FINAL PROJECT REPORT **YEAR**: 1 of 1 (final report delayed 1 year)

Project Title: CSI in the orchard: finding the killers of 4 key apple pests

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Budget 1:
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Item	Year 1: 2006
Salaries	$13825
Benefits	$ 7122
Wages	$0
Benefits	$0
Equipment	$0
Supplies	$0
Travel	$0
Miscellaneous	
Total	**$20947**

Footnotes: Supported Eugene Miliczky

Budget 2:
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Item	Year 1: 2006
Salaries	$21243
Benefits	$ 2897
Wages	$0
Benefits	$0
Equipment	$0
Supplies	$0
Travel	$0
Miscellaneous	
Total	**$24140**

Footnotes: Supported Pablo Palmandez

Understanding the causes of pest insect mortality is critical to discovery of the best bio-rational methods to control these pests. Unlike death from disease or parasitoids, unwitnessed predation cannot be measured with confidence - the predator eats the evidence. The most unbiased measure of predation is direct observations of predators eating the prey (pest) of concern or direct measurement of a predator's consumption history based on physical or biochemical gut content analysis. This study was designed to perfect methods to determine key predators of four key pests of apples by detection of prey remains in the form of PCR of prey DNA in the predator's guts: codling moth, OBLR, woolly and rosy apple aphids. After a season of sampling the gut content analysis of the aphids was eliminated because the predators of these species were readily "caught in the act" through the season. The project has been plagued by difficulties in DNA extraction and contamination of reagents with amplification products. This has prompted plans to develop an antibody for codling moth. Several PCR primers were designed and optimized for the detection of codling moth and OBLR.

Objectives:

1) **Design multiplex PCR methods to specifically detect DNA of codling moth, OBLR, woolly and rosy apple aphids in predator guts**
2) **Collect predators throughout the season and the day/night cycle and use the method to estimate predation frequency and to rank predator importance**
3) **Conduct laboratory feeding studies to help interpret data collected in the field**

Significant findings:

- Primers designed for the CO-1 gene proved non-specific in the case of beetle predators of codling moth and OBLR. Additional primers were designed for the ITS-1and ITS-2 regions of codling moth and OBLR. These functioned well in multiplex format. Primers were not designed for the aphids.
- A salt-based extraction protocol was developed to work with all predator species but was especially useful for large carabids, earwigs, and spiders where multiple expensive extraction columns would be required
- DNA extraction and PCR proved to be too expensive($7/specimen) for high-throughput sample analysis. Progress was further hindered by sample contamination with PCR products. These two factors (expense and contamination by PCR product) has lead to the development of a LAMP (loop-mediated amplification) procedure which is still being optimized.
- About 2,500 predators were collected from 6 orchard sites using pitfall traps, beat tray sampling and by direct collection. Only 10% of these were analyzed by PCR.
- Feeding and digestion trials were conducted for a large, common carabid species *Pterostichus* sp. and the spiders *Cheiracanthium mildei* and *Holoena sp*. Prey signal retention exceeded 2 days in these species.

Results and discussion (by objective):

Objective 1*:* PCR primers were originally developed using the CO-1 gene based on comparative moth, beetle, lacewing, and bug sequences found in Genbank and DNA sequences for codling moth and OBLR developed in the lab. These provided high activity but many spurious bands in the carabid beetle predators. Thus primers were redesigned for internal transcribed spacer genes of the rDNA gene cluster (ITS-1 and ITS-2) based on DNA sequences for codling moth and OBLR developed in the lab. These eliminated cross reactivity problems with similar sensitivity.

Multiplex PCR methods were developed for codling moth and OBLR using ITS-2 primers. This allowed both species to be detected simultaneously in a single PCR amplification of a specimen, reducing by half the PCR portion of the expense of predator gut analysis. We also developed a rapid salt alcohol DNA extraction protocol but we still must dissect the gut from very large predators (large carabid beetles and spiders). For the large predators, the calculated expense for PCR of a single specimen was in excess of $7, $6 of which was labor. This expense prevented the analysis of the majority of collected specimens.

Objective 2*:* Predators were collected from 6 orchards using 3 major collection methods: beat tray samples, 24 hr pitfall trapping, and by visual search and collect. Six orchards were sampled and over 2,500 predators were collected. DNA analysis has just begun and is not reported here. The following narrative describes the orchard and provides a summary of predator abundance by predator type and by orchard Table 1).

Orchards:

1. Moxee fujis was a mixed variety block consisting primarily of Fujis in which various experimental insecticide treatments were applied in addition to herbicides. Our samples were taken in untreated parts of the orchard. Uncultivated land with mixed native and introduced plants surrounded this orchard on all sides. This block was heavily infested with Codling moth and we released OBLR larvae on sample trees on 2 dates.
2. Moxee small block consisted of 19 apple trees of various varieties bordered by pears and soft fruits on 3 sides and uncultivated land on the fourth. No insecticide treatments applied to the trees but early in the season the ground cover was sprayed with diazinon for ant control. and herbicides were used. This block was heavily infested with Codling moth in the second generation and we released OBLR larvae on 2 dates.
3. Mike Young's was a commercial 3 acre organic block of large golden delicious trees mostly bordered by other orchards with some exposure to uncultivated land. This orchard was very heavily infested with Rosy apple aphid but incidence of Codling moth was low due to mating disruption and repeated granulovirus applications.
4. Scott Leach's was a commercial organic block of red and golden delicious trees bordered by other orchard and an irrigation canal. The orchard had a moderate infestation of Rosy apple aphid and a low to moderate infestation of Wooly apple aphid, noticeable mostly on crown suckers. Codling moth incidence was low.
5. Wallace block was a nearly abandoned block of red and golden delicious trees bordered by other orchards and a highway. This orchard had received very little management for a number of years. The irrigation system was poorly maintained and the trees had not been pruned. Also, the orchard had not been mowed for more than a year and small trees of several species had established themselves in the understory. Codling moth and San Jose scale infestations were both ~100% on the fruit and there was a low to moderate infestation of Wooly apple aphid. Experimental trapping/monitoring of codling moth was being conducted in this orchard.
6. The Garza Office block is a former commercial organic orchard consisting of red and golden delicious trees now being used for experimental codling moth treatments. It was surrounded by other orchards and weedy, uncultivated land. We sampled in control plots where coding moth

levels were as high as 50% fruit damage. There was also a low level of Wooly apple aphid, primarily around the bases of the trees.

Table 1. Summary of 2006 predator collection data for CSI project. Abbreviations for expected prey: CM = codling moth; LR = leafroller; RAA = Rosy apple aphid; WAA = Wooly apple aphid. Abbreviations for type of sample: PT = pitfall trap; BT = beat tray; HC = hand collection; CB = cardboard band/bundle; SN = sweep net. Relative abundance of predators indicated by: - absent; + low abundance; ++moderate abundance; +++ high .

Orchard Number						
Parameter	1	2	3	4	5	6
Expected Prey	CM, LR	CM, LR	RAA	RAA, WAA	CM, WAA	CM, WAA(?)
Types of Sample	PT, BT, HC,CB	PT, BT, HC, CB	PT, BT, HC, SN	BT, HC	PT, BT, HC, CB	PT, BT, HC
Sampling period	June - August	June - October	June – July	May - July	July - October	August – October
Predator abundance	Moderate	Moderate to high	High	High	High	Moderate
# predators taken	100 – 200	> 500	> 500	300 -400	> 500	300 – 400
	Relative abundance of predator taxa collected					
Lacewings	+	+	+++	+++	++	-
Predatory bugs	+	++	+++	+++	+++	-
Lady beetles	+	+	++	++	++	-
Syrphid flies	-	-	++	++	+	-
Earwigs	+	+++	++	-	+	-
Ground beetles	++	+++	+++	-	+++	++
Rove beetles	+	++	+	-	+	+
Ants	+	+	-	++	-	-
Spiders	++	+++	++	++	++	++
Daddy-longlegs	-	+	+	-	+	+

Note on revised scope of objective 2 . Collections of predators in trees infested with Rosy apple aphid showed a very strong bias toward three predator groups in this order of abundance: lacewings>ladybeetles>syrphid flies> all others (mostly predatory bugs). Observations make it clear that these species are in curled leaves eating the aphids arguing against expensive assays of these predators. No primers were designed for the two aphids species and no PCR studies were conducted.

Objective 3. Evidence of predator feeding on our key pests must be interpreted based on knowledge of feeding frequency and digestion rates. We completed 2 laboratory feeding and digestion studies , a large carabid beetle, Pterostichus species, and one spider, Chieracanthium mildei. Predators were fed a single large codling moth larvae and allowed to digest up to 48 hr (beetle) or 96 hr (spider). Results were unambiguous, both these predators digest very slowly and prey signal is largely retained after 2 to 4 days (See Figure 1).

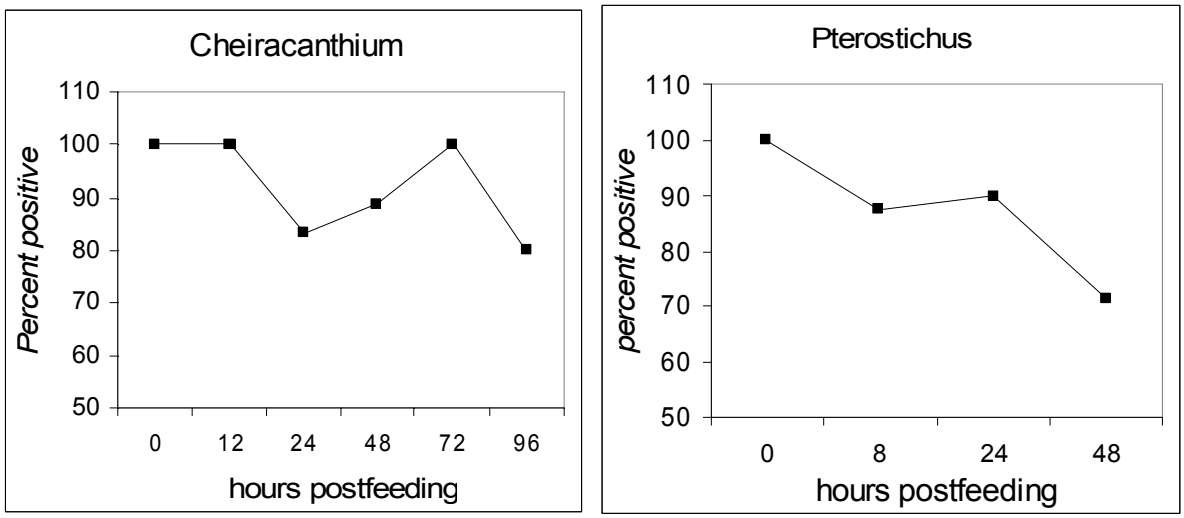


Fig. 1. Digestion pattern of a spider (C*heiracanthium milde*i) and a large carabid beetle (*Pterostichus* sp.) following feeding on a single large codling moth larvae. No signal was observed in controls.

Studies of insects in the field showed a strong association of positives with time of the season. Carabids collected early in summer showed no codling moth in their guts while those from late summer into fall, when codling moth are wandering, showed a high percentage. However, this percentage fell off dramatically later in fall. This is depicted in figure 2.

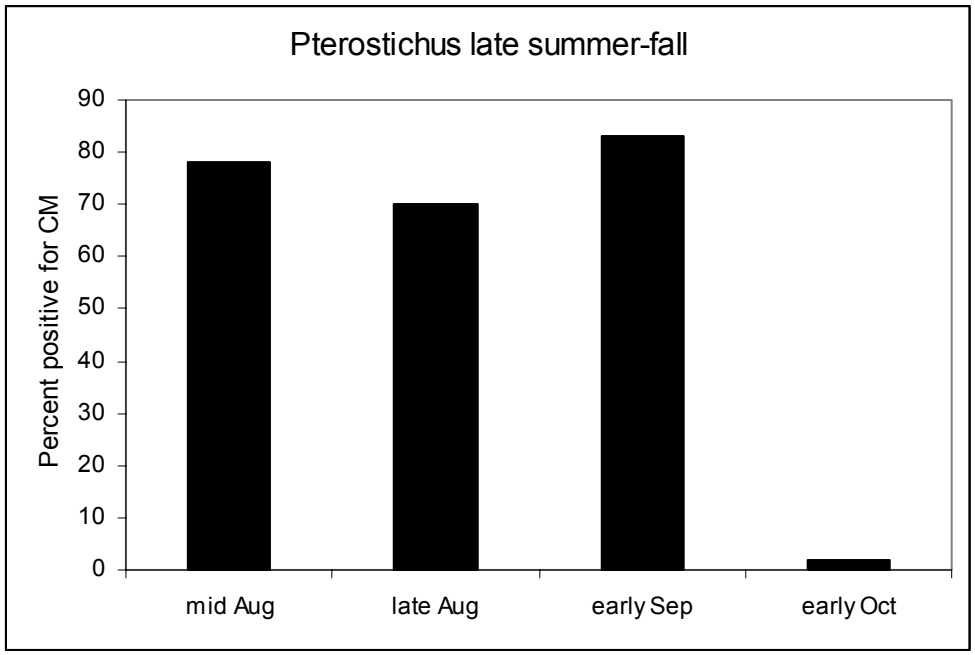


Fig.2. Late summer pattern gut contents of the carabid, Pterostichus, assayed for codling moth. A sharp decline is evident in late fall.

A similar pattern may be present with spiders and other predator groups but we have collected less data on these species. However, from individuals collected from Moxee and Wallace (high CM sites) during late August to early September, positives for codling moth in guts were high. Figure 3 summarizes data from over 50 predators (3 spiders types and earwigs).

Overall, our results suggest significant predation by both carabids (only the large carabids) and selected spiders and our data remains largely limited to the late summer early fall period when codling moth larvae are exiting fruit and seeking cocooning sites. See discussion of predator behavior below.

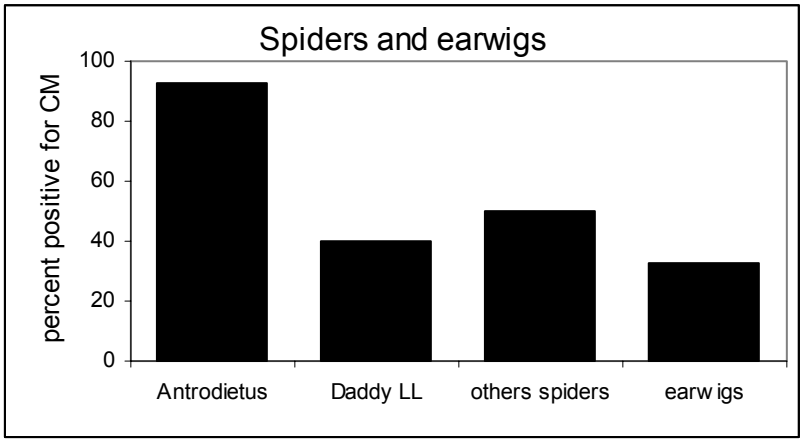


Fig.3 Gut content summary for the spider Antrodietus, the Opilionids (daddy long legs), 3 other spiders combined, and earwigs. (n>10 for each bar).

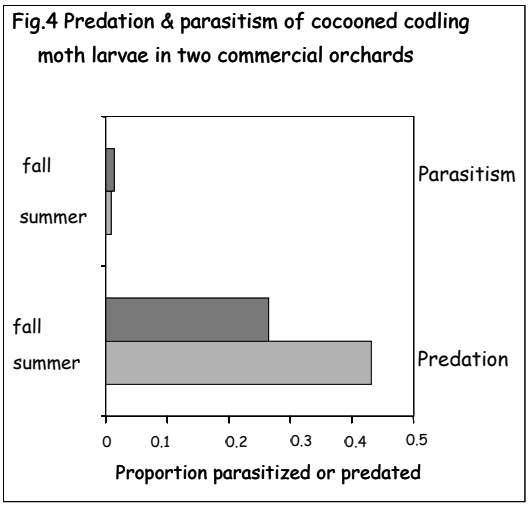
The future of predator gut-content analysis: methods and sampling issues

Recent studies in press suggest that PCR for gut analysis suffers several problems not found with the use of monoclonal antibodies (Fournier et al., submitted Oecologia). These are the high expense for both materials and labor for DNA extraction and the PCR assay itself and problems with repeatability of the PCR and cross contamination in labs doing high throughput PCR by PCR product. These problems do not exist with ELISA, but ELISA using monoclonal antibodies suffers from the high cost and time associated with developing the monoclonal(s). There are two alternatives to these problems which we are explorint

First, polyclonal antibodies need to be reconsidered. The original ecological applications of insect predator gut content analysis employed polyclonal, not monoclonal, antibodies whose non-specific fraction was removed by cross precipitation with non-target antigen (predator and non-target prey proteins). Since that time molecular biologists have helped develop and use polyclonal antibody produced against small antigens (short polypeptides) which can show high tissue and species specificity. This approach offers the potential to have high antibody specificity without investment of the time and money to develop monoclonals. Insect hemolymph contains several proteins (hexamerins, arylphorins, tyrosine oxidases) which are highly abundant and display regions of high divergence providing target domains for developing polyclonal antibodies.

A second approach is to detect DNA in predator guts using recently developed non-PCR methods. Of particular interest is the isothermal method called LAMP (loop mediated amplification procedure) which employs a polymerase enzyme that self-cycles at 60 C, and a chemical reaction that produces a visible precipitate (or fluorescent dye) allowing the determination of positive detection without the need to run a gel or visualize with a real-time instrument. Proponents of the method claim it is much more tolerant of dirty samples (potentially dramatically reducing extraction costs) and is equal to or more sensitive than PCR. This last point raises again the concern for cross contamination, but because no gels are run and the results can be seen through the closed tube, cross contamination and lab-wide contamination should be dramatically reduced. We have already developed two primer sets for the LAMP amplification of codling moth ITS-2. A third advantage of LAMP is that instead of using 40 base pairs of primer sequence the method employs four complex primers that cover 120-150 bases of the target sequence dramatically reducing the likelihood of cross reactivity with non-target sequences.

The final concern of gut content analysis is the issue of sampling the predator community. Recent studies in the laboratory have show that the carabid predators *Pterostichus* and *Harpalus*, the spider predators *Holoena,* and 3 other spider species captured in pitfall traps have shown that these predators do not consume codling moth larvae in their cocoons during exposures of 5 or more days. However, upon presentation of live, active codling moth larvae, all of these species showed aggressive and rapid attack and consumption of the larvae. These results together with evidence of predation of codling moth cocoons in the field in other studies (Figure 4), suggest that our pitfall trap captures may not be representative of key predators of codling moth.



Analysis of gut contents of the predators collected herein will be expanded under the apple IPM project (Jones, Horton and Unruh) and will include evaluation of the peptide-polyclonal and LAMP approaches. Improved understanding of predators that attack codling moth in their cocoons will also be evaluated in the IPM project using video surveillance of sentinel larvae.