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

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ABSTRACT
Field cage studies were conducted to compare retention times between two inexpensive proteins, non fat dry milk (NFDM) and chicken egg whites, on glassy-wing sharpshooter (GWSS), *Homalodisca coagulata* and *Hippodamia convergens*. Each marker was applied to the insects by either directly spraying the insects with a conventional spraying device or by exposing the insects to pre-marked leaf tissue. Subsequently, the recaptured insects were analyzed by either an anti-NFDM or an anti-egg white enzyme-linked immunosorbent assay (ELISA) to detect the presence of each respective marker. Data indicate that both protein markers were retained well on both insect species, regardless of the application method. Generally, the topical marking procedure yielded higher ELISA values than the insects marked by contact exposure; however, both methods were sufficient for marking almost 100% of each population for > 2 weeks.

INTRODUCTION
Glassy-wing sharpshooter (GWSS), *Homalodisca coagulata* (Say) feeds on a variety of plants, and in the process transmits the bacterium, *Xylella fastidiosa*, which is the causal agent of Pierce's disease (PD) (Varela 2001). The spread of PD by GWSS now threatens the grape and ornamental industries of California. 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 first phase of our research plan consists of optimizing a mark-capture procedure for GWSS and its natural enemies that will facilitate future studies of intercrop dispersal. Historically, most studies of insect dispersal have relied on the mark-release-recapture (MRR) technique (Hagler & Jackson, 2001). Typically, mass-reared insects or insects collected *en masse* from the field are marked in the confines of the laboratory and then released at a specific site(s) in the field (i.e., at a central point). The insects are then recaptured using various spatial and temporal sampling schemes to quantify their movement. Unfortunately MRR studies use a relatively small portion of the population and recapture even a smaller proportion of the population (i.e., usually < 1.0%), thus making extrapolations about dispersal to the population level less reliable. The information gained from dispersal experiments could be significantly improved if a large proportion of the insect fauna (e.g., the simultaneous marking of GWSS and its natural enemies) could be marked directly in the field (e.g., mark-capture type experiments) and if several distinctive markers were available for studying intercrop movement of insects.

The development of a protein marking technique (Hagler 1997ab, Hagler & Jackson 1998, Blackmer et al., 2004) solved many of the problems associated with other marking techniques for MRR studies. The procedure is simple, sensitive, safe, rapid, inexpensive (for MRR type studies), invisible, and stable (Hagler & Jackson 1998). Moreover, several distinct proteins are available which facilitate the simultaneous marking of different cohorts of individuals (Hagler 1997a, Hagler & Naranjo 2004). We demonstrated that parasitoids (*Eretmocerus* spp. and *Encarsia formosa*) can be easily marked internally with vertebrate immunoglobulin (IgG) proteins by incorporating the various proteins into a honey diet or marked externally (*Trichogramma* sp.) with a fogging device (Hagler 1997b, Hagler *et al.* 2002). However, the major limitation of this technique is that the IgG proteins are too costly for mark-capture type studies. Recently, we discovered two inexpensive proteins that have potential as markers for mark-capture studies. The proteins are casein (from non-fat dry milk) and chicken egg whites (Egg Beaters™ or All Whites™). In collaboration with Vincent Jones we have developed anti-casein and anti-egg white 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 report, we investigated the feasibility of marking GWSS and *Hippodamia convergens* using two different application procedures. The first method for marking the insects consisted of spraying the markers on the insects in the field using a conventional hand sprayer (e.g., direct contact exposure). The second method for marking the insects consisted of exposing the insects to plant tissue that had previously been sprayed with each protein (e.g., residual contact exposure).

OBJECTIVES

The overall objectives of our research are to:

1. Quantify GWSS and natural enemy dispersal patterns in a complex landscape and
2. Determine which factors influence their dispersal. To accomplish these objectives we must first develop a 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.

RESULTS

Direct Contact Marking Method

Dozens of nylon-meshed sleeve cages (66 X 70-cm, 18-cm dia.) were placed on randomly selected citrus branches located at the Agricultural Operations Research Station in Riverside, CA. Adult GWSS and *H. convergens* were then introduced into each cage and sprayed with a 5.0% solution of non-fat dry milk (NFDM) or chicken egg whites (All Whites™). A single cage from each marking treatment was randomly selected on 12 different sampling dates for up to 35 days after marking. All of the surviving GWSS and *H. convergens* in the randomly selected cages were assayed by an anti-NFDM or an anti-egg white ELISA to detect the presence of each respective protein mark.

Residual Contact Marking Method

Randomly selected citrus branches located at the Agricultural Operations Research Station in Riverside, CA were sprayed with a 5.0% solution of NFDM or chicken egg whites. The branches were allowed to dry for several hours, and then nylon-meshed sleeve cages were placed on the branches. Adult GWSS and *H. convergens* were then introduced into each cage. The sampling scheme was the same as the one described above. All of the surviving GWSSs and *H. convergens* in the randomly selected cages were assayed by an anti-NFDM or an anti-egg white ELISA to detect for the presence of each respective protein marker.

The ELISA results for the protein marked GWSS are given in Table 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 than the residual contact marking method. Generally, the markers were retained on 100% of the GWSS for ≈ 2 and 3 weeks by the residual and topical marking procedures, respectively. The ELISA results for the protein-marked *H. convergens* are given in Table 2. *H. convergens* ELISA reactions were very similar to the reactions yielded by GWSS.

CONCLUSIONS

In the first phase of our research described here, we showed that protein markers can be 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. Next, we will use this technique to investigate the landscape-level movement of GWSS (nymphs and adults) and its natural enemies. We propose to use the mark-capture system to simultaneously quantify the intercrop dispersal of GWSS and its natural enemies. Specifically, we will spray large areas (e.g., field plots, whole trees, bushes, etc.) with inexpensive proteins using conventional spray equipment. In turn, insects that are hit by the protein solutions or that eat or walk on plant material containing protein residues will obtain enough protein to be detected by protein-specific ELISAs. Because the two marking ELISAs (chicken egg whites and NFDM) do not cross-react, we can apply the materials to two different host plants in close proximity to one another. Then, insects can be collected using temporal and spatial sampling schemes and analyzed for the presence of each respective protein marker to determine not only the insect's point of origin but the timing and extent to which portions of the population move among different plant species.

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Table 1. The mean (±SD) ELISA readings and the percentages of protein-marked GWSS scoring positive for the presence of chicken egg white or non fat dry milk for up to 35 days after marking. GWSS were scored positive for the presence of each marker if the ELISA value exceeded the mean negative control value by 3 standard deviations.

Application Method	Days After Marking	Egg White Marker			Non Fat Dry Milk Marker		
		Number Assayed	Mean ELISA Reading	Percent Positive	Number Assayed	Mean ELISA Reading	Percent Positive
Residual Contact	1	31	0.49 (0.3)	100.0	8	0.38 (0.2)	100.0
	3	7	0.46 (0.4)	100.0	10	0.38 (0.2)	100.0
	5	19	0.94 (0.4)	100.0	4	0.43 (0.1)	100.0
	8	15	0.71 (0.3)	100.0	5	0.20 (0.1)	100.0
	12	26	0.57 (0.4)	88.5	36	0.36 (0.2)	100.0
	13	7	0.52 (0.3)	100.0	5	0.28 (0.3)	100.0
	15	26	0.31 (0.2)	100.0	6	0.27 (0.3)	83.3
	17	13	0.40 (0.2)	100.0	15	0.11 (0.1)	66.7
	19	13	0.17 (0.2)	76.9	5	0.11 (0.1)	40.0
	21	3	0.10 (0.1)	66.7	6	0.08 (0)	66.7
	34	0	---	---	3	0.06 (0)	33.3
	35	13	0.12 (0.1)	46.2	1	0.15 (NA)	100.0
	Negative Controls	25	0.05 (0.01)	0	20	0.04 (0.01)	0
Topical Contact	1	22	1.62 (0.1)	100.0	16	0.43 (0.1)	100.0
	3	12	1.26 (0.6)	100.0	20	0.40 (0.1)	100.0
	5	8	1.13 (0.5)	100.0	1	0.46 (NA)	100.0
	8	13	1.26 (0.4)	100.0	2	0.64 (0.1)	100.0
	12	16	1.23 (0.5)	100.0	8	0.45 (0.2)	100.0
	13	3	0.66 (0.2)	100.0	3	0.41 (0.2)	100.0
	15	3	0.30 (0.1)	100.0	0	---	---
	17	22	0.46 (0.3)	100.0	6	0.38 (0.3)	66.7
	19	7	0.34 (0.3)	100.0	2	0.40 (0.1)	100.0
	21	1	0.07 (NA)	100.0	1	0.04 (NA)	0.0
	34	7	0.16 (0.1)	57.1	10	0.19 (0.2)	80.0
	35	4	0.16 (0.2)	50.0	1	0.49 (0.3)	100.0
	Negative Controls	20	0.05 (0.01)	0	20	0.04 (0.01)	0

Table 2. The mean (±SD) ELISA readings and the percentages of *Hippodamia convergens* scoring positive for the presence of chicken egg white or non fat dry milk for up to 35 days after marking. *H. convergens* were scored positive for the presence of each marker if the ELISA value exceeded the mean negative control value by 3 standard deviations.

Application Method	Days After Marking	Egg White Marker			Non Fat Dry Milk Marker[1]		
		Number Assayed	Mean ELISA Reading	Percent Positive	Mean ELISA Reading	Number Positive	Percent Positive
Residual Contact	1	19	0.83 (0.3)	100.0			
	3	19	0.63 (0.2)	100.0			
	5	18	0.29 (0.1)	100.0			
	8	15	0.31 (0.2)	100.0			
	12	12	0.37 (0.3)	75.0			
	13	12	0.49 (0.2)	100.0			
	15	15	0.25 (0.2)	86.7			
	17	5	0.37 (0.2)	100.0			
	19	0	---	---			
	21	3	0.23 (0.2)	66.7			
	34	0	---	---			
	35	18	0.23 (0.3)	94.4			
	Negative Controls	63	0.04 (0.01)	0			
Topical	1	15	1.25 (0.2)	100.0	0.33 (0.1)	17	100.0
	3	26	0.96 (0.3)	100.0	0.34 (0.2)	27	100.0
	5	26	0.62 (0.3)	100.0	0.21 (0.1)	12	100.0
	8	18	0.75 (0.3)	100.0	0.25 (0.3)	2	100.0
	12	33	0.55 (0.3)	100.0	0.17 (0.1)	48	100.0
	13	17	0.23 (0.2)	100.0	0.26 (0.2)	17	100.0
	15	4	0.21 (0.3)	75.0	0.21 (0.2)	8	100.0
	17	20	0.33 (0.2)	100.0	0.25 (0.2)	2	100.0
	19	23	0.24 (0.2)	100.0	0.05 (0.1)	1	33.3
	21	4	0.35 (0.1)	100.0	0.20 (0.2)	20	90.9
	34	23	0.25 (0.1)	100.0	0.11 (0.1)	7	58.3
	35	8	0.27 (0.2)	100.0	---	---	---
	Negative Controls	39	0. 04 (0.01)	0	30	0.04	0.01

[1/]The retention of nonfat milk by contact application was not investigated for *H. convergens*.

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