DESCRIPTION:	Determination of Key Predators of Pear Psylla using Molecular Bioassays for Pray Detection
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# **Determination of Generalist Predators of Pear Psylla**

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# Introduction

Prediction of biological control of pear psylla has been hampered in the past by our lack of understanding of the relative feeding rates and preferences of the various generalist predators found in pear orchards. While studies have shown a suite of generalist predators (lacewings, earwigs, facultative mirid predators) can be found in pear orchards, there has been no clear pattern as to the relative importance of each type of predator and if this importance shifts as the season progresses.

Initial steps to develop a molecular probe for the DNA of pear psylla were successfully conducted by a post-doctoral researcher in the lab, Nuria Agusti, in collaboration with Dr. Tom Unruh, USDA-ARS, Yakima, WA. The probe was used to successfully detect the DNA of pear psylla in the guts of generalist predators for ca. 24-36 hours after ingestion of the psylla. As such, we should be able to use the probe to determine what percentage of each generalist population of predators has consumed at least one psylla within the previous 24 hours. While the technique does not allow us to determine the number of psylla eaten per predator, the percentage of any population at any given time that is consuming psylla can be determined and combined with existing data on relative feeding rates under laboratory conditions. The hope is that the combination of feeding rates, percentage actively feeding in the field, and the number of the generalist predators within an orchard will provide additional directions for focusing biological control studies.

In 2001, the number of generalist predators within various orchards was determined and assigned to various broad categories (e.g. spiders, earwigs, or to specific genera (Campylomma – a mirid predator). These numbers were tracked over time and one finding was the fact that spiders often comprised more than 50% of the generalist predators, but this proportion varied tremendously between orchards. While the probe was used successfully with the insect predators and preliminary data for spiders also looked promising, the spider specimens of the spiders was not probed at this time because of difficulties in identification. One reason for the delay has been the wide diversity of species and families of spiders found within pear orchards that are not under broad-spectrum insecticide regimes. No one family was determined to dominate the species complex between orchards or over time. However, these data did suggest that spiders might play a previously unstudied source of predation that might prove key to understanding and predicting biological control of psylla.

The objectives for 2002 included a more systematic determination of the generalist predators across orchards using fixed sampling approaches rather than the approach used in 2001 which focused on more orchards under varied conditions to determine the range of potential predators. Similarly, the hope was to finish the probing of samples from 2001 and 2002 with the molecular probe to determine the relative feeding rates.

# **Materials and Methods**

Visual surveys were conducted in the early part of the season by foliar inspections within pheromone test plots in hopes of identifying orchards experiencing differences in psylla populations. Difficulties in orchard selection arise given that orchards with high psylla populations by definition have failed to achieve effective biological control, sometimes due to disruption from OP insecticides eliminating the potential biological control agents. Thus, surveys of orchard under high-pressure situations might fail to identify predators that in fact might be successful in biological regulation of psylla populations.

Conversely, orchards without significant pear psylla populations also cannot have high predators counts since the orchard lacks populations to support the predators. So, our quest was to try and identify orchards as they passed through transitional stages or were in intermediate levels of pressure from pear psylla.

Predator collections were conducted in hourly blocks from approximately 7am-10am. Collecting stopped when yield of the sample collections dropped which was usually by 10 am. Collections were made by one or two teams of 2 people each, each team generating about 50 tap collections / hour. The actual number of taps was noted for each hour's collection so that yield per tap could later be calculated. A "tap" consisted of a rapid three whacks of a limb with a circular beat collection tray held underneath. One tap sample was taken per tree and all arthropods on the collection tray were collected. Our collections were divided into hourly segments so we could go back and detect any broad temporal distribution of predator activity (early vs mid morning). Predators were individually placed in epindorf tubes in the field (to prevent predation between individuals) and tubes then placed on dry ice to prevent further digestion of stomach contents and possible psylla DNA degradation. Tubes transferred to a  $-80^{\circ}$ C freezer on return to the lab. Specimens were sorted, labeled, and identified to broad taxonomic categories at a later date.

Psylla samples were conducted by collecting a total of 400 leaves as follows: 8 leaves per top shoot from each of 5 topshoot from each of 10 trees across a predator sample area (approximately 2-3 acres). Leaves were bagged on a per tree basis and placed in an ice chest for transport back to the lab. They were then inspected and number of psylla was recorded.

Six sites in two areas (Sacramento and Ukiah) were sampled for generalist predators and pear psylla. The sites in Sacramento included a range of management inputs from minimal management (Biagi), limited managed (Jaime – codling moth control with pheromone, no additional psylla treatments), and transitional organic (Peck (North and South) and Eagle Point). The orchard in Ukiah was identified late in the season as a site with high pear psylla numbers presumably due to disruption from OP

applications. Quantitative samples were taken from mid-June to late August with up to 3 samples from any one orchard. Repeated samples were taken in some orchards over time (Peck South, Eagle Point) over more broad periods to see if broad changes had occurred as well as across several weeks (Ukiah) to see if similar results were obtained over a short time frame.

Identification of the spider populations has continued to prove elusive and we are currently working with faculty at UC Berkeley to train staff within the lab via a short course in spider morphology and taxonomy. The hope is to have the taxonomic expertise transferred to the lab for future studies. Until the spider samples are properly identified, the samples cannot be ground up for digestion and probing with the molecular probes. All samples (spiders and insects) will be run during the same time period with the probe so as to avoid potential protocol errors biasing our results (e.g. samples run correctly for the mirids, but not the spiders).

# Results

#### Pear Psylla

The results of the pear psylla counts are shown in Figures 1 and 2 for all orchards in the 2 counties. Despite a focus on orchards that were either 1) under minimal management or 2) organic (transitional or certified), the number of pear psylla remained very low for all samples. Visual surveys early in the season had failed to indicate pear psylla population, which was supported by more quantitative counts as the season progressed. Thus, the number of pear psylla per leaf remained below 0.1 psylla per leaf for all orchards except Eagle Point (0.48) in Sacramento County, and the percent of leaves infested remained below 6% infested leaves for the orchards. However, one orchard in Ukiah, which was identified late in the season as having pear psylla problems, was sampled twice and yielded more than 5 psylla per leaf with more than 85% of the leaves infested. Thus, our initial objective of sampling a range of orchards with low to very high densities was achieved, but we failed to find the intermediate levels desired. The lack of pear psylla could obviously result from several factors including 1) a lack of an initial population early in the season 2) effective biological control 3) environmental conditions or 4) some other undetected factor.

#### Predator Distributions

In contrast to 2001 (range 42-83%), the percentage of the total spider contribution was ca. 24% in 2003. True bugs (e.g. Anthocorids, Reduviids, *Campylomma*) proved to be greater at 33% on average across the orchards (Figure 3). Twenty percent of the generalist predators were lacewings, which have been implicated in psylla control, but are often not terribly reliable given their propensity to disperse as adults. However, the results were similar to 2001 in that the pattern varied between sites considerably.

In some orchards such as Eagle Point, the spider complex, which did not appear to be dominated by any one family, comprised ca. 44% of the total generalists (Figure 4). By definition, the relative proportion of two other groups, the mirid (true bugs) and lacewings were only 2% of the total, respectively. Two other orchards in the same region

(Biagi and Jaime) varied tremendously in the composition with only 5% of the samples being lacewings in Biagi, but almost 32% in the Jaime orchard (Figures 5 and 6). Spiders again were fairly common in both sites at 30% for Biagi and 43% for Jaime.

Comparison of some of the samples from the Peck orchards (North (Figure 7) and South (Figures 8 and 9)) did not show any starting changes over time or over the orchard with the proportions for most groups (e.g. spiders 11-23%), lacewings (19-25%), true bugs (25-46%)). For the orchard from Ukiah (Figure 10), no clear differentiation was seen in terms of the types of predators in that all of the general groups were recovered, but the percentage of the true bug complex was unusually high with 59% of the total being mirids true bugs.

However, the percentage data provide only part of the story in that the absolute values and ratios may prove more important. Predator number per tap ranged from 0.22-1.13 with the highest predator counts found in the Ukiah orchard with the highest pear psylla counts (Figure 11). If the predator counts are converted to predator: prey ratios, then the patterns change considerably. Because the molecular probes of the predators have not been completed, only total predator counts are expressed as a ratio to illustrate an approach. The predator: prey ratios were the highest for all Sacramento sites at 4.7 to 25.6, whereas the lowest ratio was observed for the Ukiah site at 0.2 despite having the greatest number of predators.

#### Conclusion

The results of this study can only be interpreted as preliminary at best and much of the analysis remains to be completed (e.g. molecular assays and taxa identification). These data will hopefully be coupled with some newer on-going studies that are targeting use of stable isotopes as indicators of feeding preferences by the predators that can shift between plant feeding and feeding on animal prey such as Campylomma. Based on isotopic ratios, it can be determined if facultative predators are shifting their preference as the season progresses from feeding on pear tissues to feeding on prey like psylla. One potential outcome might be that the maintenance of predators like *Campylomma* is possible in pear orchards when pear psylla numbers are low. The potential for use of *Campylomma* as predators appears feasible since no clear data exists to support any negative impact on pear production in contrast to apple systems.

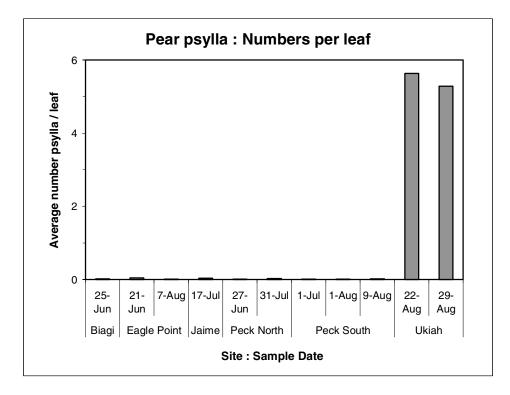


Figure 1. Number of pear psylla per leaf for 5 sample orchards in Sacramento area (Biagi, Eagle Point, Jaime, Peck(North and South Orchards), and Ukiah area.

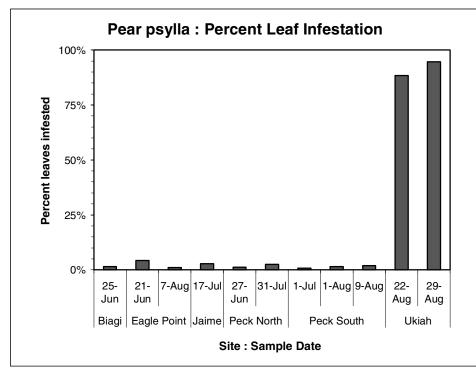


Figure 2. Number of pear psylla per leaf for 5 sample orchards in Sacramento area (Biagi, Eagle Point, Jaime, Peck(North and South Orchards), and Ukiah area.

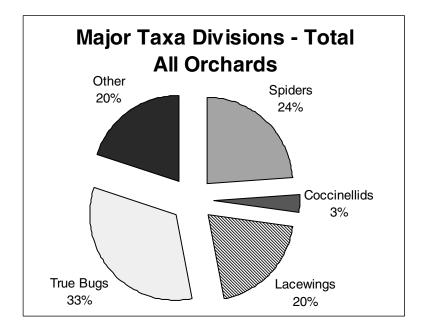


Figure 3. Relative distribution of generalist predators for all orchards

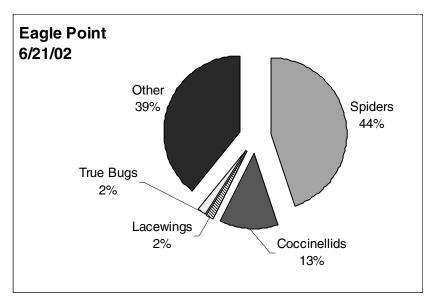


Figure 4. Relative distribution of generalist predators in Eagle Point (organic), Sacramento, CA.

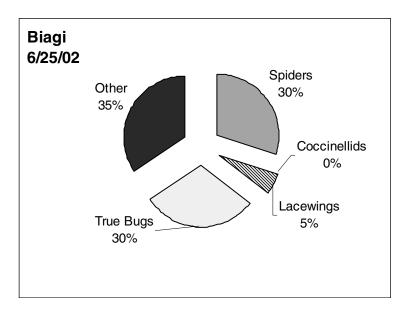


Figure 5. Relative distribution of generalist predators in Biagi (minimal management), Sacramento, CA.

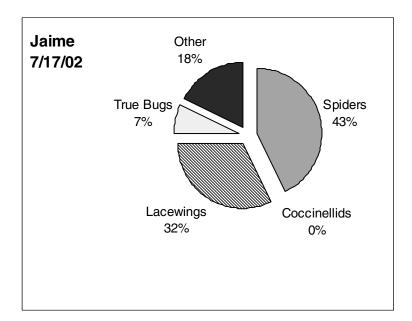


Figure 6. Relative distribution of generalist predators in Jaime (pheromone treated), Sacramento, CA.

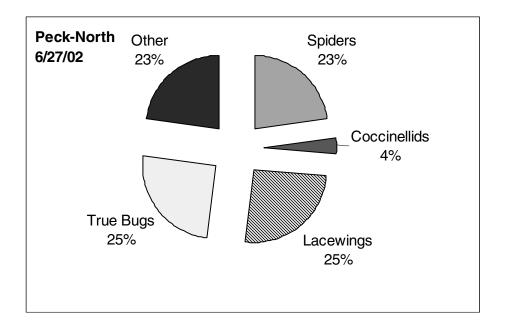


Figure 7. Relative distribution of generalist predators in Peck North (transitional organic), Sacramento, CA, June 27, 2003.

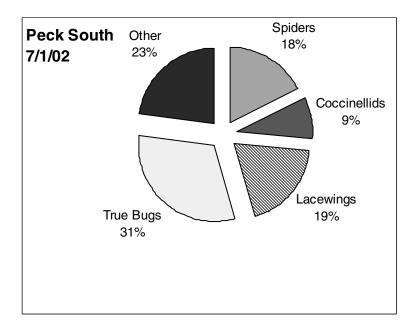


Figure 8. Relative distribution of generalist predators in Peck South (transitional organic), Sacramento, CA, July 1, 2003.

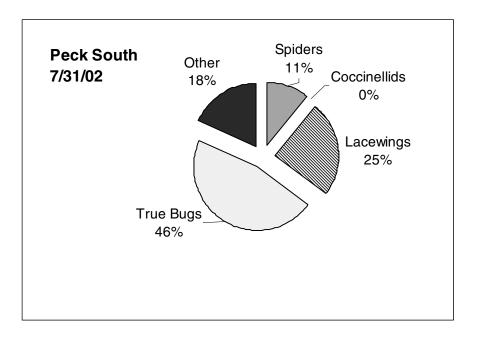


Figure 9. Relative distribution of generalist predators in Peck South (transitional organic), Sacramento, CA, July 31, 2003.

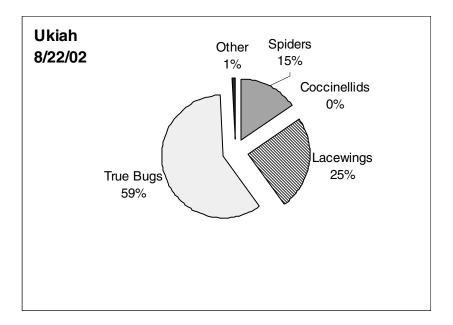


Figure 10. Relative distribution of generalist predators in Ukiah (OP treated), Sacramento, CA, Aug. 22, 2003.

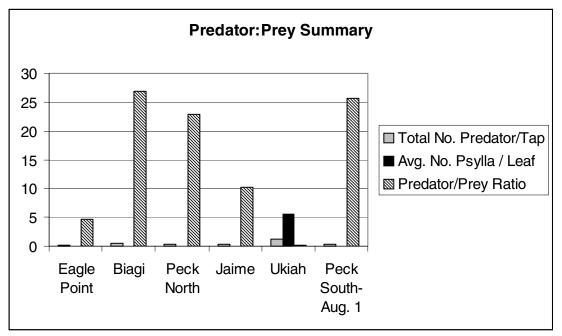


Figure 11. Predator and prey counts and predator:prey ratios for 5 orchards.