PROGRESS TOWARD DEVELOPING A NOVEL IMMUNOLOGICAL APPROACH FOR QUANTIFYING PREDATION RATES ON THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
We just completed the first year of a multi-year research project dedicated to quantifying predation rates on GWSS nymphs and adults and qualifying predation on eggs. There are enough protein/antibody complexes commercially available that each GWSS in a field cage can be marked with a specific protein. We marked two GWSS adults and two GWSS nymphs, each separately with a unique protein and released them into small field cages (N=60) placed in a citrus orchard for 8 hours. Each cage also contained a sentinel GWSS egg mass and an assemblage of six potential GWSS predators. The experiment contained a day and night treatment. Observed mortality for each GWSS life stage and predator species was determined by simply counting the number of survivors remaining in each cage after 8 hours. Results showed that GWSS adults were preyed upon three times more frequently than nymphs and mostly during the day light cycle. Ultimately, the gut contents of each predator will be analyzed by four protein-specific ELISAs to determine how many GWSS each individual predator consumed (note: we are currently conducting these assays). Additionally, the gut contents of each predator will be examined by a GWSS egg-specific sandwich ELISA to determine the frequency of predation on GWSS eggs.

INTRODUCTION
Very little information exists on predaceous natural enemies of GWSS because identifying the feeding choices and amount of prey consumed by generalist predators is very difficult. Predators and GWSS are small, elusive, cryptic, and the predators may feed exclusively at night (Pfannenstiel & Yeargan, 2002). Moreover, predators do not leave evidence of attack. Perhaps the most frequently used experimental approach for evaluating natural enemies in the field are through studies conducted in field cages (Luck *et al.*, 1988). Such studies require manipulation of either the natural enemy or the targeted prey population(s) within the cage (e.g., the removal or introduction of the organism of interest). Mortality of the pest can be estimated based on the presence or absence of the pest (Luck *et al.*, 1988). Such studies have documented the qualitative impact of manipulated predator assemblages on many types of pests, but they do not provide quantitative information on predation rates or evidence of which predator in the assemblage is exerting the greatest biological control. Often the only direct evidence of arthropod predation can be found in the stomach contents of predators. Currently, the state-of-the-art predator stomach content assays include enzyme-linked immunosorbent assays (ELISA) for the detection of pest-specific proteins (Hagler, 1998) and PCR assays for the detection of pest-specific DNA (Symondson, 2002).

ELISAs have been widely used to identify key predators of certain pests, including GWSS (Hagler *et al.*, 1992; Hagler & Naranjo, 1994; Fournier *et al.*, submitted). The simplicity and low cost of conducting an ELISA lends itself to the efficient screening of hundreds of field-collected predators per day. However, polyclonal antibody-based ELISAs often lack species specificity and monoclonal antibody (MAb)-based ELISAs are too technically difficult, costly, and time consuming to develop for wide scale appeal (Greenstone, 1996). Moreover, pest-specific ELISAs share the same limitation as the other predator evaluation methods; the quantification of predation rates is impossible (see Hagler & Naranjo, 1996 for a review). PCR assays using pest-specific DNA probes might be less expensive to develop (Greenstone & Shufran, 2003), but PCR assays are also not quantifiable and they are more costly, technical, tedious, and time consuming to conduct than ELISAs (de Leon et al., In Press).

The many shortcomings of each method of predator assessment described above were the impetus for us to develop a technique to quantify predator activity. The technique combines our previous research using pest-specific MAb-based ELISAs to detect predation (Hagler *et al.*, 2003) with protein marking ELISAs we developed to study arthropod dispersal (Hagler & Jackson, 1998). Previously, we described a technique for marking individual GWSS, each with a unique protein (Hagler et al., 2004). In turn, the gut contents of predators were examined by a multitude of protein-specific ELISAs to

determine how many GWSS were consumed and which predator species consumed them. The advantages of immunomarking prey over prey-specific ELISAs are: (1) prey-specific antibodies (or PCR probes) do not need to be developed, (2) the protein-specific sandwich ELISAs are more sensitive than the indirect prey-specific ELISAs, (3) a wide variety of highly specific protein/antibody complexes are available, (4) the specificity of each antibody to its target protein facilitates the labeling of many individual pests and examination of the gut contents of every predator in the assemblage by a myriad of protein-specific ELISAs, and (5) all of the proteins and their complimentary antibodies are commercially available at an affordable price.

OBJECTIVES

1. Quantify predation on GWSS nymphs and adults.
2. Qualify predation on GWSS eggs.
3. Determine the circadian feeding activity of the predators.

Results obtained from this research will enhance our basic understanding of predator-prey interactions and aid in evaluating the efficacy of generalist predators for a conservation or an inundative biological control program.

RESULTS

We improved the detection capability of a MAb-based ELISA developed to detect GWSS egg protein in the guts of predators (Hagler et al., 2003; Fournier et al., submitted). Preliminary feeding studies revealed that the conventional indirect ELISA was not very effective at detecting GWSS egg remains in predator guts. To this end, we developed a more sensitive sandwich ELISA (e.g., we conjugated our GWSS-specific MAb to horseradish peroxidase). A comparative study of the efficacy of both ELISA formats was conducted on the green lacewing, *Chrysoperla carnea.* Feeding trials were conducted to determine how long GWSS egg antigen can be detected by ELISA in a predator's gut after consumption, and if consumption of alternative prey items affects the sensitivity of the ELISA for detecting GWSS. The predator tested was third-instar *C. carnea*. *Chrysoperla carnea* was selected for this study because it: (1) is commonly found in California, (2) is a voracious predator, and (3) has been directly (e.g., direct focal observation) (Kent Daane, pers. obs.) and indirectly (e.g., by gut content ELISA) observed feeding on GWSS eggs in the wild (Fournier et al., submitted).

The sandwich ELISA was much better at detecting GWSS egg remains in lacewing guts, particularly for those individuals that were provided with supplemental prey after consuming GWSS eggs (Figure 1). Specifically, the sandwich ELISA format consistently yielded higher ELISA reactions and a higher percentage of positive responses for GWSS remains in lacewing guts. Moreover, the sandwich ELISA had a much longer prey detection interval than the indirect ELISA (Figure 1). We are now confident that we can readily detect GWSS egg remains in field collected predators for at least 8 hours (e.g., the length of time that the predators were in the field cages) after feeding.

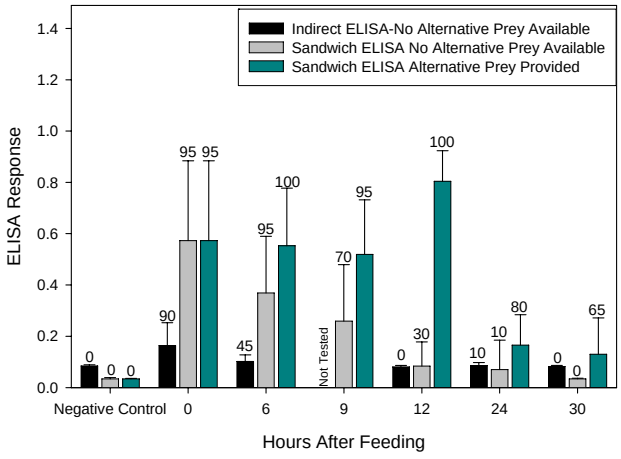

Figure 1. ELISA results testing for the presence of GWSS egg antigen in the gut of *C. carnea* using an indirect and sandwich ELISA format. Following the consumption of 3 GWSS eggs, *C. carnea* were held for 0 to 30 hours in Petri dishes that did not contain additional prey (indirect and sandwich ELISA format) or in Petri dishes that contained an unlimited supply of pink bollworm eggs (sandwich ELISA format only). The numbers above the error bars are the percentage of individual positive GWSS egg remains.

During the summer of 2005, multi-faceted field cage studies were conducted to quantify predation on GWSS nymphs and adults and qualify predation on GWSS eggs. Additionally, the degree of interguild (e.g., predation on GWSS) and intraguild predation (e.g., predation on the predators inhabiting the assemblage) occurring in the assemblage was assessed during day and night light cycles by simply conducting a visual count of the number of dead insects in each cage after 8 h. The field cages contained an arthropod assemblage consisting of six species of predaceous insects (Figure 2) and the various life stages of GWSS. The GWSS introduced into each cage included a sentinel egg mass; two 2nd or 3rd instar nymphs marked with rabbit IgG or chicken egg white, respectively; and two adults marked with soy milk or nonfat dry milk, respectively. The visual insect counts revealed that approximately 28% and 9% of the GWSS adults and nymphs were preyed upon, respectively. Moreover, 2.5% (big-eyed bug, *Geocoris punctipes)* to 17.5% (lady beetle, *Hippodamia convergens*) of the generalist predators introduced into the cages were also preyed upon (Figure 2). With the exception of *H. convergens*,

predation was more common during the day light cycle than the night light cycle. In the near future (this fall), we will determine which predators in the assemblage fed on the various life stages of GWSS. Specifically, we will conduct five highly specific post mortem gut content ELISAs on each individual predator. Egg predation events will be detected using an established GWSS egg-specific ELISA (Hagler et al., 2003; Fournier et al., submitted) nymph predation events will be detected using rabbit IgG and chicken IgG specific ELISAs (Hagler, 1997), and adult predation events will be detected using soy and nonfat dry milk specific ELISAs (Jones et al., submitted).

CONCLUSIONS

Although it is widely accepted that predators play a role in pest regulation, we still have an inadequate understanding of, and ability to predict their impact in cropping systems. Frequently parasitoids are given major credit for suppressing pest populations; however, the impact that predators have on suppressing GWSS populations goes unrealized due to the difficulties of assessing arthropod predation as discussed above. The prey marking technique described here circumvents many of the shortcomings of the current methods used to study predation. Over the next two years we will analyze the gut contents of thousands of predators using five separate ELISAs to qualify and quantify predation events on GWSS eggs, nymphs and adults. Ultimately, this information can be used to improve the efficacy of conservation and inundative biological control of GWSS.

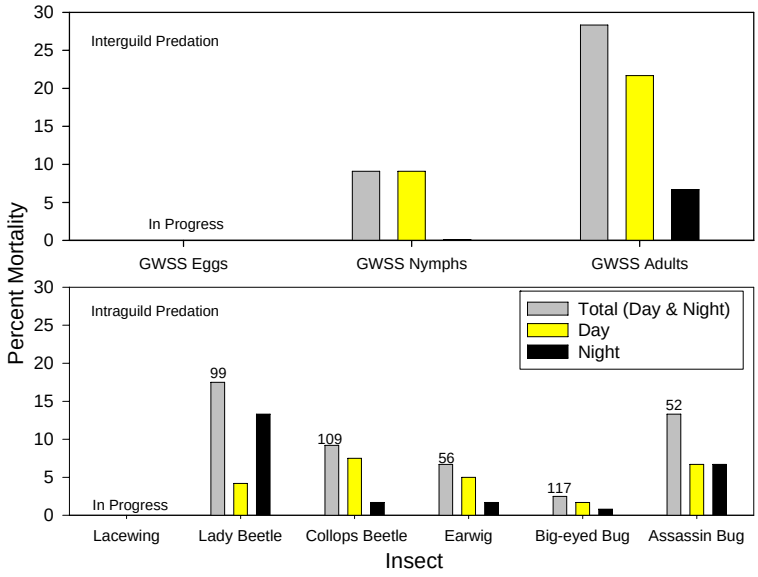

Figure 2. Percent mortality of GWSS nymphs and adults inhabiting the field cages (e.g., interguild predation) (**Top graph**). Percent mortality of the predator assemblage inhabiting the field cages (e.g., intraguild predation) (**Bottom graph**). Note: the percent mortality for the GWSS egg stage and lacewing larvae could not be determined visually directly in the field due to their cryptic nature. We are currently assessing their mortalities as we go through the cage samples in the laboratory.

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