INTERIM FINAL REPORT

Project Title: A biochemical approach to quantifying pear psylla predation in the field

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Funding History:
Year initiated: 2001 ($4,000)
2002 ($28,600)
2003 ($28,600)
This project was funded August to August and year 3 is only 30% completed

2004 Objectives: As this project is funded through August of 2004, this final report is premature and is thus entitled Interim final report. We plan on submitting another report in 2005. We have three objectives for 2004 and 2005:

1. Complete digestion profiles for *Orius*, one coccinellid, and an ant predator. We also will add replicates to our existing digestion profiles. These digestion profiles remain important in interpreting results of field studies and for choosing the best primers for the
2. Develop a time budget for feeding by key predator species. We attempted this effort in the field in 2002 and failed due to the small size of predators and the low densities at which they occur. We failed again to execute this in laboratory microcosms in 2003 but will attempt to do that for *Anthocoris, Deraeocoris*, and a lacewing.
3. Use ELISA and PCR to provide estimates of predation frequency by key predators. We now have doubts that a time specific predation rate may be beyond the reach of current technology. However, we are confident that comparative predation rate or intensity data can be collected. We are currently analyzing field specimens from 1999 collected by D. Horton. We will continue to analyze the field data we have collected in 2001-2003 and work with specimens collected by Horton collected in 2003, and that his group will collect in 2004 (see Horton proposal).

OBJECTIVES 2003

1. Complete digestion studies on Lacewings emphasizing short digestion times and evaluate longer amplicons for the bugs (completed)

2. Utilize PCR method to estimate predation rates of pear psylla by of *Anthocoris, Campylomma, Chrysoperla, Deraeocoris* and *Formica* in pear orchards (ongoing).

3. Describe daily rhythm of feeding by predators in laboratory microcosms to support molecular estimates of predation rates in the field (incomplete).

Significant findings in 2002-2003

(These findings are refinements of 2002 and represent 16 months of effort)

1. Compared Monoclonal Antibody and DNA digestion rates in 4 predator species highlighting species differences. Found digestion rates of protein versus DNA highly variable among species
   - Anthocorus and Deraeocoris digest prey much more slowly that lacewings
   - The minute pirute bug, *Orius* sp. showed complete digestion of prey DNA at ingestion
   - The two lacewings differed significantly from each other in digestion patterns of protein

2. Demonstrated importance of size of DNA target for PCR on estimating predation rate and showed method superior to use of monoclonal antibodies
   - A. tomentosus digest prey (halflife) in 1 day with 110 -280 bp amplicons
   - A. tomentosus digest prey (halflife) in 6 hours with 1800 bp amplicon
   - lacewings digest prey remains (110-280 bp and MABS) in less than 2 hrs
   - lacewings have completely digested prey at ingestion for large amplicons (700bp)

3. Existing PCR technology remains too unreliable to accurately estimate predation rates
   - Need more complete DNA extraction and stabilization methods.
   - Need improved primer designs to maximize detection of rare DNA molecules
4. Both PCR and ELISA represent useful tools to estimate comparative predation activity
   - Suitable for comparing insects in high host environments versus low host environments
   - Analysis of field populations ongoing

Results and Discussion

Many past studies have characterized psylla abundance from a combination of leaf counts and beat tray data and predator abundance from beat trays and from these data made inferences on predator importance. This includes some of my own data presented previously to the Winter pear and Tree fruit commissions and earlier data collected by Bob Fye and Brad Higbee at the YARL. Similarly there is a collection of studies of how many psyllas various predators can eat in the laboratory (Fye, Brunner, and others). Unfortunately, only a few studies conducted under very restrictive conditions have experimentally estimated predation of psylla by specific free-roaming predators in the field (Unruh and Higbee, 1994). In sum, we do not yet know which the most important predators of pear psylla are and when they are abundant enough to control psylla below damaging levels in the field.

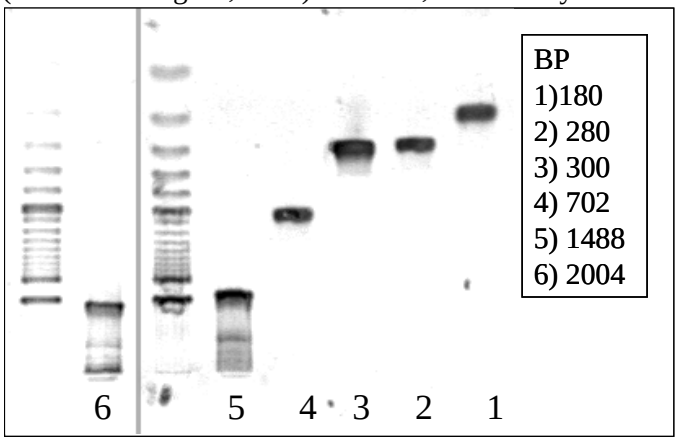


Figure 1. PCR amplification products for primer sets designed to amplify a range in size of segments from the mitochondrial CO1 gene of pear psylla. These primers only amplify psylla and not other insect groups hence they can be used for detection of psylla remains in the guts of predators.

2001: Over the last 6 years we have been studying methods to detect if a predator has eaten a psylla -including specific monoclonal antibodies made against psylla proteins (Horton et al. 1997) and DNA primers that specifically amplify psylla DNA (Agusti, Unruh and Welter, 2003). Dr. Agusti spent 4 months in my laboratory in the winter of 2000-20001. During that period we developed primers that specifically amplify DNA of pear psylla, which can be used to detect "forensic" evidence of psylla in the gut of predators. These primers amplify small pieces of psylla DNA (64- 280 bp). After Dr. Agusti's departure in spring of 2001 we designed primers that amplify larger stretches of DNA (amplicons 300-2,000 bp) as reported in February 2002. (Figure 1from 2002 is reproduced on the left). From this work we have begun to learn details about the digestion biology of key predators.

2002-2003: We completed comparisons between the monoclonal antibody (MAB hereafter) and 3 of the PCR amplicons (280, 702, and 1,800 base pairs, hereafter PCR_{280} etc.) for the predatory bugs, *Anthocoris tomentosus* and *Deraeocoris brevis* and the lacewings, *Chrysopa carnea* and *Chrysoperla rufilabris* (See figures 2-5 below). Surprisingly, the retention time of psylla signal in the gut of Anthocorus and Deraeocoris bug predators is quite long, more than 24 hr. Also, our results with the

bugs show that larger segments of prey DNA are digested more rapidly than smaller segments of DNA. In other words, we can estimate the time since the last meal with greater precision using larger, more rapidly digested, target DNA. The value of short retention times of the prey signal is clear when you consider that some predators may eat more than 1 psylla per hour. A short pulse or signal life is required to have a realistic estimate of predation rate.

The most vivid differences between species is the very rapid digestion of both prey DNA and protein in lacewings compared to the bugs. PCR_{280} and MABs show almost identical digestion rates within *Anthocoris tomentosus and Deraeocoris brevis* with a half life of detection of about 1440 minutes (24 h) but not completely reaching zero (psylla detected) in 2 days when all digestion studies were terminated. In contrast, *Chrysopa carnea* and *C. rufilabris* digested psylla extremely rapidly with a PCR_{110} half life of an hour in *C. rufilabris* and about 2 hours in *C. carnea*. A bizarre result that we are still trying to understand was the extremely rapid digestion of protein in *C. rufilabris*. While protein digestion in *C. carnea* was congruent with DNA digestion, it was much more rapid than small DNA amplicons in *C. rufilabris*. Furthermore, it seemed highly variable, actually rising with time, despite high sample sizes.

The digestion biology of the lacewings differs importantly from the predatory bugs (but see comments below on the mirid, *Orius)*. Both groups inject digestive enzymes into the prey but the lacewings have an incomplete digestive tract as nymphs thus no prey signal is excreted. Furthermore, exo-digestion of the prey, by injected enzymes, is much more thorough and rapid by the lacewings (as evidenced by cadaver examinations) and is especially notable in *C. rufilabris* which digests most of the prey tissues visible the host.

These relationships point out a key strength of the DNA-based method over monoclonal antibodies. Our 2002-3 data indicate that amplicon size must be appropriate the digestion biology of each predator species to be biologically meaningful. We can design DNA primers that best fit a species' digestion biology to more precisely estimate the time since last feeding. In contrasts psylla proteins (as antigens for the antibodies) are likely to be digested at roughly the same rate, independent of protein type.

However, our studies with the minute pirate bug, *Orius tristis* (Miridae) shows that we cannot always adjust PCR amplicon sizes to the digestion biology of the predator. In pilot studies with *Orius* we found that DNA of the prey is apparently completely digested prior to ingestion. That is, *Orius* injects fluids into the prey liquefying it and afterwards sucks in the contents. In our assays, when *Orius* leaves the prey (removes its stylets for 1 minute or more) and is tested immediately, without internal digestion, there is no prey signal in its gut (n=14). In contrast, prey protein can still be detected using our MAB.

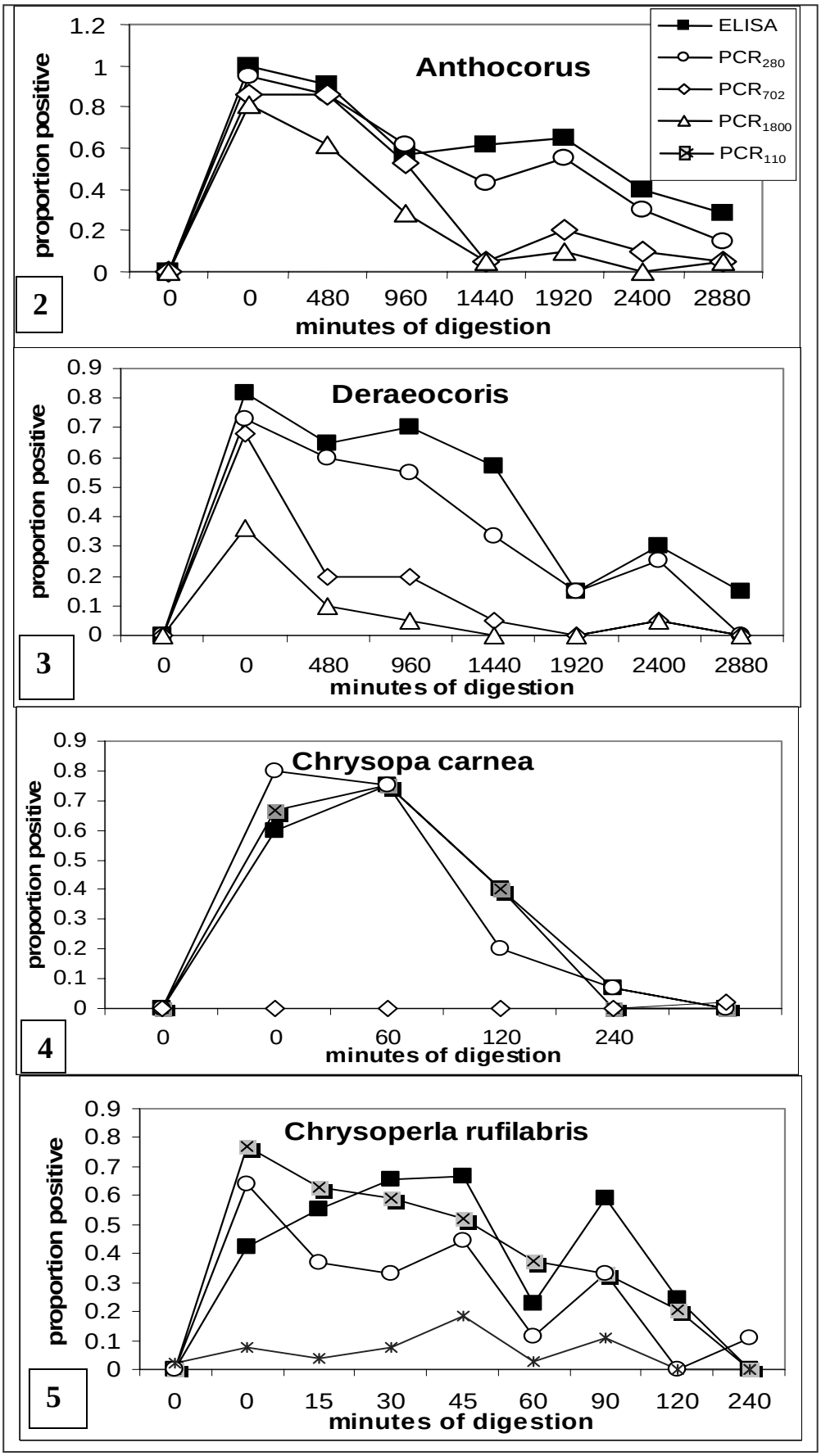
Captions for Figures 2-5. Digestion of psylla protein as measured by ELISA using a psylla MAB and digestion of psylla DNA as measured by PCR of several different sized amplicons

2: For *Anthocoris tomentosus*, ELISA and PCR280 amplicons showed similar digestion profiles that were quite long (Half life of 24 hours) and larger amplicons digested more rapidly.

3. For *Deraeocoris brevis,* patterns are similar to *Anthocoris* with the exceptions that larger amplicons are digested even more rapidly and have lower baselines at zero digestion time.

4. For *Chrysopa carnea* digestion occurred very rapidly, within 4 hours for both protein and DNA (ELISA and PCR methods). Remarkable is that both PCR 110 and 280 amplicons mirror the ELISA digestion curve but larger amplicons (700 and 1800 show no amplification at all)

5. For Chrysoperla rufilabris digestion was even more rapid than in the other lacewing, occurring within 2 hours. There appeared to be high and inexplicable variability in the ELISA pattern with digestion rate estimates sometimes being negative through time. More expected is the digestion curves for the 2 PCR amplicons, 110 and 280. Here the more rapid digestion of the 280 amplicon compared to 110 is what is predicted on d is most consistent with the bug predators.


Anthocorus
proportion positive
minutes of digestion
ELISA
PCR280
PCR702
PCR1800
PCR110
2
Deraeocoris
3
Chrysopa carnea
4
Chrysoperla rufilabris
5