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A protein-based approach to mark arthropods for mark-capture type research

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Abstract

A series of studies was conducted to test methods for marking a wide variety of arthropods with inexpensive proteins for mark-capture dispersal research. The markers tested included egg albumin protein in chicken egg whites and casein protein in bovine milk. The first study qualified the effectiveness of the two marks on more than 50 arthropod species inhabiting cotton via two application procedures. The application methods included: (1) a topical plus residue protein application, and (2) a residue-only protein application. Both protein marks, regardless of the method of application, were readily retained on the arthropod assemblage over the duration of the study. The second study determined how rapidly insects acquire chicken egg albumin protein after contact exposure to cotton tissue sprayed with an egg whites solution. Under laboratory conditions, the vast majority of adult *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and *Lygus hesperus* Knight (Heteroptera: Miridae) acquired the mark after 5 min, and immature *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) acquired the marker after 40 min. The third study determined how rapidly *H. convergens* and *L. hesperus* acquire bovine casein protein after contact exposure to either alfalfa, *Medicago sativa* L. (Fabaceae), or lesquerella, *Lesquerella fendleri* (Watson) (Brassicaceae), plants sprayed with a bovine milk solution. These insects rapidly acquired the casein mark from the plant residue under field conditions. A final study determined how long *H. convergens* retain casein protein after 24-h exposure to alfalfa and lesquerella plants containing a 7-day-old residue of bovine milk. Approximately 95% of the *H. convergens* maintained the casein mark for 2 days after removal from each type of plant.

Introduction

The success of quantifying arthropod dispersal patterns is often dependent on having a reliable method to mark the arthropod of interest. Over the years, researchers have used many types of substances to mark arthropods (Southwood, 1978; Hagler & Jackson, 2001; Lavandero et al., 2004; Goubault & Hardy, 2007). The most suitable marker for any given study is strongly influenced by whether a mark-release-recapture (MRR) or mark-capture study is needed. Of these, the mark-capture method is the most difficult because the arthropods must be marked directly in the field. By process of elimination, the most useful markers for mark-capture studies are materials that are inexpensive and can be easily applied to vast areas directly in the field by broadcast spray application. Paints, dyes, and dusts are the most common and least expensive types of arthropod markers available for MRR, but these are usually not well suited for mark-capture type studies.

Rare or trace elements (e.g., rubidium, strontium, and cesium) are arguably the most effective markers for mark-capture type studies (Berry et al., 1972; Stimmann et al., 1973; Van Steenwyk et al., 1978; Fleischer et al., 1986; Armes et al., 1989; Akey et al., 1991). Of these, rubidium is by far the most frequently used trace element marker for

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insects. Foliar applications of RbCl have been frequently applied to various plant species to label a diverse array of insects directly in the field (Frazer & Raworth, 1974; Stimmann, 1974; Shepard & Waddill, 1976; Graham et al., 1978a,b; Alverson et al., 1980; Corbett et al., 1996; Tillman et al., 2007). However, analytical methods to determine presence of trace elements in insects are relatively costly, time consuming, and requires technical expertise (Akey et al., 1991; Hagler & Jackson, 2001).

A major breakthrough for marking insects for dispersal studies was the development of the so-called protein marking or immunomarking procedure. The method consists of marking arthropods with an exogenous protein. In turn, the protein marker is detected in or on the arthropods by an anti-protein-specific enzyme-linked immunosorbent assay (ELISA). The first generation of protein markers included rabbit IgG and chicken IgG proteins (Hagler et al., 1992; Hagler, 1997). These proteins have been effective for marking a wide variety of insects for MRR-type research (Hagler et al., 1992, 2002; DeGrandi-Hoffman & Hagler, 2000; Blackmer et al., 2004; Hagler & Naranjo, 2004; Peck & McQuate, 2004; Buczkowski & Bennett, 2006; Jasrotia & Ben-Yakir, 2006; Janke et al., 2009). However, the major drawback with IgG protein marks is that they are too expensive for mark-capture studies. Recently, second generation protein-specific ELISAs were developed to detect egg albumin protein contained in chicken egg whites, soy protein in soy milk, and casein protein contained in bovine milk. Each ELISA is highly sensitive and specific to its targeted protein and, most importantly, the proteins are readily available at only a fraction of the cost of IgG proteins (Jones et al., 2006).

In this article, we evaluate various aspects of the acquisition and persistence of casein and egg albumin markers on a wide variety of arthropods under laboratory and field conditions. We also discuss the potential advantages and limitations of the protein marking procedure for marking arthropods for large-scale mark-capture type dispersal studies.

Materials and methods

First study – Acquisition of protein marks by an arthropod assemblage

Study site. Field cage studies were conducted to determine the acquisition of egg albumin and bovine casein protein marks by an assemblage of arthropods. Each protein was administered to the arthropod complex using two different approaches. The field cages were erected during the summer of 2004, at a 1.2-ha field site located at the USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ, USA. The field was planted with the commonly used full season cotton, *Gossypium hirsutum* L. (Malvaceae), cultivar 'Delta Pine 5415'. The cotton was grown using standard agronomic practices. No insecticides were applied to the field. This field site was purposefully selected for this study because it contained a relatively low population of arthropods, due to it being surrounded by a large industrial park within the Phoenix metropolitan area. This feature allowed us to manipulate, to a certain degree, the arthropod populations introduced into each of the field cage treatments. Field cages ($1.83 \times 1.83 \times 1.83$ m) were erected in randomly located areas within the study site and the roughly 90 cotton plants enclosed in each cage were sprayed with one of the two proteins described below.

Test arthropods. We augmented the arthropod population in each field cage to insure that we had enough arthropods to conduct a lengthy study. Arthropods were collected en masse on 29 June for the chicken egg white study, and 12 August 2004 for the bovine milk study, using standard 38-cm diameter sweepnets from densely populated cotton and alfalfa fields located at the University of Arizona Maricopa Agricultural Center, Maricopa, AZ. The arthropods were placed into large paper bags, sealed, and transported to the study site. Half of the sweepnet samples were released into two of four field cages on the same day they were collected for the topical plus residual mark application treatment (see below). The other half of the bagged arthropod samples were stored overnight in a refrigerator (4 °C) and then released the next day into the remaining two field cages for the residual mark-only application treatment (see below).

Protein markers. Two protein-rich materials, chicken egg whites (All Whites™; Papetti Foods, Elizabeth, NJ, USA) and non-fat dry milk (Kroger, Cincinnati, OH, USA) were evaluated as potential marks for the arthropod complex introduced into each cage.

Chicken egg whites evaluation: Half of the sweepnet samples were released in equal numbers into two randomly selected field cages described above on 29 June 2004. The arthropods were given a 24-h acclimation period to settle in the cages before application of 1 l of a 10% (vol/vol) solution of chicken egg whites on 30 June using a 17-l backpack sprayer (Spray Doc™; Gilmour Gardening Innovation, Gilmour, Somerset, PA, USA). Four hours later, after the spray solution had dried (note: the temperature was ca. 40 °C), the other half of the arthropod sweepnet samples collected the day before was released into the two remaining cages. This design resulted in arthropods in the first two cages potentially picking up the marker via either direct contact with the spray solution

or by residual contact after the marker solution dried; those in the latter two cages could only acquire the mark by residual contact.

Non-fat dry milk evaluation: The materials and methods for the non-fat dry milk evaluation were the same as those described above, except the trials were started on 12 August 2004. We applied the non-fat dry milk markers at the same rate of 1 l of a 10% (wt/vol) solution to each cage on 13 August and introduced the arthropods in the residual marking cages 4 h after the foliage had dried.

Arthropod sampling procedure. Arthropods were collected from each cage every other day starting on the day of the protein application. The day 0 samples were collected 4 h after the arthropods were exposed to the markers. Two sampling procedures were used to insure that we collected enough arthropods on each date. First, three whole-plant samples were collected on each sample date from each cage using a large 100-cm long × 75-cm wide canvas sleeve cage (note: fewer whole-plant samples were taken toward the end of the study because of the destructive sampling process). The day before each plant sample was taken, we placed three canvas sleeve cages at the base of three randomly selected plants per cage. The bottom of each sleeve cage was closed around the base of the plant with a drawstring and the top of the sleeve cage was left open at the base of the plant. On each sampling date, the top of the sleeve cages were rapidly pulled over the entire plant and closed at the top with a drawstring. The base of each cotton plant was cut just below the bottom drawstring and the plants were frozen at −80 °C within 10 min after collection. Relatively few arthropods were collected from the whole-plant samples; therefore we also collected ca. 30 arthropods from each cage by hand on each sample date. The hand-collected arthropods were collected haphazardly. That is, we collected the first 30 arthropods that we encountered in each cage. An individual hand-collected arthropod was placed into a clean 1.5-ml microtube and frozen at −80 °C within 10 min after collection.

Plant sampling procedure. Cotton leaf samples were collected on each sample date from the three whole-plant samples described above to determine the persistence and uniformity of the protein spray applications on the plant foliage. Five leaves were randomly selected from the top one-third, middle one-third, and bottom one-third of each of the three whole-plants collected from each cage. A single, randomly selected 6.0-mm diameter sample of leaf tissue was taken from each leaf using a disposable cork borer. The disposable cork borer was a 6.0-mm diameter soda straw (Kroger) with the cutting edge trimmed off between each leaf sample to prevent cross contamination between samples. Arthropod and cotton leaf tissue samples were collected from each cage every other day until all the plants were removed from the cages 28–30 days after marking.

Arthropod and plant sample preparation. Samples were placed into individual 1.5-ml microcentrifuge tubes containing 750-µl Tris buffered saline (TBS) (pH 7.4). The samples were then soaked at 27 °C for 1 h at 100 r.p.m. on an orbital shaker. Individual samples were assayed for the presence of each protein mark using the anti-chicken egg albumin or anti-casein ELISA described by Jones et al. (2006).

Protein-specific ELISAs. Anti-egg albumin ELISA: An indirect anti-chicken egg albumin ELISA was performed on each sample collected from the cages sprayed with chicken egg whites. An 80-µl aliquot of each sample was placed in an individual well of a 96-well ELISA plate (Falcon Pro-Bind™ No. 353915; Becton Dickinson Labware, Franklin Lakes, NJ, USA). Each well of the assay plate was incubated for 1 h at 37 °C. The contents of each well were discarded and washed 5× with a phosphate buffer saline-tween 20 (0.5% tween, pH 7.4) (PBST) solution (No. P3563; Sigma Chemical Company, St. Louis, MO, USA). Then, 360-µl of a PBS–BSA (1.0% BSA, pH 7.4) (Sigma No. P3688) solution was added for 1 h at 27 °C or overnight at 4 °C to each well to prevent non-specific binding. The blocking solution was discarded and the plate was washed 2× with PBST. An 80-µl aliquot of rabbit anti-chicken egg albumin (ovalbumin) (Sigma No. C-6534) diluted 1:8 000 in a buffer solution consisting of PBS–BSA (1%) and Silwet L-77 (Setre Chemical Company, Memphis, TN, USA) (1.3 µl ml^{-1}) was added to each well for 1 h at 37 °C. Plates were washed 5× as described above and an 80-µl aliquot of goat anti-rabbit IgG (whole molecule) (Sigma No. A-6154) conjugated to horseradish peroxidase diluted 1:2 000 in the PBS–BSA-Silwet buffer described above was added to each well of the ELISA plate for 2 h at 37 °C. Plates were washed 5× with PBST and an 80-µl aliquot of TMB substrate (No. TMBW-0100-04, TMB 1 Component HRP Microwell Substrate; BioFX, Owings Mills, MD, USA) was added to each well for 10 min at 27 °C. Following substrate incubation, the ELISA optical density (OD) of each well was measured with a microplate reader (SpectraMAX 250; Molecular Devices, Sunnyvale, CA, USA) set at 650 nm.

Anti-casein ELISA: An indirect anti-casein ELISA was performed on each sample collected from the cages sprayed with non-fat dry milk. An 80-µl aliquot of each sample was placed in a well of an ELISA plate and incubated for 1 h at 4 °C. The contents of each well were

discarded and washed 2× with PBST. Then, 360 µl of a 25% chicken egg white solution diluted with TBS was added to each well to block non-specific binding sites on the plates. Each plate was incubated for 1 h at 4°C. The blocking solution was discarded and washed 2× with PBST. An 80-µl aliquot of sheep anti-bovine casein (No. K20025S; Meridian Life Sciences, Saco, ME, USA) diluted 1:2 000 in a buffer solution consisting of 25% chicken egg white solution in TBS was added to each well for 1 h at 4 °C. Plates were washed 5× with PBST and an 80-µl aliquot of mouse anti-goat/sheep IgG (Sigma No. A-9452) conjugated to horseradish peroxidase diluted 1:4 000 in a 25% egg white solution in the TBS buffer was added to each well for 1 h at 4 °C. Plates were then washed 5× with PBST, an 80-µl of substrate was added to each well for 10 min, and read as described above.

Data analysis. Arthropod and cotton leaf tissue samples serving as negative ELISA controls were collected from unmarked cotton fields located at the University of Arizona Maricopa Research Farm (ca. 56 km from the study site) and assayed by the ELISAs described above. Individual arthropod and leaf tissue samples collected from each cage were scored positive for the presence of the respective markers if the ELISA OD reading exceeded the mean negative control reading by three standard deviations (Hagler, 1997). A diverse array of arthropods (e.g., more than 50 species) were collected over the course of the study, but there was often a huge discrepancy from day-to-day in both type and number of arthropods collected from each cage. To simplify the data presentation, we pooled the samples from each cage receiving the same mark and method of application by just the four major arthropod orders we encountered over the duration of the study. Each arthropod was first scored positive or negative by ELISA for the presence of each respective mark and the percentage of positive immunoreactions for each order was determined. Mean ELISA OD values were also calculated for those individuals scoring either positive or negative for each major grouping to show the discrepancy in OD yielded between marked and unmarked samples. Note that the marking procedure for the cotton plants was the same for the topical contact and residual contact treatments. Therefore, the ELISA data obtained from each of the four cages was combined (n = 60 leaf disks per region of the plant per sample date; note: the sample sizes decrease late in the study from removal of the whole-plant samples).

Leaf area determination. The growth of the cotton plants over the course of the study (30 and 28 days for the chicken egg albumin and bovine casein studies, respectively) was quantified by measuring the total leaf area of randomly selected cotton plants surrounding the field cages. The total leaf area was measured using a Li-3100 Area Meter (LiCor, Lincoln, NE, USA). The mean (± SEM) leaf area (cm^2) was calculated for 10 randomly selected plants on 29 June and 28 July for the chicken egg whites retention study and on 11 August and 5 September for the non-fat dry milk retention study.

Second study – Temporal acquisition of chicken egg albumin by insects

Test insects. A study was conducted to determine how rapidly insects acquire chicken egg albumin after contact exposure to cotton leaf tissue sprayed with a 10% chicken egg white solution. The test insects included adult *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), adult *Lygus hesperus* Knight (Heteroptera: Miridae), and third instar *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). These insects were selected because they represent a strict predator with chewing mouthparts, an omnivore with piercing/sucking mouthparts, and a strict herbivore with chewing mouthparts, respectively. *Hippodamia convergens* were purchased from a supplier of beneficial organisms (Nature's Control, Medford, OR, USA), *L. hesperus* were collected from an alfalfa field located at the University of Arizona Maricopa Research Farm, and *T. ni* were obtained from a colony maintained at the Western Cotton Research Laboratory, Phoenix, AZ, USA.

Plant marking procedure. Cotton plants were grown in a greenhouse using the method of Hagler et al. (2004). Individual cotton plants, ca. 80-cm tall (ca. 20 leaves per plant), were uniformly sprayed with 35 ml of a 10% chicken egg white solution using a standard hand sprayer (e.g., the type of spray bottle used for window cleaning). The cotton plants were allowed to dry outside for 4 h at ca. 40 °C. After drying, randomly selected leaves were pulled from the plant, cut with a clean razor blade, and fit precisely inside a 3.5-cm diameter Petri dish (insect arena). Individual insects were placed in arenas for 5, 10, 20, 40, 60, 120, 240, or 480 min (n = 30 individuals per time interval). After each holding interval, the insect was removed from the arena, placed into a 1.5-ml microcentrifuge tube, and immediately frozen at −80 °C.

Sample preparation. Two sample preparations were made for each insect. The first sample consisted of soaking each intact individual in 750 µl of TBS for 1 h on a laboratory shaker set at 37 °C and 100 r.p.m. Then, an 80-µl aliquot of each insect sample was pipetted into a well of an ELISA plate and analyzed by the anti-chicken egg albumin ELISA described above. This sample procedure was designed to

detect an external protein mark on each insect. Then, each intact insect sample was thoroughly macerated with a tissue grinder in the remaining 670 µl of buffer solution and an 80-µl aliquot of sample was pipetted into a well of another ELISA plate and analyzed by ELISA. This sample procedure was designed to detect both an external and internal mark (e.g., protein in the gut that might have been ingested by feeding on the marked plant tissue). Additionally, three cotton leaf samples (6.0 mm diameter leaf disks) were collected from each arena and assayed by ELISA to determine the uniformity of the mark on the cotton leaf surface.

The positive ELISA threshold values for the insect and cotton plant samples were calculated as described above using unmarked samples. Individual insect and plant samples were scored positive or negative by ELISA for the presence of the egg albumin mark to determine the percentage of positive ELISA reactions yielded for each cotton leaf and insect sample. Mean ELISA OD values were calculated for each group that scored positive and negative, respectively, to depict the discrepancy between marked and unmarked samples.

Third study – Temporal acquisition of bovine casein by insects

Study site. A series of field cage tests were conducted to determine how rapidly adult *H. convergens* and *L. hesperus* acquire bovine casein after exposure to either alfalfa, *Medicago sativa* L. (Fabaceae) or lesquerella, *Lesquerella fendleri* (Watson) (Brassicaceae) plants previously sprayed with a 100% whole milk solution. The field sites included a 1.6-ha alfalfa and a 1.6-ha lesquerella field located at the University of Arizona Maricopa Agricultural Center. The plants in each field were ca. 0.5 m tall and grown using standard agronomic practices. A randomly selected 5 × 5-m portion of each field was sprayed evenly with 1.0 l of whole milk on 12 March 2007, using a commercial backpack sprayer (MD155DX Mist Duster gas-powered backpack sprayer; Maruyama, Denton, TX, USA). The milk residue on the plants was allowed to dry for 2 h at ambient temperature (ca. 28 °C). After the marker dried, >50 randomly selected plants from each crop were enclosed in individual sleeve cages. The sleeve cages (1 m long × 0.5 m diameter) were constructed from nylon tulle (mesh size 1 mm^2; Tempe Sales, Tempe, AZ, USA) and had an opening on each end. The bottom of each cage was tied at ground level around the base of an individual plant with a permanent zip-tie. The top of the sleeve cage was grabbed and pulled up over the top of each plant and eight adult *H. convergens* and eight adult *L. hesperus* were placed in each sleeve cage and the top of each cage was tied with another zip-tie. The insects were allowed to roam freely within each cage for 0.25, 0.5, 1, 2, 4, or 24 h. After each time interval, five randomly selected caged plants were cut at their base (just below the bottom zip-tie), and frozen within 10 min at −20 °C. Frozen insects were processed by removing the caged plants from the freezer and carefully searching the entire contents of each cage for each of the *H. convergens* and *L. hesperus* that had been introduced into each cage. Alfalfa and lesquerella leaf samples were also collected from each cage to determine the uniformity and persistence of the mark on the plants. Five leaves were selected from each plant (n = 25 per time interval) using the disposable cork borer method described above. The five leaf tissue samples were collected from evenly spaced distances on the plant starting from the bottom fifth of the plant and ending on the top fifth of the plant.

Sample preparation. Individual samples were placed in 1.5-ml microtubes containing 750 µl of TBS and soaked at 27 °C for 2 h on a laboratory shaker (100 r.p.m.). An 80-µl aliquot of each sample was pipetted into a well of an ELISA plate and analyzed by the anti-casein ELISA described above.

Data analysis. The positive ELISA threshold values for the insect and plant samples were calculated as described above using unmarked samples. Individual insect and plant samples enclosed in each cage were scored positive or negative by ELISA for the presence of the casein mark to determine the percentage of positive ELISA reactions. Mean ELISA OD values were calculated for each group that scored positive and negative to depict the discrepancy between marked and unmarked samples, respectively.

Fourth study – Persistence of casein on *Hippodamia convergens*

Study site. A study was conducted to determine the persistence of casein on *H. convergens* after individuals were exposed for 24 h to alfalfa and lesquerella plants containing a 7-day-old residue of milk and then removed from the treated plants for 1, 2, or 7 days. Several nylon mesh sleeve cages were erected in the 5 × 5-m plots described above 1 week after the initial application of the 100% whole milk solution. Then, 10 adult *H. convergens* were released into each cage for 24 h. After 24 h, five caged plants were cut at their bases, taken immediately to the laboratory, and leaf tissue samples collected from each cage were sampled, processed, and assayed by the anti-casein ELISA described above. The remaining sleeve cages were removed from the alfalfa and lesquerella fields and the *H. convergens* collected from these cages were placed individually into 3.5-cm diameter Petri dishes. Each dish contained a surplus of pink bollworm eggs [*Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)] for food and a wetted sponge for water. The beetles were then

placed in an incubator set at 27 °C, 30% r.h., and L16:D8 photoperiod. Individuals were held in the Petri dishes for 1, 2, or 7 days after removal from the sleeve cages. After each holding interval, the live insects were frozen at −20 °C and assayed for the presence of the mark by the anti-casein ELISA.

Data analysis. The positive ELISA threshold values for *H. convergens* and alfalfa and lesquerella leaf samples were calculated as described above using unmarked samples. Individual samples were scored positive or negative by ELISA for presence of the casein mark to determine the percentage of positive ELISA reactions. Mean ELISA OD values were calculated for each group that scored positive and negative to depict the discrepancy between marked and unmarked samples, respectively.

Results

First study – Acquisition of protein marks by an arthropod assemblage

Persistence of egg albumin on cotton leaf tissue. The negative control cotton leaf samples collected from outside of the marked areas yielded a mean (± SEM) ELISA

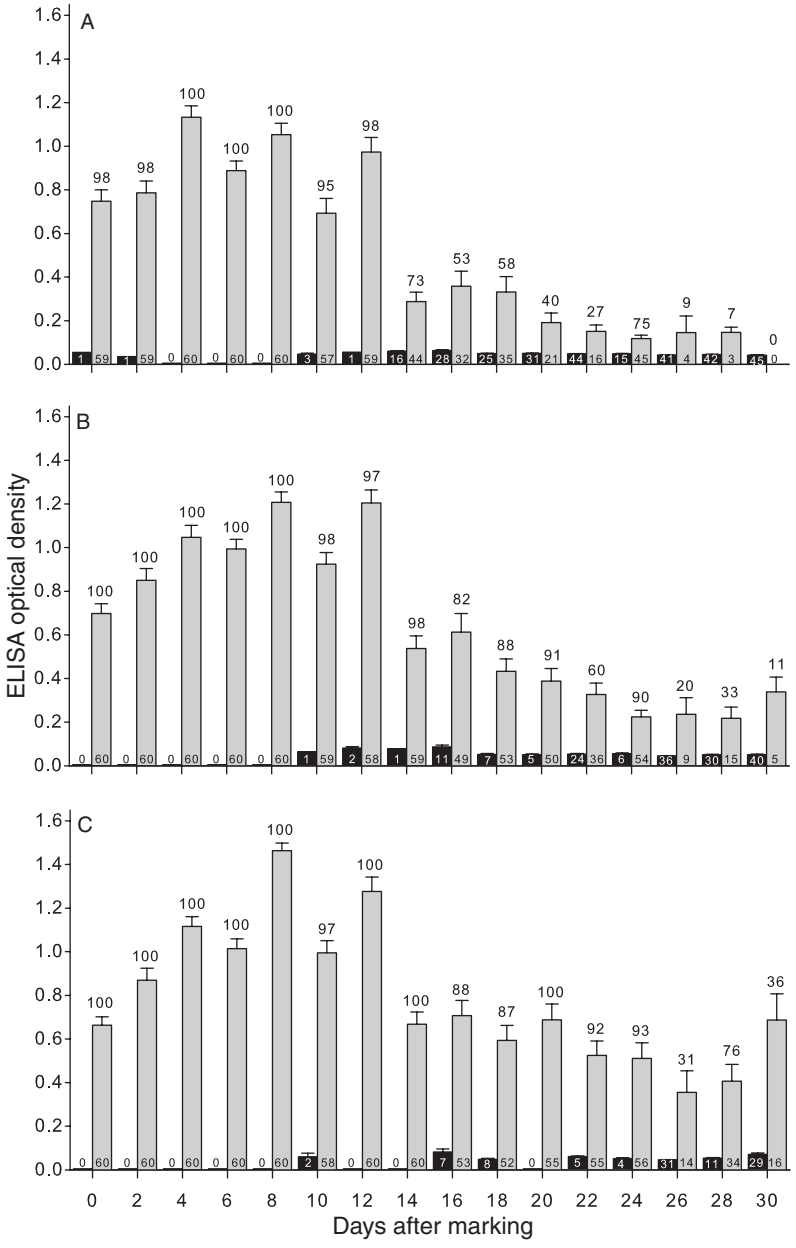

Figure 1 Mean (+ SEM) ELISA optical density values and percentage of cotton leaf disks scoring positive (% positive is given above the error bars of each gray bar) for the presence of chicken egg albumin after exposure to a topical spray of a 10% All Whites™ solution. The leaf disks were collected from the (A) top, (B) middle, and (C) bottom one-third of each cotton plant, respectively. The black bars represent the values yielded by those cotton disks that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

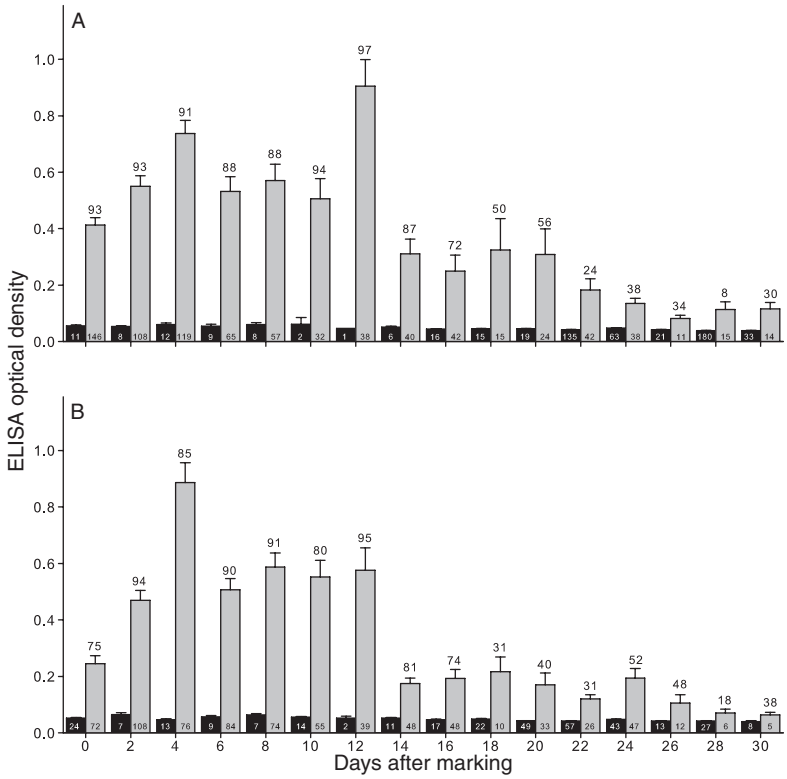

Figure 2 Mean (+ SEM) ELISA optical density values and percentage of arthropods scoring positive (% positive is given above the error bars of each gray bar) for the presence of chicken egg albumin after (A) exposure to a topical spray plus a residue treatment of a 10% All Whites™ solution, or (B) contact exposure to a residue treatment of 10% All Whites™. The black bars represent the values yielded by those arthropods that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

OD of 0.043 ± 0.008 (n = 60), thus establishing a positive ELISA threshold value of 0.067 for those leaf samples sprayed with egg whites (Figure 1). Almost every cotton leaf disk sample collected for up to 12 days after marking, regardless of region of the plant that it was collected from, yielded a positive immunoreaction. The plant leaf disks collected from the upper third of the cotton plants had a sharp decline in ELISA reactivity, and subsequently a decline in the percentage of positive ELISA reactions at ≥14 days after marking. However, the mark was retained well on the leaves located on the lower two-thirds of the plants for 24 days after marking. Overall, the leaf samples collected in the cages scoring negative by ELISA yielded values of 0.048 ± 0.002, 0.053 ± 0.003, and 0.058 ± 0.007, whereas those scoring positive yielded values of 0.685 ± 0.051, 0.732 ± 0.056, and 0.801 ± 0.055 from the top, middle, and bottom portions of the cotton plants, respectively. In short, the positively mark plant tissues yielded ELISA OD values >10× higher than the negative control threshold value of 0.067.

Acquisition of chicken egg albumin spray + residue on the arthropod assemblage. A total of 1 345 arthropods, representing more than 50 species were examined for the presence of the mark. Mean ELISA OD values for those individuals yielding a positive immunoreaction ranged from 0.90 on day 12 to 0.08 on day 26, whereas those individuals scoring negative by ELISA yielded an average OD reading of <0.05 over the course of the study (Figure 2A). The egg albumin mark was detected on about 90% of individuals for up to 14 days after marking and on 60% of the individuals tested over the course of the study. The arthropods scoring negative by ELISA yielded an overall mean OD value of 0.044 ± 0.0004, whereas those scoring positive yielded a mean OD value of 0.468 ± 0.016. The four most common arthropod orders encountered in the field cages that were exposed to the topical spray plus residue egg white mark included Coleoptera, Heteroptera, Hymenoptera, and Araneae (Table 1).

Acquisition of chicken egg albumin residue by the arthropod assemblage. A total of 1 066 arthropods were examined for the presence of the egg white residue mark. Mean OD values for those individuals yielding a positive immunoreaction ranged from 0.89 ± 0.07 on day 4 to 0.06 ± 0.01 on day 30, whereas those individuals scoring negative by ELISA consistently yielded an average reading of <0.05 (Figure 2B). The residual egg albumin mark was detected

Table 1 The number assayed (n) and percentage (%) of individuals from the most common arthropod orders encountered that scored positive by ELISA for the presence of egg albumin protein after exposure to either a topical spray plus residue treatment or a spray residue only treatment of a 10% solution of Chicken Egg All Whites™

	Topical + residue treatment[1]								Residue treatment[2]							
	Coleoptera		Heteroptera		Hymeno-ptera		Araneae		Coleoptera		Heteroptera		Hymeno-ptera		Araneae	
Days	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	17	100.0	74	98.6	21	76.2	20	95.0	12	100.0	46	69.6	2	100.0	18	83.3
2	16	100.0	61	96.7	16	93.8	13	76.9	18	100.0	55	100.0	16	62.5	9	100.0
4	16	93.8	49	91.8	37	89.2	19	94.7	21	95.2	27	100.0	23	69.6	5	60.0
6	26	92.3	20	95.0	12	50.0	12	100.0	21	90.5	40	92.5	12	75.0	13	92.3
8	15	86.7	27	92.6	8	62.5	7	85.7	15	86.7	29	100.0	21	81.0	8	100.0
10	7	71.4	16	100.0	1	100.0	7	100.0	28	78.6	18	100.0	4	75.0	12	58.3
12	9	100.0	15	100.0	2	50.0	5	100.0	7	100.0	15	100.0	6	100.0	7	85.7
14	5	60.0	25	92.0	2	100.0	10	90.0	18	72.2	26	88.5	1	0.0	6	66.7
16	9	55.6	25	100.0	9	11.1	10	60.0	14	71.4	24	91.7	4	50.0	12	41.7
18	7	71.4	10	50.0	4	50.0	4	25.0	5	60.0	11	27.3	2	0.0	11	27.3
20	5	40.0	12	75.0	16	31.3	9	77.8	7	71.4	20	45.0	15	26.7	10	40.0
22	10	20.0	14	71.4	130	13.8	10	60.0	16	37.5	13	53.8	22	18.2	12	66.7
24	24	75.0	7	57.1	22	13.6	4	50.0	14	71.4	13	38.5	13	23.1	1	100.0
26	6	66.7	6	33.3	8	37.5	2	50.0	0	NA	11	54.5	3	0.0	8	75.0
28	16	6.3	8	25.0	19	15.8	6	66.7	3	0.0	9	33.3	3	33.3	6	16.7
30	8	37.5	4	50.0	13	53.8	1	0.0	0	NA	2	50.0	7	57.1	1	0.0
Total	196	72.4	373	89.5	320	37.8	139	81.3	199	79.4	359	81.3	154	52.6	139	66.2

[1]The most common members of each order encountered in the field cages where the arthropods were treated to a topical spray + residue treatment of protein included: *Hippodamia convergens* (23% of the beetle population), *Collops vittatus* (Say) (16%), and *Systena mitis* (LeConte) (10%) for the beetles; *Geocoris punctipes* (Say), *Geocoris pallens* Stål (47%), *Rhinacloa forticornis* Reuter (16%), *Nabis alternatus* Parshley (12%), *Lygus hesperus* (11%), and *Zelus renardii* Kolenati (5.6%) for the true bugs; *Solenopsis xyloni* McCook (98%) for the ants; and *Misumenops celer* Hentz (77%) for the spiders.

[2]The most common members of each order encountered in the field cages where the arthropods were subjected to a residue-only treatment of protein included: *C. vittatus* (23%), *H. convergens* (16%), and *S. mitis* (16%) for the beetles; *G. punctipes* and *G. pallens* (40%), *L. hesperus* (24%), *N. alternatus* (13%), and *R. forticornis* (10%) for the true bugs; *S. xyloni* (93%) for the ants; and *M. celer* (71%) for the spiders.

on 70% of the arthropods examined over the duration of the study. The arthropods scoring negative by ELISA yielded an overall mean OD value of 0.047 ± 0.001, whereas those scoring positive yielded an overall mean OD of 0.423 ± 0.016. A summary of the percentage of positive ELISA reactions obtained by the most common arthropod orders encountered is given in Table 1.

Persistence of topical bovine casein on cotton leaf tissue. The daily mean (+ SEM) ELISA OD values and the percentages of positive immunoreactions for cotton leaf samples sprayed with non-fat dry milk are presented in Figure 3. The negative control cotton leaf disks collected from outside of the marked areas yielded a mean ELISA OD of 0.053 ± 0.011 (n = 270), thus establishing the positive ELISA OD threshold value of 0.086 for those leaf samples sprayed with milk. Almost every cotton leaf disk collected for up to 4 days after marking contained casein residue. Overall, 41, 72, and 68% of the leaf samples collected from the top, middle, and bottom regions of the plants, respectively, contained casein residue.

Acquisition of bovine casein spray + residue on the arthropod assemblage. A total of 1 037 arthropods, representing more than 50 species, were assayed for the presence of the 10% bovine casein mark. Mean ELISA OD values for those individuals yielding a positive immunoreaction ranged from 0.45 ± 0.024 on the day of application to 0.14 ± 0.037 on day 24, whereas those individuals with a negative reaction consistently yielded an average reading of <0.05 over the course of the study (Figure 4A). The casein mark was detected on 59% of the arthropods examined. The arthropods collected in the cages scoring negative by ELISA yielded an overall mean OD value of

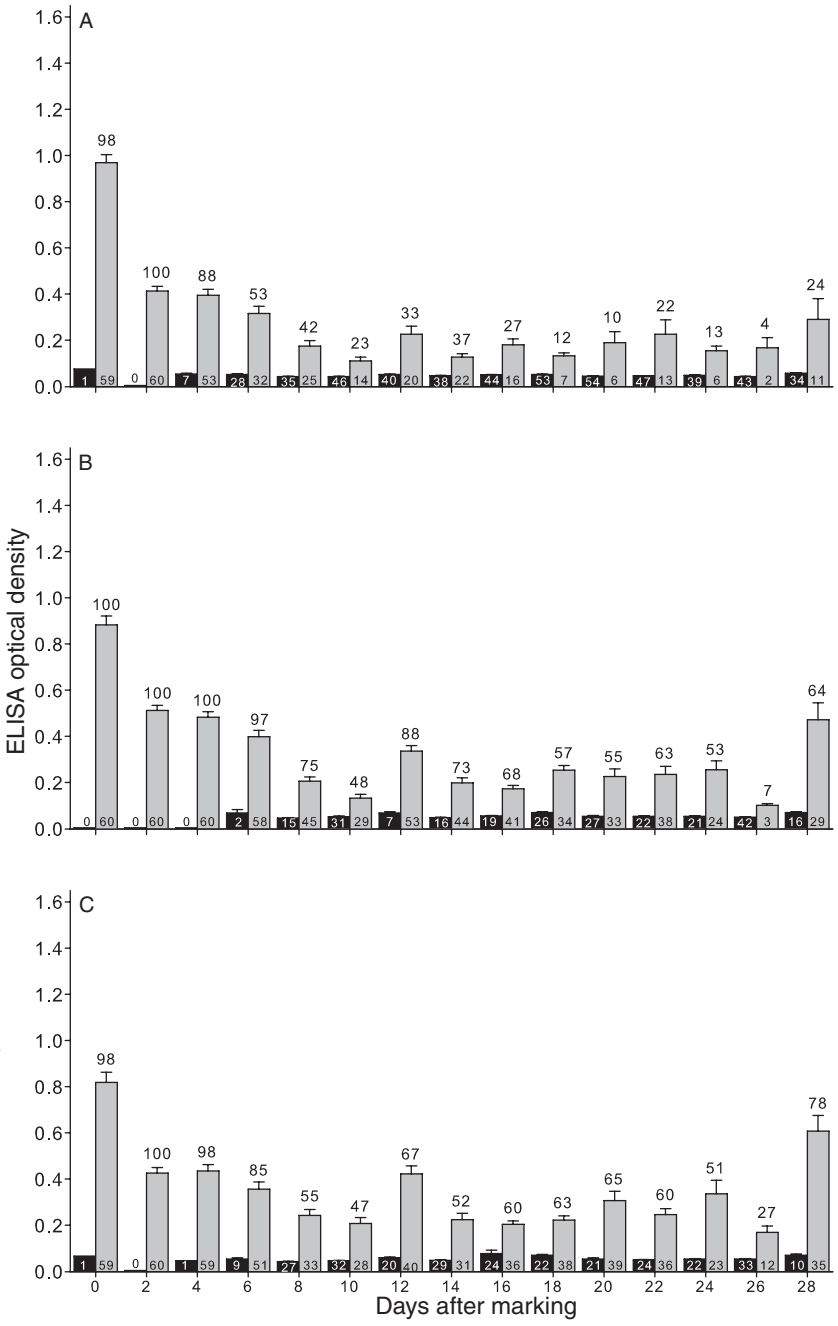

Figure 3 Mean (+ SEM) ELISA optical density values and percentage of cotton leaf disks scoring positive (% positive is given above the error bars of each gray bar) for the presence of bovine casein after exposure to a topical spray of a 10% non-fat dry milk solution. The leaf disks were collected from the (A) top, (B) middle, and (C) bottom one-third of each cotton plant, respectively. The black bars represent the values yielded by those cotton disks that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

0.048 ± 0.0006, whereas those scoring positive yielded a value of 0.322 ± 0.012. A summary of the percentage of positive ELISA reactions obtained by the most common arthropod orders encountered is given in Table 2.

Acquisition of bovine casein residue on the arthropod assemblage. A total of 920 arthropods were assayed for the presence of the casein mark. Mean OD values for those individuals yielding a positive immunoreaction ranged from 0.426 ± 0.267 on day 26 to 0.090 ± 0.009 on day 6, whereas those individuals with a negative reaction consistently yielded an average reading of <0.06 over the course of the study (Figure 4B). The mark was detected on 45% of the arthropods tested over the duration of the study. The arthropods collected in the cages scoring negative by ELISA yielded an overall mean ELISA OD of

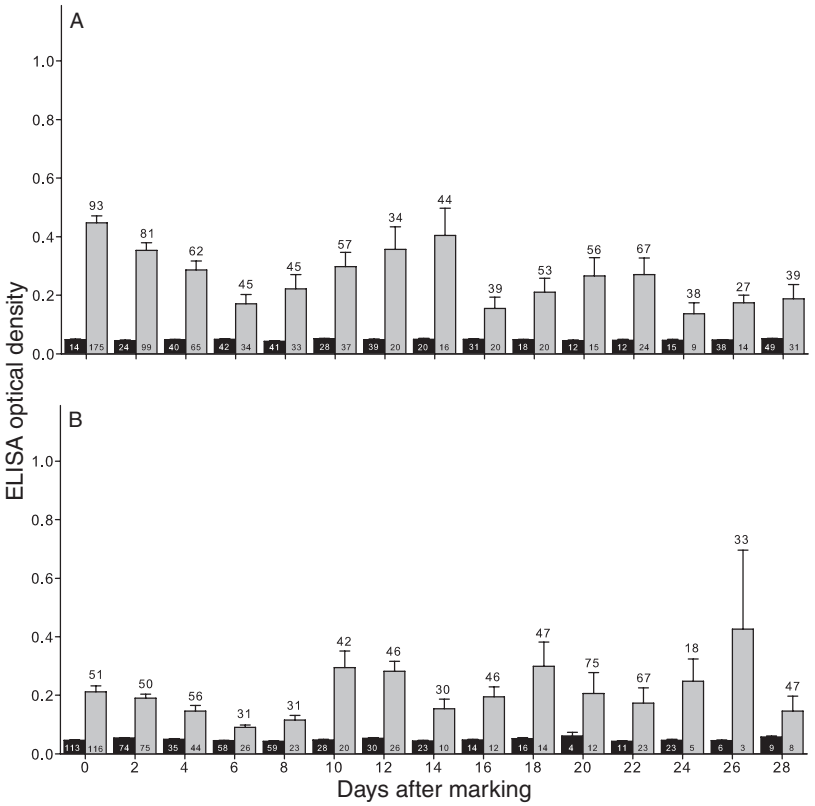

Figure 4 Mean (+ SEM) ELISA optical density values and percentage of arthropods scoring positive (% positive is given above the error bars of each gray bar) for the presence of bovine casein protein after (A) exposure to a topical spray plus a residue treatment of a 10% non-fat dry milk solution, or (B) contact exposure to a residue treatment of 10% non-fat dry milk. The black bars represent the values yielded by those arthropods that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

0.048 ± 0.0005, whereas those scoring positive yielded a mean value of 0.196 ± 0.009. A summary of the percentage of positive ELISA reactions yielded by the most common arthropod orders encountered is given in Table 2.

Second study – Temporal acquisition of chicken egg albumin by insects

Almost every cotton leaf disk sampled (n = 3 per arena) from each of the Petri dish arenas (n = 480 arenas) contained egg albumin. This indicates that each cotton leaf arena contained a consistent and even distribution of protein mark on its surface. Specifically, 99.7% (n = 1440) of the cotton leaf disk samples contained egg albumin and they yielded an overall mean ELISA OD of 0.993 ± 0.011.

The temporal acquisition of the chicken egg albumin residue from the plant surface to the insects that were first soaked in ELISA buffer are provided in Figure 5. The mark was acquired by ≥90% of the *H. convergens* and *L. hesperus* after only 5 min exposure to the marked leaf surface (Figure 5A and B). The acquisition of egg albumin by immature *T. ni* was slower, but the majority of individuals obtained the mark after a 20- to 240-min exposure period (Figure 5C). The detection of the mark on the same insect samples that were then macerated in the ELISA buffer yielded poor results. For example, the overall percentage of positive ELISA reactions for *H. convergens*, *L. hesperus*, and *T. ni* samples soaked in buffer was 87.5, 91.3, and 68.8%, respectively, whereas the overall percentage for the same samples subsequently macerated in the buffer was 10.7, 30.0, and 3.8%.

Third study – Temporal acquisition of bovine casein by insects

Plant tissue. The temporal stability and distribution of the bovine casein mark on alfalfa and lesquerella plants are presented in Figure 6. The negative control alfalfa and lesquerella samples yielded low mean ELISA OD values of 0.050 ± 0.011 (establishing a critical positive ELISA threshold value of 0.083) and 0.044 ± 0.002 (establishing a critical value of 0.050), respectively. The bovine casein mark applied as a 100% topical solution of whole milk was retained on 100% of the alfalfa and 79% of the lesquerella leaves tested over the duration of the study. Overall, the lesquerella leaf samples scoring negative yielded an average ELISA OD of 0.052 ± 0.007, whereas the positive alfalfa and lesquerella samples yielded average values of 0.787 ± 0.349 and 0.340 ± 0.252, respectively.

Table 2 The number assayed (n) and percentage (%) of individuals from the most common arthropod orders encountered that scored positive by ELISA for the presence of bovine casein protein after exposure to either a topical spray plus residue treatment or a spray residue only treatment of a 10% milk solution

	Topical spray + residue treatment[1]								Residue treatment[2]							
	Coleoptera		Hetero-ptera		Hymeno-ptera		Araneae		Coleoptera		Heteroptera		Hymeno-ptera		Araneae	
Days	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0	46	100.0	63	85.7	30	86.7	9	100.0	21	76.2	38	23.7	113	50.4	5	60.0
2	24	87.5	54	72.2	6	83.3	12	75.0	14	85.7	22	54.5	69	36.2	8	62.5
4	21	61.9	50	64.0	7	14.3	8	50.0	12	16.7	23	52.2	13	38.5	3	66.7
6	16	56.3	32	40.6	13	15.4	5	40.0	15	26.7	24	29.2	30	26.7	4	25.0
8	15	53.3	24	54.2	5	40.0	10	30.0	15	33.3	17	47.1	9	22.2	13	30.8
10	17	76.5	15	80.0	20	20.0	5	80.0	7	14.3	12	58.3	0	NA	9	33.3
12	4	25.0	13	84.6	12	8.3	11	27.3	5	80.0	9	66.7	12	16.7	5	40.0
14	2	50.0	11	90.9	12	8.3	6	33.3	5	20.0	8	62.5	3	0.0	6	50.0
16	3	33.3	16	43.8	21	47.6	4	0.0	4	75.0	8	50.0	3	33.3	3	33.3
18	10	50.0	8	50.0	1	0.0	8	75.0	8	50.0	9	33.3	2	0.0	4	100.0
20	3	33.3	15	60.0	1	0.0	4	50.0	1	100.0	5	80.0	1	0.0	1	100.0
22	6	66.7	5	80.0	4	50.0	6	66.7	9	55.6	2	100.0	7	42.9	4	75.0
24	1	0.0	7	57.1	6	0.0	3	66.7	1	100.0	13	15.4	2	0.0	2	0.0
26	3	33.3	28	39.3	6	0.0	4	25.0	1	0.0	7	42.9	1	0.0	0	NA
28	2	0.0	21	71.4	51	29.4	3	0.0	1	0.0	13	53.8	0	NA	1	100.0
Total	173	71.7	362	65.7	195	35.4	98	52.0	119	49.6	210	43.3	265	38.9	68	48.5

[1]The most common members of each order encountered in the field cages where the arthropods were treated to a topical spray plus residue treatment of protein included: *Systena mitis* (54%), *Collops vittatus* (18%), and *Hippodamia convergens* (5%) for the beetles; *Lygus hesperus* (39%), *Geocoris punctipes* and *Geocoris pallens* (32%), *Zelus renardii* (12%), and *Nabis alternatus* (6%) for the true bugs; *Solenopsis xyloni* (96%) for the ants; and *Misumenops celer* (62%) and *Metaphidippus* spp. (27%) for the spiders.

[2]The most common members of each order encountered in the field cages where the arthropods were subjected to a residue-only treatment of protein included: *S. mitis* (56%) and C. *vittatus* (7%) for the beetles; *L. hesperus* (42%), *N. alternatus* (16%), *G. punctipes* and *G. pallens* (14%), and *Z. renardii* (12%) for the true bugs; *S. xyloni* (99%) for the ants; and *M. celer* (46%) and *Metaphidippus* spp. (10%) for the spiders.

Hippodamia convergens. The temporal acquisition of bovine casein by *H. convergens* exposed to marked alfalfa and lesquerella plants is given in Figure 7. The negative control *H. convergens* yielded a mean ELISA OD of 0.056 ± 0.010 (n = 101), thus establishing a critical positive threshold value of 0.086. Overall, 89% (n = 283) and 96% (n = 247) of the *H. convergens* collected from the marked alfalfa (Figure 7A) and lesquerella (Figure 7B) obtained casein regardless of the duration of exposure to the plant tissues. Moreover, the mark was acquired by 90 and 95% of the *H. convergens* within 15 min of exposure to the marked alfalfa and lesquerella plants, respectively.

Lygus hesperus. The negative control *L. hesperus* yielded a mean OD of 0.054 ± 0.010 (n = 59), thus establishing a critical positive threshold value of 0.084. Overall, 76% (n = 299) and 91% (n = 253) of the *L. hesperus* acquired the mark, regardless of the duration of exposure to the marked plants. Moreover, the mark was acquired by 62 and 95% of the *L. hesperus* within 15 min of exposure to the marked alfalfa (Figure 7C) and lesquerella plants (Figure 7D), respectively.

Fourth study – Persistence of casein on *Hippodamia convergens*

Plant tissue. The percentage of alfalfa and lesquerella plants marked with the bovine casein protein at the onset of the study (e.g., the beginning of the *H. convergens* 24 h contact with 7-day-old residues of milk on plant tissue) is given in Figure 6 (see the dark gray vertical bars at day 7). The average (± SEM) ELISA OD for the leaf tissues yielding a positive reaction was 0.720 ± 0.080 (n = 30) and 0.313 ± 0.098 (n = 11) for the alfalfa and lesquerella, respectively. Moreover, the percentage of positive plant samples at the onset of the study was 100 for alfalfa and 37 for lesquerella (Figure 6).

Hippodamia convergens. The retention of the 7-day-old casein residues on *H. convergens* after a 24-h exposure

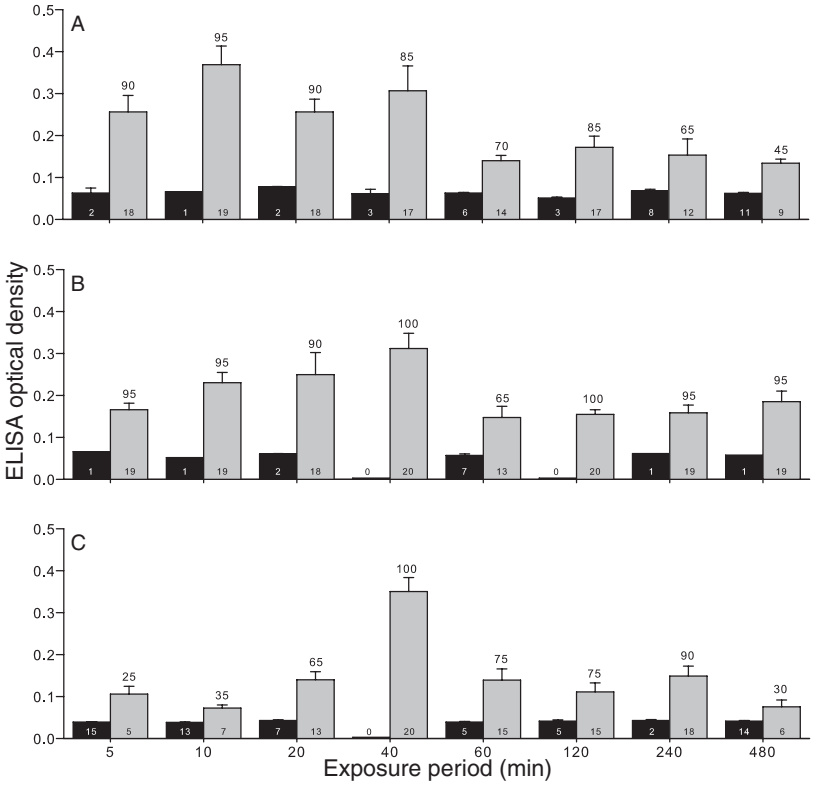

Figure 5 Mean (+ SEM) ELISA optical density values and percentage of (A) *Hippodamia convergens*, (B) *Lygus hesperus*, and (C) *Trichoplusia ni* scoring positive (% positive is given above the error bars of each gray bar) for the presence of chicken egg albumin after variable exposure time to cotton leaf tissue sprayed with a 10% All Whites™ solution. The black bars represent the values yielded by those insects that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

period to marked alfalfa or lesquerella are given in Figure 8. The frequency of positive ELISA reactions for the presence of the mark was high (e.g., 88–100%) for up to 2 days after removal from the marked plants. The proportion of individual *H. convergens* marked with the protein decreased to 58 and 24%, 7 days after the insects were removed from the marked alfalfa and lesquerella, respectively (Figure 8).

Discussion

Proteins in readily available food products such as chicken eggs or cow's milk offer a promising alternative to conventional arthropod markers for mark-capture research (Hagler & Jackson, 2001; Jones et al., 2006; Horton et al., 2009). The inexpensive proteins were applied to crops using conventional spray equipment. The markers were then detected on the crops and their arthropod inhabitants using protein-specific ELISAs. The range of formulations of egg whites (e.g., liquid and powdered) and milk (e.g., whole, 2%, skim, and powdered) that can be used offers many options for administering the mark to the arthropod(s) of interest. The protein-specific ELISAs have not shown cross-reactivity with any plant type, arthropod species, water type (e.g., dH_2O or tap H_2O), or spray additive (e.g., TBS, EDTA, or Sylgard 309) tested to date, which is useful if a particular study might require multiple distinctive marks subjected to a wide variety of field conditions (Jones et al., 2006; Horton et al., 2009). Moreover, the ELISAs are simple, sensitive, inexpensive, and have been standardized for mass production (e.g., >1 000 samples per day).

The first study showed that both proteins were retained for ≥4 weeks on the vast majority of leaves sampled from the lower two-thirds of the cotton plant foliage. The height and total leaf area of the cotton plants nearly doubled in size over the duration of the study (see below), which could explain the gradual decline in frequency of positive ELISA reactions from the leaves sampled from the upper third of the plant over the course of the study (e.g., sampling of new cotton plant tissue). Data also indicated that, at the concentration tested, that egg albumin was retained on the cotton leaf tissue at a higher concentration and for a longer period of time than bovine casein. The study also revealed that egg albumin was obtained more frequently by arthropods than bovine casein. This could be attributed to a combination of factors. First, as mentioned above, the cotton leaf samples exposed to egg white albumin consistently contained a higher concentration of protein than the leaf samples exposed to milk casein. This likely led to

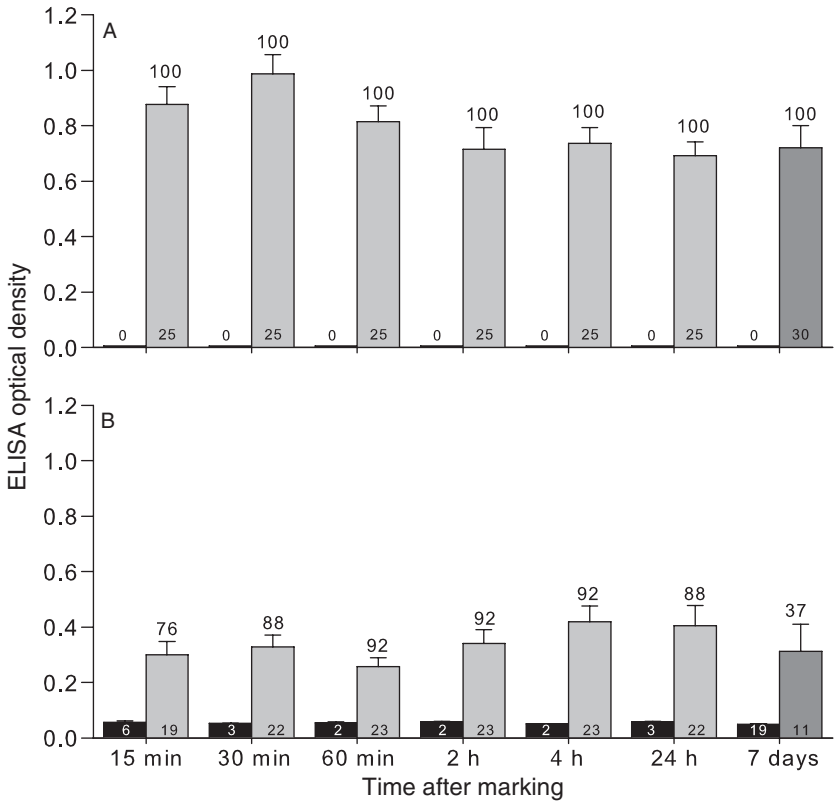

Figure 6 Mean (+ SEM) ELISA optical density values and percentage of leaf disks of (A) alfalfa and (B) lesquerella scoring positive (% positive is given above the error bars of each gray bar) for the presence of bovine casein after exposure to a topical spray of a 100% whole milk solution. The black bars represent the values yielded by those leaf disks that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The darker gray bars at day 7 indicate the mean OD of the leaf tissue that *Hippodamia convergens* were exposed to during the fourth experiment (see the text for further details). The number inside each bar is the sample size.

the higher incidence of egg-marked arthropods as a result of direct contact with the egg-marked plant tissue. Second, the egg whites might be a more effective arthropod mark than casein. A previous study showed that 80 and 20% of pear psylla, *Cacopsylla pyricola* (Förster), were successfully marked after contact exposure to egg whites and milk, respectively (Jones et al., 2006). That difference would be magnified in this study because the rate of milk applied was half the concentration used by Jones et al. (2006), and dose of marker will obviously affect marking rates (VP Jones, unpubl.). Third, the plant architecture at the time of each specific protein application could have influenced the outcome of the study. The cotton plants had average leaf surface areas of 2 093 ± 398 and 4 025 ± 804 cm^2 at the start of the egg albumin (29 June) and bovine casein (11 August) evaluations, respectively. Obviously the smaller plants sprayed with egg whites received a more thorough and uniform application of protein than the larger plants sprayed with the same volume of milk.

Although the first and third study revealed that the majority of arthropods contained a protein mark over the course of each experiment, we emphasize that the temporal stability of these proteins was not truly tested on the arthropod assemblage. Specifically, the second and third study, and Jones et al. (2006) showed that arthropods can rapidly self-mark with protein after contact with protein-marked plant tissue.

The temporal stability of only the casein mark was tested, and only to a limited extent, in the fourth study. This study revealed that the majority (i.e., >90%) of *H. convergens*, when exposed for 24 h to a 7-day-old residue of bovine casein-marked alfalfa or lesquerella plant tissues, will retain the mark for 2 days after removal from the marked plant tissues. However, the mark retention declined to 58 and 24% of the beetles tested 1 week after removal from the marked alfalfa and lesquerella, respectively. Again, it is highly probable that these inexpensive proteins would be retained longer on arthropods if they were either directly sprayed with protein or exposed to a higher concentration protein residue. Further studies are needed to directly compare the residual activity of the two protein marks on arthropods exposed to different concentrations of protein marks under variable biotic and abiotic conditions.

Marking arthropods with inexpensive proteins has enormous potential as a tool for mark-capture and MRR research. However, there are caveats to this procedure that should be considered before using it for dispersal studies. First, protein marks may pass laterally

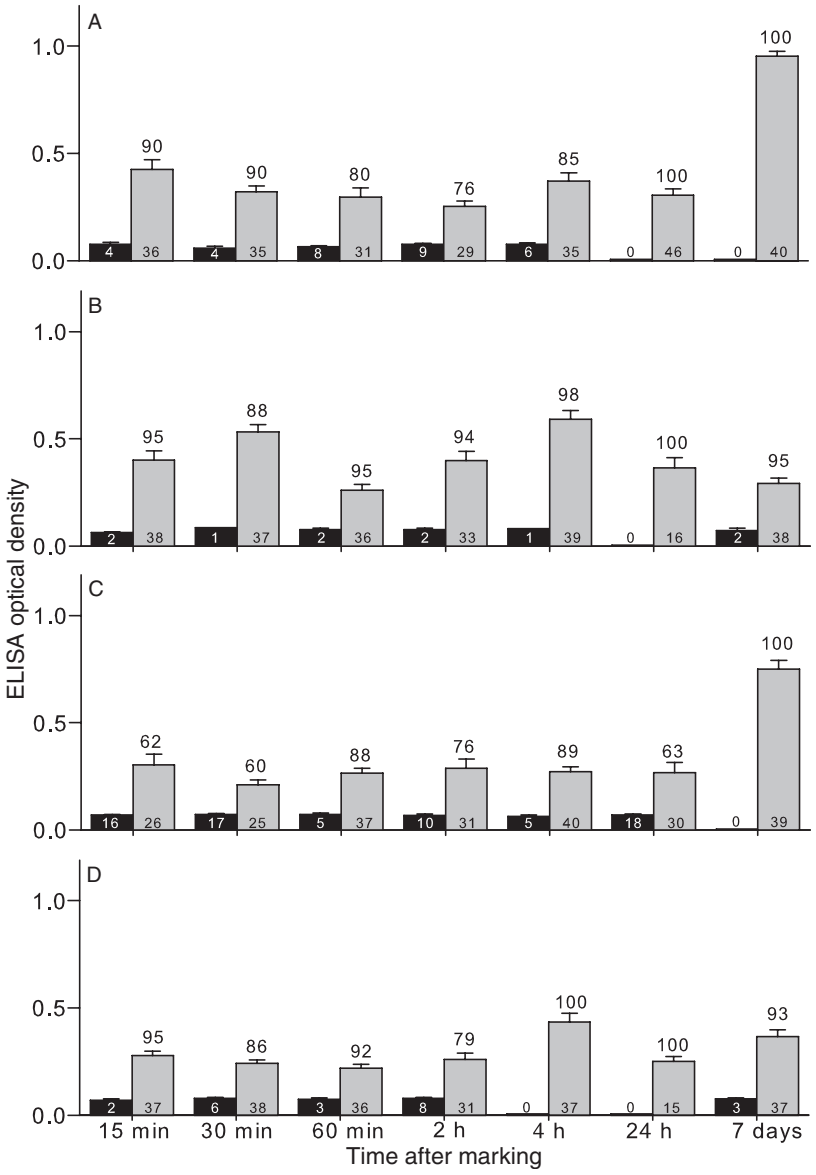

Figure 7 Mean (+ SEM) ELISA optical density values and percentage of (A, B) *Hippodamia convergens* scoring positive (% positive is given above the error bars of each gray bar) for the presence of bovine casein protein after contact exposure to whole milk applied to (A) alfalfa and (B) lesquerella plants, and of (C, D) *Lygus hesperus* scoring positive for bovine casein after contact exposure to a residue of whole milk applied to (C) alfalfa and (D) lesquerella plants. The black bars represent the values yielded by those insects that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

by external contact from marked to unmarked individuals. Rabbit IgG-marked honey bees (Degrandi-Hoffman & Hagler, 2000) and termites (Buczkowski et al., 2007) have transferred the mark to nest mates, and large groups of externally marked pink bollworm moths transferred IgG to unmarked moths, but individual male moths did not readily transfer the protein on unmarked females during mating (Hagler & Miller, 2002). Second, due to the sensitivity of the marking procedure, extreme caution is needed to avoid spray drift of protein markers to unintended areas (Jones et al., 2006). Third, abiotic factors such as rain, humidity, and wind (if used as a dust) might alter the effectiveness of the markers (Jones et al., 2006). For instance, Jones et al. (2006) showed that the egg mark is more soluble that the milk mark. Therefore, milk marker likely has a greater rain-fastness than egg marker. Fourth, the method used to recapture protein-marked arthropods must be considered. A variety of methods (e.g., hand collection, sweepnet, sticky card, and fan trap) has been used to capture protein-marked insects for MRR-type studies with no apparent negative effect on the precision of the marking procedure (Hagler & Miller, 2002; Hagler et al., 2002; Blackmer et al., 2004, 2006). However, for mark-capture research the collection devices (e.g., sweepnet, d-vac net, etc.)

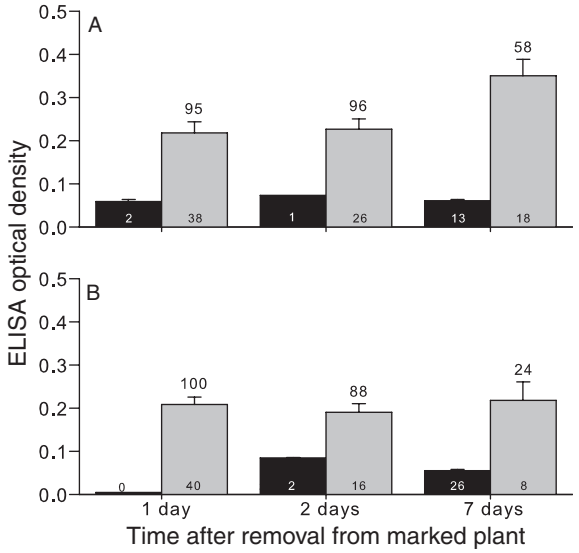

Figure 8 Mean (+ SEM) ELISA optical density values and percentage of *Hippodamia convergens* scoring positive (% positive is given above the error bars of each gray bar) for the presence of bovine casein after removal from (A) alfalfa and (B) lesquerella plants marked with whole milk. The black bars represent the values yielded by those insects that scored negative by ELISA, the gray bars represent those that scored positive by ELISA. The number inside each bar is the sample size.

will become contaminated by direct contact with marked plants and are unsuitable collection devices in those areas directly treated with the protein mark. At present, the only useful methods for sampling arthropods within protein-marked areas are adhesive traps (Jones et al., 2006), beating trays with adhesive liners (Horton et al., 2009), or handpicked collections. Fifth, it is possible that protein marks may pass up the food chain to predators feeding on marked prey. Egg albumin was not detected by indirect ELISA in spiders that had eaten a chicken egg albumin-marked pear psylla (Horton et al., 2009). However, rabbit IgG protein was readily detected by sandwich ELISAs in the guts of predators that consumed IgG-marked prey items (Hagler, 2006; Buczkowski & Bennett, 2007; Mansfield et al., 2008). The detection of marked prey in predator guts may be attributed to the amount of protein on the marked prey, the size of the predator, the type of ELISA used to detect the protein (e.g., indirect or sandwich ELISA), or a combination of these factors (see Hagler, 1998, 2006; and Fournier et al., 2006, for a review of the caveats of predator gut content analyses). Lastly, studies are needed to investigate the possible side effects that each type of protein mark might have on arthropods. Such studies are underway to investigate the effect that various proteins have on insect survival and flight behavior.

In summary, the second generation of protein markers described here provides a means for researchers to mark arthropods for large-scale mark-capture type dispersal research. The key advantages of the procedure are that it is inexpensive, relatively simple, and reliable, and vast areas can be marked with conventional spray equipment. Moreover, the specificity of the various ELISAs provides the tools (e.g., multiple marks) necessary to monitor intercrop dispersal patterns. Finally, the ELISA procedure is adaptable for high throughput of field-collected samples.

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