PROGRESS TOWARD QUANTIFYING LANDSCAPE-SCALE MOVEMENT PATTERNS OF THE GLASSY-WINGED SHARPSHOOTER AND ITS NATURAL ENEMIES USING A NOVEL MARK-CAPTURE TECHNIQUE

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ABSTRACT
Here we present the results of the first year of our research targeted at quantifying the landscape-level movement patterns of GWSS and its natural enemies. We showed that protein markers can be rapidly acquired and retained on insects for several weeks after marking directly in the field. Specifically, we sprayed a large citrus plot and a large olive tree plot with different inexpensive proteins using conventional air blast sprayer. In turn, insects that were hit by the protein solutions or that were exposed to marked plant tissue obtained enough protein to be detected by a protein-specific ELISA. Because the various protein specific ELISAs do not cross-react, we can apply the various proteins to different host plants in close proximity to one another. This marking technique provides the necessary tool to distinguish GWSS and its natural enemies so that studies of dispersal, migration, longevity, and density can be conducted. Additionally, different protein markers can be used to identify insect movement from different areas within a crop or from different crops.

INTRODUCTION
Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) feed on a variety of plants, and in the process transmit the bacterium, *Xylella fastidiosa*, which is the causal agent of Pierce's disease (PD) (Varela, 2001). Due to the polyphagous feeding habit and high dispersal capability of GWSS, control of this pest will require an areawide management approach. Such an approach requires extensive knowledge of the host plant preferences and dispersal characteristics of GWSS and its natural enemies. Unfortunately, very little is known about the dispersal characteristics of GWSS (Blua & Morgan, 2003; Blackmer *et al*,. 2004) and its associated natural enemy complex. This is due, in part, to the lack of an effective technique for studying insect dispersal at the landscape level.

The development of a protein marking technique (Hagler, 1997ab; Blackmer *et al*., 2004) solved many of the problems associated with other marking techniques for marking insects. The procedure is simple, sensitive, safe, rapid, inexpensive (for MRR type studies), invisible, and stable (Hagler & Jackson, 2001). Moreover, several distinct proteins are available which facilitate the simultaneous marking of different cohorts of individuals (Hagler 1997a; Hagler & Naranjo, 2004). Recently, we identified several inexpensive proteins that can be used to mark insects for mark-capture type studies. The proteins are casein (from non-fat dry milk), chicken egg whites (Egg Beaters™ or All Whites™), and soy milk (Silk™ Soymilk). In collaboration with Vincent Jones we have developed anti-casein, anti-egg white, and anti-soy enzyme-linked immunosorbent assays (ELISA) to each of these proteins. In turn, these ELISAs can be used to detect the presence of each protein on protein-marked insects. In this paper, we report on the efficacy of this marking procedure for marking GWSS and its natural enemies directly in the field for mark-capture type studies.

OBJECTIVES
1. Quantify GWSS and natural enemy dispersal patterns in a complex landscape.
2. Determine which factors influence their dispersal.

To accomplish these objectives we must first develop a reliable mark-capture protein marking technique and quantify the protein marking retention intervals for the targeted insects. Field application of better mark-capture techniques will enhance our understanding of the area-wide dispersal patterns of GWSS and its natural enemies. The first phase (year 1 of 2) of our research consisted of optimizing a mark-capture procedure for GWSS and its natural enemies that will facilitate future studies (years 2 and 3) of intracrop and intercrop dispersal. Here we described three experiments that were conducted to validate the efficacy of the protein marking procedure on GWSS and one of its potential natural enemies, *Hippodamia convergens*.

RESULTS

Experiment 1

The first experiment was conducted to determine the retention time of two different proteins, non-fat dry milk (NFDM) and chicken egg whites (CEW) on GWSS and *Hippodamia convergens* under field conditions. Here we tested the efficacy of two marking procedures. The first procedure was a residual contact marking method. Randomly selected citrus branches were sprayed with a 5.0% solution of NFDM or CEW (All Whites™). The branches were allowed to dry for 2 h, and then 15 nylon-meshed sleeve cages (66 X 70-cm, 19-cm dia.) were placed on the branches. Adult GWSS (≈ 20 per cage) and *H. convergens* (≈ 30 per cage) were then introduced into each cage. A single cage was randomly selected on 12 different sampling dates for up to 35 days after marking. All surviving GWSS and *H. convergens* in the randomly selected cages were assayed by an anti-NFDM or an anti-CEW ELISA to detect the presence of each respective protein marker. The second procedure was a direct contact marking method. Fifteen nylon-meshed sleeve cages were placed on randomly selected citrus branches. Adult GWSS (≈ 20 per cage) and *H. convergens* (≈ 30 per cage) were then introduced into each cage and sprayed with a 5.0% solution of NFDM or CEW. The sampling scheme and assays were as described above.

The ELISA results for the protein marked GWSS are given in Figure 1. Data indicate that both marking procedures, regardless of the type of protein marker used, were retained well on GWSS. As expected, the topical marking procedure yielded higher ELISA values and had longer retention times than the residual contact marking method. The markers were retained on 100% of the GWSS for ≈ 2 and 3 weeks by the residual and topical marking procedures, respectively. *H. convergens* ELISA reactions were very similar to the reactions yielded by GWSS (data not shown).

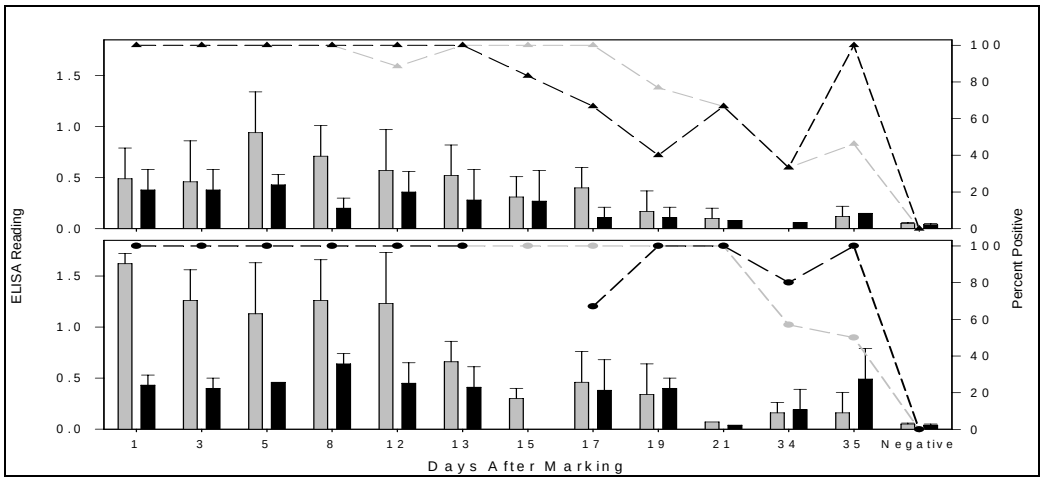

Figure 1. The mean ± SD ELISA values (vertical bars read from the left y-axis) and percentage of GWSS (line plot read from the right y-axis) scoring positive for the presence of CEW (gray bars) or NFDM (black bars). The top graph represents the insects marked by contact exposure and the bottom graph represents the insects marked by topical spray. GWSS were scored positive for the presence of each marker if the ELISA value exceeded the mean negative control value by 3 standard deviations (note: the day 15 NFDM topical spray samples were lost).

Experiment 2

The second study was conducted to determine the efficacy of the marking procedure under realistic open field conditions. The field site was a commercial farm located near Porterville, CA. The field was ≈20 acres, split equally into ≈10 acres of 8-year-old olive trees and 16-year-old navel orange trees. An 8-m wide fallow border divided the two crops. Eight nylon-meshed sleeve cages were placed uniformly in the field. Three sleeve cages were placed in each of the crops and two cages were attached to six ft poles and placed in the fallow border region. Adult *H. convergens* (note: GWSS were not used in this experiment due to very low populations at the study site) were then introduced into each cage (n=30/cage) the day before the fields were sprayed with their designated protein solution (see below). On Sept. 9, 2004 ≈3 acres of the olive field were sprayed with a 5.0% solution of NFDM @ 100 gal/acre and ≈3 acres of the orange grove were sprayed with a 5.0% solution of CEW @ 250 gal/ac using a 500 gal conventional air blast sprayer. Individual beetles were removed from each sentinel cage on Sept. 10 (n=10), Sept. 17 (n=10), and Sept. 24 (n=the surviving beetle population) and assayed by an anti-NFDM and an anti-CEW ELISA to detect for the presence of each respective protein mark.

The ELISA results for *H. convergens* marked directly in the field using a commercial spray rig are given in Figure 2. Markers were retained well on the beetles, regardless of the marker used or the crop that the marker was applied to for two weeks after application. In a few instances, we obtained false positive ELISA reactions (e.g., beetles collected from the unsprayed fallow field or from the crop where the specific marker was not applied). In almost all instances, the false positive

reactions were barely above the threshold value used (mean + 3SD of negative control beetles) to score a positive reaction. The occasional false positive ELISA reactions were probably due to spray drift of the markers or human error which can occur while conducting an ELISA.

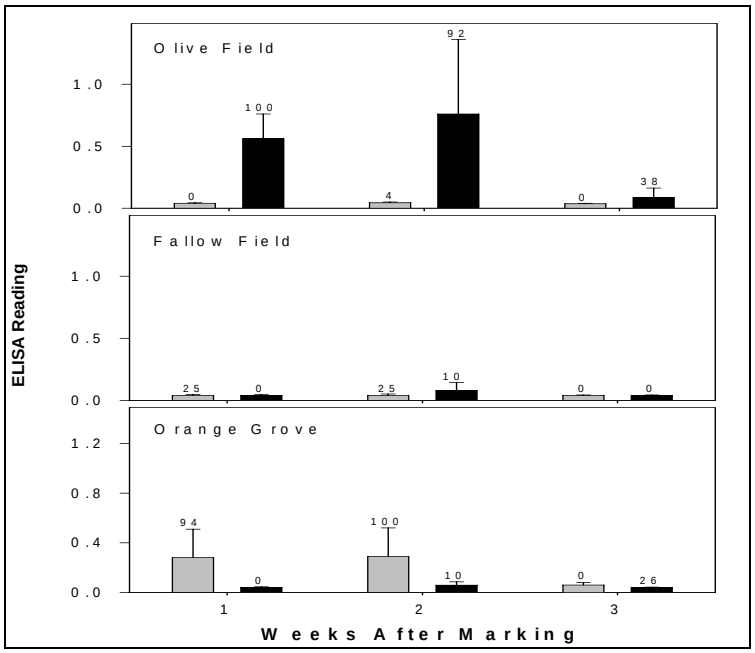

Figure 2. The mean (±SD) ELISA readings of protein-marked *H. convergens* held in sentinel cages. The grey bars are the CEW ELISA reactions and the black bars are the NFDM ELISA reactions. The numbers above each error bar are the percentage of positive ELISA responses for each treatment.

Experiment 3

The third study was a laboratory study conducted to determine how long it takes for an insect to become marked after residual contact exposure to marked plant tissue. The insect used in this study was adult *H. convergens*. Individual greenhouse grown cotton plants, ≈80-cm tall (≈ 20 leaves per plant), were sprayed with 35 ml of a 10% CEW solution using a standard hand sprayer. The cotton plants were allowed to dry for 1 h at 45°C. After drying, randomly selected leaves were pulled from the plant and cut to fit inside a 3.5-cm Petri dish (insect arena). An individual beetle was placed in an arena for 5, 10, 20, 40, 60, 120, 240, or 480 min. After each holding interval, the beetles were assayed by an anti-CEW ELISA to detect the presence of the marker.

The ELISA results for the protein marked *H. convergens* are given in Figure 3. Data indicate that the majority of beetles acquired the mark by residual contact within 5 minutes after exposure.

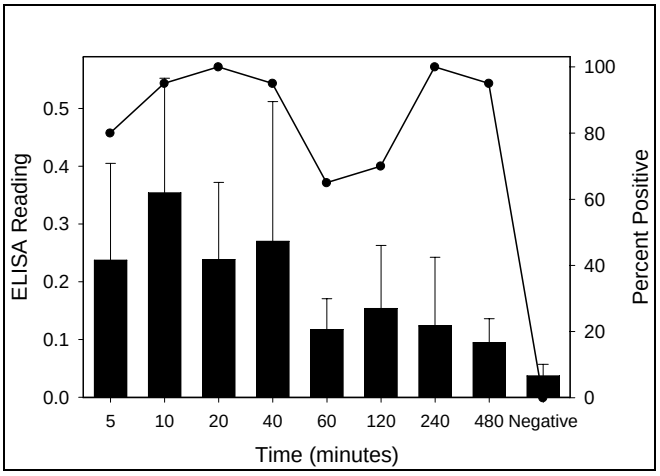

Figure 3. The mean ± SD ELISA values (vertical bars read from the left y-axis) and percentage of *H. convergens* (line plot read from the right y-axis) scoring positive for the presence of chicken egg whites (n = 20 per time interval).

CONCLUSIONS

In the first phase of our research described here, we showed that protein marks can be rapidly acquired and retained on insects several weeks after marking in the field. This marking technique provides the necessary tool to distinguish GWSS and its natural enemies so that studies of dispersal, migration, longevity, and density can be conducted. Additionally, different protein markers can be used to identify insects released at different times, in different areas, or in different crops. Currently (e.g., summer/fall of 2005), we are using this technique for several different projects to investigate the landscape-level movement of GWSS (nymphs and adults) and its natural enemies.

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