AN IMMUNOLOGICAL APPROACH FOR QUANTIFYING PREDATION RATES ON THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

The gut contents of 376 individual predators were assayed for glassy-winged sharpshooter (GWSS) remains using a multitude of ELISAs designed to detect predation on various GWSS life stages. We found that almost 10% of the predators examined contained GWSS remains in their guts. We recorded 10, 17, and 20 predation events on the GWSS egg, nymph, and adult stages, respectively. Of the predators examined in this study, *Collops vittatus* (20.6%) and *Hippodamia convergens* (16.7%) had the highest percentage of individuals positive for GWSS remains. Approximately 10% of five of the other predator species and none of the earwigs tested contained GWSS remains.

INTRODUCTION

Predaceous natural enemies can be important regulators of arthropod populations (Luff, 1983). However, accurately identifying key predators of most pests is very difficult because predators and their prey can often be small, elusive, and cryptic. Hence, visual field observations of predation are extraordinarily difficult to obtain. Perhaps the most frequently used experimental approach for evaluating predaceous 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. Mortality of the pest can be estimated based on the presence or absence of the pest over time (Smith & De Bach, 1942; 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 immunoassays (typically ELISA) for the detection of pest-specific proteins (Hagler & Naranjo, 1996) and PCR assays for the detection of pest-specific DNA (de León *et al.*, 2006).

ELISAs using pest-specific monoclonal antibodies (MAbs) have been widely used to identify key predators of certain pests, including the glassy-winged sharpshooter (GWSS) (Fournier *et al.*, 2006). The simplicity and low cost of ELISA lends itself to the efficient screening of hundreds of field-collected predators per day (Hagler & Naranjo, 2005). However, MAb development is technically difficult, costly, and time consuming 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 than ELISAs (pers. obs.). These difficulties have resulted in a dearth of information on the quantitative impact that generalist predators have on suppressing pest populations.

The many shortcomings of each method of predator assessment described above were the impetus for us to develop a technique to more efficiently quantify predator activity. The technique combines our previous research using pest-specific MAb-based ELISAs to detect predation (Fournier *et al*., 2006) with protein marking ELISAs developed to study arthropod dispersal (Hagler *et al.*, 2002; Jones *et al*., 2006). In this study we erected 40, 1-m long field cages on selected citrus branches. We then placed (using a paper clip) a sentinel GWSS egg mass (ca. 6 to 12 eggs per mass) on the underside of a randomly selected leaf in each cage along with two individuals each of *Hippodamia convergens*, *Collops vittatus*, *Chrysoperla carnea*, *Labidura riparia*, *Geocoris punctipes*; and one individual each of *Sinea confusa* and *Zelus renardii*. One hour later, we released two GWSS adults and two nymphs into each cage. The four mobile GWSSs were each marked with a different protein before they were introduced into each cage. After 6 hours, each citrus branch was cut at its base, just below each cage, and immediately frozen on dry ice. Each predator was then analyzed by four protein-specific ELISAs to determine if they contained marked GWSSs in their guts. Additionally, the gut contents of each predator was examined by a GWSS egg-specific sandwich ELISA (Fournier *et al*., 2006) to determine the frequency of predation on GWSS eggs.

OBJECTIVES

We are in the final phase of a multi-year research project dedicated to:

1. Quantifying predation rates on GWSS nymphs and adults
2. Qualifying predation on GWSS eggs. Using a novel multiple prey marking technique (Hagler, 2006) and a GWSS egg-specific MAb (Fournier *et al*., 2006) we simultaneously examined the gut contents of a total of 376 predators from seven predator species for the presence of five GWSS prey items (e.g., GWSS egg protein, 2 protein marked nymphs and 2 protein marked adults).

RESULTS

The recovery rate of the predators ranged from 55% for earwigs and lacewings to 97.5% for the assassin bugs and lady bugs (Table 1). The low recovery rate of earwigs and lacewings is likely due to their ability to escape from the cages (e.g., earwigs), they were the victims of interguild predation (e.g., lacewings) (Hagler, 2006), or they were overlooked in the sorting process (e.g., lacewings).

Of the 376 predators examined by ELISA, a total of 37 predatory events (9.8% of all the predators examined) were recorded in this study. Of these, 2.7% (n=10) were the result of predation on the GWSS egg stage. Unfortunately, due to the limitations of using a pest-specific ELISA (as mentioned above), we cannot determine if the positive reactions were due to predation on one or more eggs. The number of predation events recorded for the GWSS nymph and adult stages were 17 (4.5% of the population) and 20 (5.3% of the population), respectively. Since these predation events were each detected with a specific protein ELISA, we are confident that these results represent the first quantified results of predation using molecular gut content methods (e.g., immunological or DNA based). Interestingly, of the 376 predators assayed, only three predators yielded more that one positive ELISA reaction. Specifically, one lady beetle yielded a positive reaction for at least one GWSS egg and an adult, one assassin bug (*S. confusa*) yielded a positive reaction for both of the marked GWSS nymphs released into its cage, and one flower beetle yielded a positive reaction for both marked GWSS adults released into its cage.

Table 1. Predator gut content ELISA results yielded by the 376 individuals assayed for the presence of GWSS eggs, nymphs, and adults.

Species (common name)	Total Number Released	Total Number Recovered (%) After 6 h	# Positive (%) for GWSS Egg Stage	# Positive (%) for GWSS Nymph Stage	# Positive (%) for GWSS Adult Stage	Total (%) by Species
Hippodamia convergens (lady beetle)	80	78 (97.5%)	4 (5.1%)	4 (5.1%)	5 (6.4%)	**13 (16.7%)**
Collops vittatus (flower beetle)	80	68 (85.0%)	2 (2.9%)	7 (10.3%)	5 (7.4%)	**14 (20.6%)**
Chrysoperla carnea (lacewing)	80	44 (55.0%)	1 (2.3%)	0 (NA)	2 (4.5%)	**3 (6.8%)**
Labidura riparia (earwig)	80	44 (55.0%)	0 (NA)	0 (NA)	0 (NA)	**0 (NA)**
Geocoris punctipes (big-eyed bug)	80	64 (80.0%)	3 (4.7%)	3 (4.7%)	2 (3.1%)	**8 (12.5%)**
Sinea confusa (assassin bug)	40	39 (97.5%)	0 (NA)	2 (5.1%)	3 (7.7%)	**5 (12.8%)**
Zelus renardii (assassin bug)	40	39 (97.5%)	0 (NA)	1 (2.6%)	3 (7.7%)	**4 (10.3%)**
Total (%) by GWSS Life Stage	**480**	**376 (78.3%)**	**10 (2.7%)**	**17 (4.5%)**	**20 (5.3%)**	**37 (9.8%)**

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. The impact that predators have on suppressing GWSS populations goes unrealized due to the difficulties of assessing arthropod predation. The prey marking technique (Hagler, 2006) combined with a GWSS egg-specific gut content ELISA (Fournier *et al*., 2006) circumvented many of the shortcomings of the current methods used to study predation. Here, we quantified predation on GWSS nymphs and adults and qualified predation on GWSS eggs. This information and the data presented by Fournier *et al*. (in this volume) will be used to develop a comprehensive biological control program that better conserves the populations of those predators exerting the greatest control on the various GWSS life stages.

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ARE GLASSY-WINGED SHARPSHOOTER POPULATIONS REGULATED IN CALIFORNIA? LONG-TERM PHENOLOGICAL STUDIES & CONSTRUCTION OF MULTI-COHORT LIFE TABLES FOR THE GLASSY-WINGED SHARPSHOOTER IN CITRUS ORCHARDS

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ABSTRACT
Glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*; formerly *H. coagulata*) population densities have been steadily declining over a 4.5 year period in organic lemons grown in an experimental study plot at University of California, Riverside Ag. Ops. Peak adult GWSS populations in August 2006 were just 32% of those observed around August 2002. It is uncertain if egg parasitism, which has consistently averaged ~ 20% per year of GWSS egg masses, is responsible for the observed decline. Density dependent analyses of time series data are planned once data sets are large enough. Phenological observations are being complimented with recently initiated multi-cohort stage frequency life tables to provide greater insight into GWSS population dynamics and identification of life stages most susceptible to mortality factors.

INTRODUCTION
In California, there is a guild of natural enemies attacking the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*; formerly *H. coagulata*). The dominant parasitoid attacking GWSS in California is *Gonatocerus ashmeadi* followed by *G. morrilli*. *G. triguttatus* from Texas and *G. fasciatus* from Louisiana have been released in California, but widespread establishment is uncertain. Completion of recent studies investigating the effect of varying temperatures on development, survivorship, and reproductive output coupled with GIS modeling, strongly suggests that climate is a major limiting factor for *G. triguttaus* in California, while *G. ashmeadi* is more robust to California's varying climatic conditions (see article in this Proceedings). Other minor parasitoid species include *G. novofasciatus*, *Ufens* sp., and *Zagella* sp. Together, this guild of parasitoids provides an average of ~15% parasitism of GWSS eggs laid during the spring generation and ~20-25% of GWSS eggs laid during the summer generation in commercial citrus orchards. The average year round parasitism level of ~20% falls short of the 33-36% level determined necessary for successful classical biological control (Hawkins and Cornell, 1994). However, data collected from bi-weekly monitoring over the last five years from an organic commercially-managed citrus orchard in Riverside indicates that GWSS populations are declining steadily each year (Figure 1). It is uncertain what the significance is of parasitism of GWSS eggs by mymarid parasitoids to this downward population trend (Figure 2). There are at least four possible reasons for low seasonal parasitism levels in California: (1) competitive exclusion amongst members of the GWSS parasitoid guild is reducing effective biological control. (2) An extremely aggressive and efficacious natural enemy that can dominate the system to the almost total exclusion of all current parasitoids has not been established in California and is needed for successful biological control of GWSS. (3) The absence of resource subsidies such as nectar provided by flowering plants in agroecosystems may limit parasitoid efficacy because longevity and fecundity is significantly reduced when parasitoids can not access carbohydrates. Understorey management may be an important cultural strategy to benefit parasitoids if it can be demonstrated not to enhance GWSS and *Xylella* populations. (4) Climate, in particular, prolonged cool periods over winter when GWSS eggs are unavailable probably has a severe affect on parasitoid reproductive success. There are two general approaches to investigating population phenomena in the field: (1) long-term phenology studies which can be used to tease out density-dependent and density-independent factors affecting population dynamics, and (2) life tables that dissect populations by life stage to determine the magnitude of change between developmental stages, and if possible elucidation of factors impacting survivorship within life stages. Both approaches need to be conducted concurrently in the same field plots using standardized methods to better understand mechanisms underlying long-term population fluctuations for GWSS in California.

OBJECTIVES
1. Construct multi-cohort life tables for glassy-winged sharpshooter nymphs and adults in citrus orchards.
2. Continue the 3 years of bi-weekly surveys of GWSS eggs, nymphs, and adults, and associated rearing of parasitoids from harvested egg masses in citrus at Ag Ops, UC Riverside.

RESULTS
The population monitoring study and measures of percentage parasitism clearly indicate that GWSS densities have continued to decline steadily at the long-term monitoring plot (Figure 1) and percentage parasitism have remained relatively constant over this time period (Figure 2). Detection of density-dependent mortality from sequential census data such as that presented here is notoriously difficult and the results of analytical models differ in outcomes depending on assumptions made even when dummy data sets have been constructed to show density dependent mortality. One of the major problems with these