treated, the tarp was dragged to the new location. Trees were sampled using beating sheets made from sticky cards (to immobilize the insects so that they would not contaminate each other); ground cover samples were shaken over the same sort of sticky cards.

Evaluation of foliage samples from the canopy showed that roughly 63% of the leaves had enough milk present to allow an insect walking across the residue to acquire the marker. Most of the variability came from the last treatment group when only 29% of the leaves had enough milk residue to mark the insects (the first two weekly treatments had 69 and 81% of the foliage marked, respectively). The variability in the leaf residues was reflected in the insect samples taken from the canopy and in the daily samples (taken for three days running in each of the three trials) in terms of the marking found over all insect groups collected. Over all groups and all three experiments, an average of 32% of the insects and spiders collected from the canopy were positively marked. Of the predators, spiders (37.7%), *Anthocoris* (25.5%), *Orius* (22.7%), and *Deraeocoris* (20.7%) showed the greatest levels of marking when more than 20 individuals were collected. If we evaluate the marking on the first two trials only, the marking is considerably higher for spiders (56%) and *Anthocoris* (38.8%); the other predators' values changed little.

Table 1. Percentage of insects positive for the milk marker that was sprayed on the tree. Only insects where > 15 were collected in a location are presented.

Insect/location	% Marked	% Corrected for Marking Efficiency
<i>Tree canopy</i>		
<i>Anthocoris</i>	25.5	§
<i>Deraeocoris</i>	20.7	§
<i>Orius</i>	22.7	§
Spiders	37.7	§
Psylla	33.5	§
Overall	31.6	§
<i>Ground cover</i>		
<i>Orius</i>	8.55	27
Spiders	22.7	72
<i>Geocoris</i>	4.8	15
<i>Lygus</i>	12	38
Overall	10.2	32

The ground collections of foliage were taken from directly underneath the tree canopy and drip-line so that they would likely show the highest amount of drift possible. In fact, the samples did show that there was some drift down to the ground, but over that entire period only 5.6% of the leaves from the ground cover were marked. All but one of the leaves were found to be marked in the first trial, suggesting that there was an error in that particular application, possibly because of moving the tarps before the milk had dried. However, even though the leaves had enough residue to mark an insect, it would still be unlikely. Our previous studies held insects on leaves treated with different amounts of marker for a 24-hour period; but in nature it would be unlikely that the insects would remain on one of the few "hot" leaves for anywhere near that length of time. Our collections of insects within the ground cover did show some with high levels of marking, particularly spiders (22.7%) and ladybird beetles (23.1%), indicating that they probably move between the tree and ground cover frequently, particularly given that the percentage marked in the tree is relatively low. We can use the overall percentage of marked insects collected in the tree to correct for the marker's low marking ability

$$\left(\frac{100}{\text{Mean \% Marked in tree}} \times \% \text{ marked in ground cover} \right).$$

When this is done, it becomes apparent that movement between the two habitats is common (Table 1) and suggests that ground cover management should have a dramatic effect on pest suppression if the predators will switch between psylla and the aphids common in the ground cover.

1C. Marking the ground cover only with the egg marker, no tree treatment.

These experiments were performed from early June to early July. This treatment is similar to work we have done the past few years but examined whether we could decrease the rate of egg used (from 20 to 10%), and we also examined more foliage samples to assess the effects of drift up to the canopy. The canopy samples were all taken from the lowest part of the canopy and thus would have been most likely to have the greatest drift. Our canopy foliage samples showed that 5.5% of the leaves had enough drift to result in marking of insects crawling across those leaves. However, all of the positive samples came on one day, suggesting that the problem was a result of application technique or possibly a short gust of wind during one part of an application.

The percentage of marked insects that were collected from the ground cover was 89.6% over all insects and spiders collected. These results are similar to our data from the past years and allow us to reduce the rates of eggs applied. This should not only reduce costs but should also reduce the importance of drift to the canopy. The high rate of marking in the ground cover also means that the correction factor is only 1.1 for insects that originated in or visited the ground cover but did not acquire the mark there.

Examination of the insects we collected in the tree showed that 36% of the *Anthocoris* were marked as originating in or visiting the ground cover (Table 2). This is roughly twice the percentage of *Deraeocoris* (17.8%) and spiders (15.2%). Green lacewings were also marked 11.7% of the time. Psylla also showed some marking (12%), suggesting that they were collected from the lower part of the canopy or that they fell to the ground and then crawled back up to the tree.

Table 2. Percentage of insects positive for the egg marker that was sprayed on the ground cover. Only insects where > 15 were collected in a location are presented.

Insect/Location	% Marked	% Corrected for Marking Efficiency
<i>Tree</i>		
<i>Anthocoris</i>	36	40.2
<i>Deraeocoris</i>	17.8	19.9
Green Lacewings	11.7	13.1
ladybeetles	6.7	7.5
Psylla	12.3	13.7
Spiders	15.2	17.0
Overall	15.36	17.1
<i>Ground Cover</i>		
Green Lacewings	95.6	§
Geocoris	79.4	§
Ladybeetles	95	§
Lygus	88.2	§
Nabids	100	§
Orius	91.6	§
Spiders	81.5	§
Overall	89.6	§

1D. Marking the ground with egg marker and the tree with milk marker.

These treatments were performed from mid-July to mid-August. The foliage samples from the tree showed no drift from the egg marker applied to the ground, but only 42.5% of the leaves in the tree were positive for the milk marker. In the ground cover, 100% of the leaves were positive for the egg marker, but 22.5% of the leaves in the ground cover also showed positive for the milk marker. All of the milk-positive collections from the ground cover came in August, with none in the mid-July collections. The foliage samples thus suggest that we should have seen valid results with the egg marker (movement from ground to tree) but possibly erroneous results from the milk marker (tree to ground).

The insects collected from the ground cover averaged 97% positive for the egg marker (Table 3), similar to our results in 1C above and in previous years. Predator samples collected from the tree were similar to 1C, with *Deraeocoris* being marked 25% of the time and spiders 22.1% of the time. Surprisingly, psylla were marked at 31.3% of the time, again suggesting that there was a drift problem (unlikely according to the foliage samples) or that they fell to the ground and crawled back up to the tree.

The milk marker applied to the canopy marked an average of 37% of the insects collected in the canopy, similar to what we observed in experiment 1B (32%). Again, with this lower level of marking we need a correction factor to help interpret the data. However, given the high level of drift from the canopy to the ground in this experiments, the results of the movement of insects from the canopy to the ground are unreliable.

Overall.

The data from this year provide us with key insights needed to improve our techniques and to help us understand movement patterns. First, in terms of improving techniques, we need to use tarps to cover

Table 3. Percentage of insects positive for milk or egg markers; milk sprayed in the tree canopy and egg on the ground cover. Only insects where >15 were collected are presented.

Insect/Location	% Marked with Egg	% Corrected for Marking Efficiency	% Marked with Milk	% Corrected for Marking Efficiency
Tree				
<i>Deraeocoris</i>	25.0	25.8	28.6	Š
Psylla	31.3	32.3	45.3	Š
Spiders	22.1	22.8	23.2	Š
Overall	27.1	27.9	37.2	Š
Ground				
<i>Geocoris</i>	90.9	Š	3.6	9.7
<i>Lygus</i>	93.4	Š	7.6	20.4
Nabid	100	Š	0.0	0.0
Orius	100	Š	4.5	12.0
Spiders	99.2	Š	7.4	19.9
Overall	97.0	Š	6.2	16.7

the cover crop when canopy sprays are applied. To make this more successful, we need to lay out the tarps below the entire treated area and allow the spray to dry completely before they are moved. This year, tarps were placed under an area of the tree and around it, and after the tree was sprayed they were moved immediately to the next tree to be treated by simply dragging them to the next location. Secondly, we need to change our experimental design to account for the difference in the ability of our milk and egg markers to mark insects as they walk over the dried residue. A way to deal with that problem is to treat the ground cover with egg and the tree canopy with milk in half our replicates and switch the treatments in the other half of the replicates (*i.e.*, tree with egg, ground cover with milk). This will be further strengthened by using the correction factors (described in 1B) to account for the differential acquisition of a mark by an insect walking over the dried residues of the different markers. This will prevent us from underestimating movement in one direction because of variability in marker efficiency. Third, our use of the wheat marker shows that we can easily determine the movement patterns for predators moving up and down the tree trunk, which in some circumstances (especially with immature stages or spiders that do not fly) may provide detailed movement patterns with a much simpler design. Finally, our studies clearly show that we need to expand our foliage samples to act as a check to determine the importance of drift and its possible impact on our findings.

Examination of the data tables also shows a marked discrepancy between the various trials in terms of species present. For example, Tables 1 and 2 show that *Anthocoris* was present in the tree canopy, but Table 3 does not. Most of this variation is a result of seasonal phenology (*Anthocoris* was rarely collected in the latter part of the season). Differences between abundances in the tree versus the ground cover may be a function of predator habitat preference (*e.g.*, *Deraeocoris* in the canopy and only rarely in the ground cover). However, our studies show that one of the key advantages of the markers (over just sampling) is that they allow us to detect movement between the ground cover and the tree canopy that occurs at times we are not sampling. For example, insects collected from the canopy, which test positive for the ground cover marker, could have picked the mark up at any time. Therefore, using markers we were able to detect a daily activity pattern where they move down into the ground cover at night and up into the tree during the day. On the other hand, traditional samples

taken at mid-day could never make that connection. A further benefit is that the markers could provide an estimate of how common that sort of movement pattern would be in the general population of insect and spider predators.

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