DESCRIPTION:	**Determination of Generalist Predators of Pear Psylla**
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Determination of Generalist Predators of Pear Psylla

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Management of pear psylla has been hampered in part by our lack of understanding of the relative importance of the different generalist predators as regulators of pear psylla populations. Many predators have been shown to feed on pear psylla, but how frequently they may choose to feed on psylla is not known at this time. Predicting effective biological control of pear psylla is one key step in developing a biologically based IPM program. If we can understand and make a reasonable prediction as to the likelihood that a population of pear psylla would be effectively regulated, then pesticide applications could be restricted to situations where control is not predicted.

Therefore, the first step is to identify which predators are actively feeding on pear psylla nymphs in the field, what proportion of a species are actively feeding, and how does this proportion change over the season. These data can be combined with counts of predators from a particular orchard and weighted by the number of psylla each predator eats under laboratory conditions. While these data will clearly not predict biological control of pear psylla with 100% accuracy, we hope that these data are one step in improving our predictive abilities.

Recently, advances were made for using molecular techniques for determining the host range and frequency of predation by generalist predators. A collaborative effort between Dr. Tom Unruh's lab in Yakima, WA and a post-doctoral researcher (Nuria Agusti) in the Welter lab developed a molecular probe to detect the presence of pear psylla DNA in the guts of generalist predators. In essence, we were able to determine if an individual predator had eaten pear psylla within the last 30 hours. This probe could be used on individual predators collected from the field under different situations to answer the questions listed above. While individuals making field decisions are not expected to use this approach, we are hoping to identify which predators merit tracking and under what conditions might they prove most useful for IPM.

Materials and Methods

Psylla samples. Psylla population samples were obtained by collecting leaves from top shoot samples. A total of 400 leaves were collected on a particular sample date and location by removing five top shoots from each of 10 dispersed trees and randomly

sampling eight leaves from each top shoot. Leaves were placed in plastic bags and transported in ice chests to the lab where they were inspected for psylla nymphs.

Samples were collected from 3 different pear- growing regions: the Sacramento Delta, the area near Fairfield, and upper Lake during June - August. In addition, three sites were sampled multiple times (see Table 6).

Predator samples. Potential psylla predators were collected from beat tray samples and sweep net samples on the same sample dates whenever possible. Field storage technique varied between samples as field protocols were developed. Multiple predators were sometimes aspirated into a single vial and immediately chilled to prevent predation and possible sample contamination. Predators were also individually placed into 1.5ml Eppendorf tubes and chilled in the field. Vials were stored at –80^{o}C upon return to the lab. Samples were recorded and sorted to family or lower level in the lab and individual specimens were placed into 1.5ml Eppendorf tubes for storage prior to DNA analysis. Given the time and hence expense of identification of samples to the species level, identification is being made a relatively superficial family level until broad groups have been tested which should allow our research to focus on more potentially important predators.

DNA analysis.

Two extraction methods were utilized to obtain DNA samples. The first technique listed was an attempt to coordinate extraction protocols with Dr. Tom Unruh in Yakima in an effort to allow our data to be cross-compared. In addition, this approach had the potential to limit our costs using a more simplified extraction procedure.

1) We attempted to extract DNA using Chelex-100 (BioRad). Individual specimens placed in 1.5 ml sterile microtubes were frozen in liquid nitrogen and then ground with a sterile mortar. Depending on specimen size, either 200 µL of 10% Chelex-100 or 400 µL of 5% Chelex-100 was immediately added to the tube. Then 8 µL or 16 µL (for a 400 µL Chelex preparation) of Proteinase K (2.5 mg/ml) was added and samples were vortexed and briefly centrifuged. Samples were stored at –20^{o}C until PCR preparation. Prior to PCR amplification, samples were thawed, vortexed for 5 seconds and centrifuged at 1300x g for 2 min. Six µL of supernate was used for the PCR reaction.

2) Successful DNA extraction resulted when individuals were prepared following the protocol of the DNeasy Tissue Kit (Qiagen). Total DNA obtained was re-suspended in 100 µL TE (10mM Tris-HCl, pH8.0, 1 mM EDTA) and stored at –20ºC.

PCR amplification reactions used primers developed by Agusti, Unruh, and Welter. A 25 µl reaction volume contained: 6 µl of re-suspended DNA, 2.5µl of 10 x buffer, 3 µl of 25mM $MgCl_2$, 0.25 µl of Taq DNA polymerase (Promega, Madison, WI), 0.25µl of 5mM dNTPs, and 1 µl of each primer 10 pmols/µl. Samples were amplified in a thermal cycler for 35 cycles at: 94ºC, 30 s; 58ºC, 30 s; 72ºC, 45 s. A first cycle of denaturation was carried out at 94ºC for 2 min, and a last cycle of extension at 72ºC for 2 min. Amplification products were all resolved eletrophoretically in 2% agarose gels. Gels were soaked in ethidium bromide for 40 minutes to identify the DNA.

Results

Pear Psylla Samples

As shown in Table 1, the orchards selected for sampling ranged from orchards that ultimately yielded detectable pear psylla (Courtland on June 22) to an abandoned orchard with greater than 65% of its leaves with pear psylla and an average count of 1.6 pear psylla nymphs per leaf (Fairfield on Aug 7). The Fairfield orchard represented an extreme case in which honeydew was present on most leaves, which made collection difficult. Because it was unclear to us what type of orchard would prove most useful (orchards with low or high psylla counts), we attempted in the first season to cover a broad range of conditions.

Predator Counts

The number of predators collected from each of 4 orchards in Fairfield or the Sacramento Delta ranged from 66 to 230 generalist predators. Because the number of tap samples was not kept consistent between orchards, the total numbers of predators cannot be directly compared. Instead, the collection process was continued if adequate numbers of predators were being captured per hour. The results for the 4 orchards in Table 2 are presented for all sites. The predators were divided into either spiders or insects, and then further divided to show the predominant predator (numerically) within each of the 2 groups. In three of the 4 sites, spiders were the predominant predator ranging from 42-83% of the total predator counts. Spiders in the families, Clubionidae and Salticidae, were common in 3 of 4 sites. A less consistent pattern was observed for the generalist insect predators with mirids being the most common in the Walnut Grove orchard, whereas anthocorids were most common in the Fairfield orchard, whereas coccinellids were the predominant predator in the Sacramento orchard.

DNA Probe Results

The results for the molecular bioassays are shown in tables 3 and 4. Table 3 are the result of predators that were brought in the field and allowed to field on pear psylla adults or nymphs within vials. These 15 individuals are only used as a possible control such that failure to detect pear psylla would suggest a problem with the bioassay protocols. Of the 15 individual predators tested, 100% tested positive as expected.

Of the 146 predators tested using the second set of protocols (Table 4), evidence of recent feeding on pear psylla ranged from 0-100% of the samples. Of the total anthocorids sampled (N=37), 51% scored positive which again suggests recent feeding on pear psylla. All of the lacewing (N=3) scored positive as well, but have not been tested sufficiently to place much confidence in this value. Many of the generalist predators, which occurred rarely (Pentatomidae) and are also facultative pests, also scored positive for feeding on pear psylla at 25% (N=4).

All except one of the 66 predators that were first extracted with Chelex failed to show any positive feeding signs (Table 5). The protocols originally developed by T. Unruh had been modified to allow for differences in predator body size, but this change

was apparently to the detriment of the assay. Given that less that 2% of the samples scored positive in sharp contrast to the data collected using the original extraction techniques of Nuria, Welter, and Unruh (Table 3 and 4), we decided not to use this technique on existing samples.

Analyses of the remainder of our generalist spider predator samples (N = 281; Table 6) still needs to be completed this winter prior to the next field season. The presence of large numbers of spiders in almost all orchards sampled compared to generalist insect predators suggests that spiders may prove more important to pear psylla control than previously expected. The fact that positive hits were scored from 2 unidentified species of spiders suggests that at least some individuals are feeding on pear psylla (Table 4) and that the bioassay may prove a useful tool for understanding spider predation.

Table 1. Results of top shoot leaf samples for pear psylla.

Orchard	Date	# leaves sampled	# leaves w/ psylla	Total # psylla	% leaves infested	Average # psylla/ leaf
Biagi	6/8	100	0	0	0	0
	7/31	400	3	3	0.8	0.01
	8/7	400	2	2	0.5	0.01
Eagle Point	6/22	400	0	0	0	0
Upper Lake	8/31	400	2	2	0.5	0.01
Pavlina	6/8	107	12	15	11.2	0.14
	6/22	400	155	278	38.8	0.70
	8/7	400	269	631	67.3	1.58
Yuki	6/19	400	7	7	1.8	0.02
	8/1	400	17	19	4.3	0.05

Table 2. General faunal characteristics of beat tray samples conducted to sample for psylla predators.

Orchard	Total	Spiders Number (% of total) Predominant families - % of spiders	Insects Number (% of total) Predominant families - % of insects
Biagi	66	55 (83%) Clubionidae – 34% Salticidae – 36%	11 (17%) Miridae – 64%
Eagle Point	90	74 (82%) Linyphiidae – 30% Araneidae – 19% Salticidae – 16%	16 (18%) Chrysopidae – 75%
Pavlina	230	97 (42%) Clubionidae – 54%	133 (58%) Anthocoridae – 33% Chrysopidae – 12% Reduviidae – 8%
Yuki	165	92 (56%) Clubionidae – 27% Salticidae – 26% Oxyopidae – 15%	73 (44%) Coccinellidae – 58%

Table 3. Results of assays screening for the presence of psylla DNA in predators intentionally fed psylla. DNA extractions were performed using protocols of the DNeasey Tissue Kit (Qiagen).

Common Name	Family	N	# positive	% positive
Minute Pirate bugs	Anthocoridae	13	13	100%
Assassin bugs	Reduviidae	2	2	100%
Spiders	unidentified	1	1	100%

Table 4. Results of assays screening for the presence of psylla DNA in field collected predators of unknown feeding status. DNA extractions were performed using protocols of the DNeasey Tissue Kit, Qiagen.

Common Name	Family	stage	N	# positive	% positive
Minute Pirate bugs	Anthocoridae	Total *-adults* *-immatures* *-unspecified*	37 *16* *10* *11*	19 *8* *10* *1*	51% *50%* *100%* *9%*
Lady bug beetles	Coccinellicae		38	3	8%
True bugs	Hemiptera	unidentified	2	2	100%
Lacewings	Chrysopidae	Total *-adults* *-immatures*	3 *1* *2*	3 *1* *2*	100% *100%* *100%*
Stink bugs	Pentatomidae		4	1	25%
Assassin bugs	Reduviidae		6	4	67%
	“Rhopalidae”		11	8	73%
Rove beetles	Staphylinidae		3	0	0%
Spiders	unidentified		2	2	100%

Table 5. Results of assays screening for the presence of psylla DNA in field collected predators of unknown feeding status. DNA extractions were performed by a method developed using Chelex-100 (BioRad).

Common Name	Family	Species	N	# positive	% positive
Minute pirate bug	Anthocoridae		1	1	100
Lady bugs	Coccinellidae	multiple	9	0	0
	Coreidae		2	0	0
Lacewings	Chrysopidae	*C. carnea* (immature)	21	0	0
		C. comanche (immature)	1	0	0
		C. oculata (adult)	1	0	0
		C. sp. nr carnea (immature)	6	0	0
		C. sp. Nr oculata (immature)	2	0	0
		Unidentified (immature)	2	0	0
Brown lacewing	Hemerobiidae	*Hemerobius sp nr pacificus* (adult)	1	0	0
Big-eyed bug	Lygaeidae	*Geocoris* sp.	1	0	0
"milkweed bugs"		"milkweed bugs"	13	0	0
green stink bug	Pentatomidae	sp.1	1	0	0
Assasin bug	Reduviidae	*Zelus* sp.	2	0	0
Rove beetle	Staphylinidae		2	0	0

Table 6. Spiders identified to family level remaining to be screened for evidence of psylla DNA.

Common name	Family	Number
Orb weavers/ garden spiders	Araneidae	19
Sac spiders/ twoclawed hunting spiders	Clubionidae	107
Hunting spiders	Gnaphosidae	3
Dwarf spiders	Linyphiidae	31
Wolf spiders	Lycosidae	5
Lynx spiders	Oxyopidae	21
Jumping spiders	Salticidae	73
Long-jawed orb weavers	Tetragnathidae	3
Crab spiders	Thomisidae	19