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A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-released insect predators

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A multiple ELISA system for simultaneously monitoring intercrop movement and feeding activity of mass-released insect predators

(*Keywords: Hippodamia convergens*, *Bemisia argentifolii*, augmentative biological control, dispersal, mark-release-recapture, quality control assessment, predation, gut content analysis)

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Abstract. We combined two protein-marking enzyme-linked immunosorbent assays (ELISA) with a predator gut content ELISA to monitor the movement and feeding activity of commercially-purchased *Hippodamia convergens* Guèrin-Mèneville (Coleoptera: Coccinellidae) under realistic field conditions during two field seasons in central Arizona. The protein-marking ELISAs were used to differentiate released *H. convergens* from the native beetles. Commercially purchased beetles marked with rabbit immunoglobulin G (IgG) were released into cotton fields and chicken IgG marked beetles were released into adjacent cantaloupe fields. Results showed that the total native beetle abundance in each crop was about the same size. The recovery rates after 15 days for the released beetles were less than 1.0% over all the releases, indicating that they dispersed readily from the release site. Of the recaptured beetles containing rabbit IgG (cotton), 82.2% were recovered in cotton and 11.8% moved to cantaloupe. Of those containing chicken IgG (cantaloupe), 66.5% were recovered in cantaloupe and 33.5% moved to cotton. A predator gut content ELISA was used to determine if there were differences in the frequency of predation of released versus indigenous *H. convergens* on the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae). The proportion of beetles containing whitefly antigens was always higher for the released beetles than for their native counterparts. Our results demonstrate an approach to combine protein marking and predator gut content ELISAs that allows the simultaneous comparison of feeding and intercrop movement of native and commercially-obtained biological control agents.

1. Introduction

There are two major issues concerning the successful implementation of predaceous insects as augmentative biological control agents. First, the released predators, whether reared in captivity or mass-collected from a different location, should retain their ability to prey on the targeted pest species (Hagler and Cohen, 1991). Second, the predators must remain within the target site (Grundy and Maelzer, 2002). Unfortunately, precise and realistic evaluations of both predator feeding behaviour and dispersal movements are difficult to attain.

Direct field observations of predator feeding are difficult because most predators and their prey are small and elusive (Fichter and Stephen, 1981; Hagler and Cohen, 1991). Post-mortem evaluations of predation are difficult because predators rarely leave evidence of attack (Hagler and Naranjo, 1996). As a result, researchers have resorted to using indirect techniques for monitoring insect predation (Luck *et al*., 1988; Sunderland *et al*., 1988). The molecular identification of prey remains in predator guts using a prey-specific monoclonal antibody-based (MAb) enzyme-linked immunosorbent assay (ELISA) is a common and precise method for evaluating predation (Greenstone and Morgan, 1989; Hagler *et al*., 1991, 1993, 1994).

Monitoring natural enemy dispersal after an inundative release is also problematic. One approach is to mark insects with some easily identifiable material prior to their release. The most commonly used materials for marking predators are various coloured fluorescent dusts (Southwood, 1978; Hagler, 1997a,b; Prasifka *et al*., 1999; Hagler and Jackson, 2001). Fluorescent dusts are convenient because they are easy to apply and to detect, however dusts are not retained well and may have adverse affects on some insects (Hagler and Jackson, 2001). A vertebrate-specific protein-marking procedure was developed to circumvent the previous disadvantages of the other marking techniques. The protein marker can be easily and rapidly applied externally or internally to both large and small insects (Hagler *et al*., 1992a, 2002; Hagler, 1997a,b; DeGrandi-Hoffman and Hagler, 2000; Hagler and Miller, 2002) and can later be detected using a protein-specific ELISA.

The techniques described here are used to determine if inundative biological control agents: (1) feed as well as their native counterparts on the targeted prey, (2) remain at their targeted site, and (3) disperse between various locations. We describe how a predator gut content ELISA (Hagler *et al*., 1993) and two protein marking ELISAs (Hagler, 1997a) can be used to evaluate an inundative predator release. The gut content ELISA detects the presence of silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring [= *Bemisia tabaci* (Gennadius) Biotype B] (Homoptera: Aleyrodidae) remains within the guts of the convergent lady beetle, *Hippodamia convergens* Guèrin-Mèneville (Coleoptera: Coccinellidae). The protein-marking ELISAs detect the presence of different protein markers on released *H. convergens*. The two protein marking ELISAs allow us to

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differentiate between native and released beetles and to monitor the intercrop movement of the released beetles. The methodology applied here may serve as a model for future studies of predator-prey interactions, predator and parasitoid dispersal patterns, and evaluation of augmentative biological control using mass-released predators and parasitoids.

2. Materials and methods

2.1. Study site

The study was conducted at The University of Arizona's Maricopa Agricultural Research Farm, located near Maricopa, AZ, USA during the summers of 1995 and 1996. The study site each year consisted of a 0.36 ha cotton plot (cv '*Delta Pine 5415*') located between two, 0.08 ha cantaloupe (cv '*Hales Jumbo*') plots (figure 1).

2.2. Model predator

Adult *H. convergens* were purchased from Nature's Control (Medford, OR, USA), a commercial supplier of beneficial insects. Adults were refrigerated for several days at 4°C until they were marked and released. We selected *H. convergens* for this study because it is known to feed on *B. argentifolii* (Hagler and Naranjo. 1994b), it is one of the few predators commercially available in large enough numbers to conduct a meaningful study, and it is indigenous to our study site.

2.3. Insect marking procedure

The beetles were removed from the refrigerator, placed into 50.0 litre plastic ice chests, and sprayed with 250 ml of a 0.5 mg/ml solution of rabbit IgG (Sigma, St. Louis, MO, USA, #I5006) or chicken IgG (Sigma, #I4881) using a standard hand sprayer. A large piece of organdy cloth was placed over each ice chest and the predators were air dried for 2 h at 35°C prior to release at the study site described below.

2.4. Predator releases

Two release trials were conducted each year in 1995 and 1996. Marked beetles were released at 19:00 h on 18 June and 9 July 1995 and on 19 June and 9 July 1996. The release sites

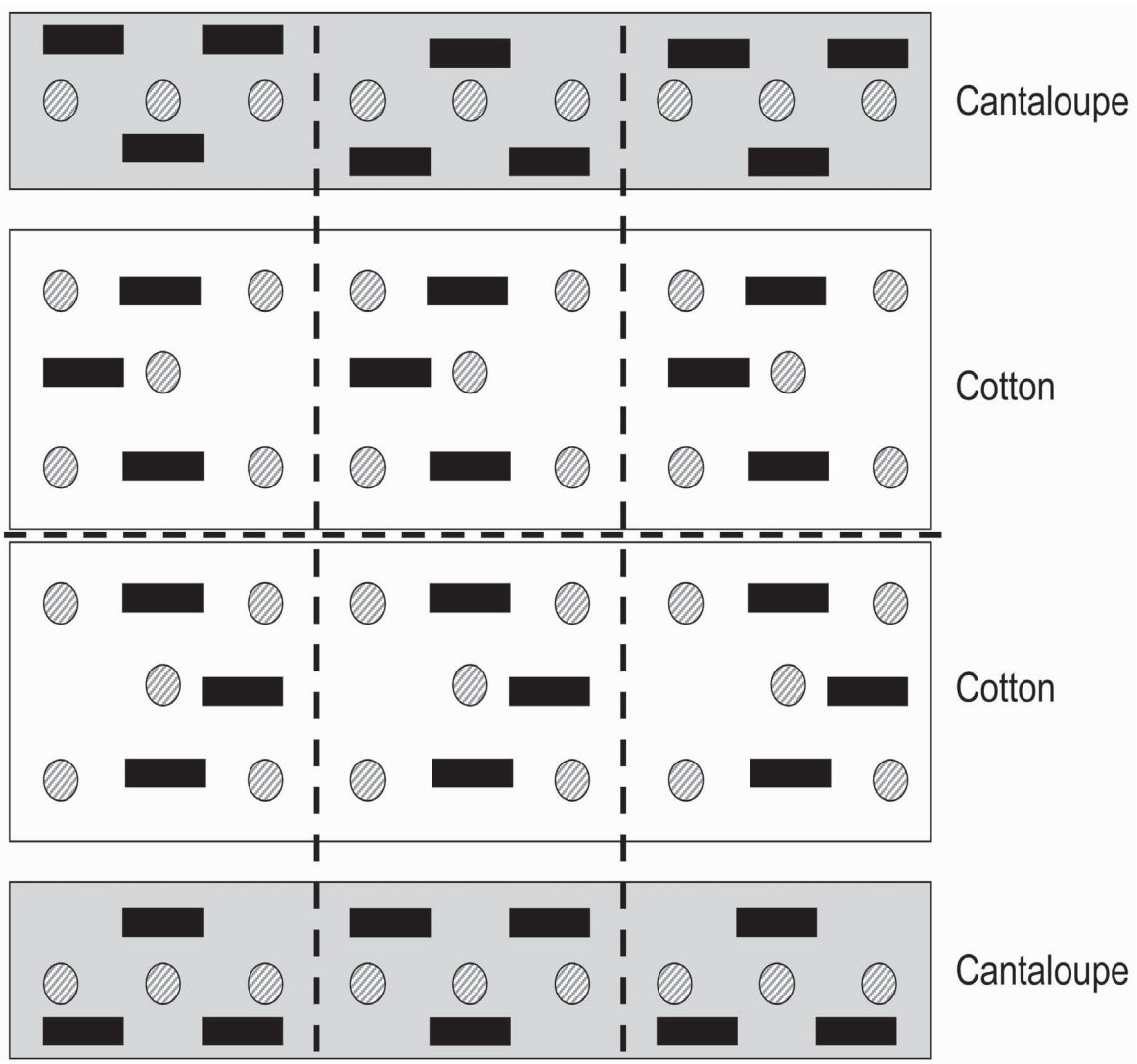

Figure 1. A diagrammatic representation of the 1995 and 1996 study site. Each grey circle represents a Hippodamia convergens *release site and each black rectangle represents a 30.5-m vacuum collection site. The vertical dashed lines represent the point that each plot was divided into thirds for the hand collections in 1996.*

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are indicated by the shaded circular regions in figure 1. For each trial, ≈2500 beetles marked with chicken IgG were released into nine equidistant locations in each of the two cantaloupe fields (45 000 total) and ≈3000 beetles marked with rabbit IgG were released into 30 equidistant locations in the cotton field (90 000 total). These release rates were calculated to equal ≈27 beetles per square meter of crop area.

2.5. Predator sampling procedures

We sampled for *H. convergens* at 3, 8, and 15 d after each release by vacuuming 30.5 m of row using a single-row tractor-mounted vacuum sampler developed by Ellington *et al.* (1984). The 30.5 m vacuum sampled sections are indicated by the black rectangle regions in figure 1. Each vacuum sample was marked indicating which crop it was collected from, placed in a 3.8 litre plastic carton, and frozen immediately on dry ice. Additional collections were made by hand in 1996 because the recapture rates were lower than desired in 1995 using only the vacuum sampler. For the hand collections, each plot was divided into thirds (six sub-plots for each crop). A single person collected beetles in each sub-plot until 50 beetles were collected or 15 min elapsed. All the predator sub-samples were pooled by date and by crop, then frozen at −70°C.

2.6. Whitefly sampling procedures

A single leaf was selected from the 5th nodal position below the mainstem terminal from 120 to 180 randomly selected plants from each crop on the day following each of the beetle releases. The leaf turn method described by Naranjo and Flint (1995) was used to estimate the number of adult whiteflies on each leaf. Each leaf was then removed from the plant and taken to the laboratory where the leaf disk method described by Naranjo and Flint (1994) was used to estimate the density of whitefly eggs and nymphs.

2.7. Predator sample preparation

Each individual beetle was macerated with a tissue grinder in 1000 μl of phosphate buffered saline (PBS, pH 7.4). A 100 μl aliquot was used to assay for the presence of whitefly egg antigen using the indirect ELISA described below (for the 1995 samples only) and two, 100 μl aliquots were used to assay for the presence of either rabbit IgG or chicken IgG using the sandwich ELISAs described below.

2.8. Predator gut content and marking ELISAs

2.8.1. Indirect gut content ELISA. An indirect ELISA was conducted on the beetles in 1995 to examine beetle gut contents for whitefly egg and adult female antigens. A 100 μl aliquot from the whole body macerated beetle was pipetted into individual wells of uncoated (clean) 96-well ELISA assay plates (Falcon Pro Bind 3915, Becton Dickinson Labware, Franklin Lakes, NJ, USA) and incubated at 4°C overnight. Each well was then incubated with a 1.0% non-fat dry milk solution for 30 min at 37°C to block non-specific binding. A 50 μl aliquot of an anti-*B. argentifolii* MAb (Hagler *et al.*, 1993) (diluted 1:500 in 1.0% non-fat dry milk) was added to the plate. The plate was incubated for 1 h at 37°C, and then washed 3 × with PBS-Tween 20 (0.05%) and 2 × with PBS. Aliquots (50 μl) of goat anti-mouse IgG/IgM conjugated to alkaline phosphatase (BioSource International, Camarillo, CA, USA, #AMI0705) diluted to 1:500 in 1.0% non-fat dry milk was added to each well. Plates were washed as noted above and 50 μl of alkaline phosphatase substrate solution (BioRad, Richmond, CA, #172-1063) was added to each well. After 2 h, the absorbance of each well was measured with a Cambridge Technology 750 microplate reader (Cambridge Technology, Watertown, MA, USA) set at 405 nm.

2.8.2. Sandwich marking ELISAs. Two sandwich ELISAs were performed on all field-collected *H. convergens* to determine if they contained any protein marker. Each of the 96 wells on the first ELISA plate was coated with 100 μl of goat anti-rabbit IgG (5.5 μg antibody/well) (Sigma, #R2004) and incubated overnight at 4°C. Similarly, each of the 96 wells on the second plate was coated with 100 μl of goat anti-chicken IgG (5.5 μg antibody/well) (Sigma, #I1161) and incubated overnight at 4°C. Each well was then incubated with a 1.0% non-fat dry milk solution for 30 min at 37°C to block non-specific antibody binding and washed as described above. A 100 μl aliquot of the macerated predator sample was placed on each plate in the wells and incubated for 2 h at 37°C. Wells were washed as described above and a 50 μl aliquot of goat anti-rabbit IgG conjugated to horseradish peroxidase (Sigma, #A6154) diluted to 1:1000 in 1.0% non-fat dry milk was added to the wells in the first plate while 50 μl aliquots of goat anti-chicken IgG conjugated to horseradish peroxidase (Sigma, #A9046) diluted to 1:1000 in 1.0% non-fat dry milk was added to the wells in the second plate. Plates were washed again as described above and 50 μl of horseradish peroxidase substrate solution was added to each well of both plates (BioRad, #172-1064). After 2 h, the absorbance of each well was measured with a Cambridge Technology 750 microplate reader set at 405 nm.

2.8.3. Negative predator controls. Commercially-purchased beetles serving as negative controls were immediately frozen (−70°C) upon arrival to the laboratory. Individual beetles were placed in 1000 μl PBS and macerated. Negative control predators were assayed for the presence of whitefly egg antigen by the indirect ELISA described above ($n = 16$/ELISA microplate). Mean (± SD) absorbance values were calculated for the negative controls. Individual field-collected predators were scored positive for *B. argentifolii* egg antigen if the absorbance value was three standard deviations above that of the negative control mean (Schoof *et al.*, 1986; Sutula *et al.*, 1986). Similarly, commercially-purchased beetles known not to contain any rabbit IgG and chicken IgG were assayed by the sandwich ELISAs described above. Field-collected beetles were scored positive for rabbit IgG or chicken IgG if the absorbance value was 3 standard deviations above that of their respective negative control mean (Hagler, 1997a,b). Those field collected predators that scored negative in both marking ELISAs were considered indigenous to the study site.

2.9. Data summary

Descriptive pie charts depicting the number of beetles collected on each sampling date that contained rabbit IgG

(released in the cotton), chicken IgG (released in the cantaloupe), and no mark (indigenous to the study site) were constructed. The crop containing the most beetles that had moved in from the other crop determined the net movement of beetles between crops. The net movement was determined first for each of the three sample dates of each release trial and then for each trial (i.e., the three sample dates for each trial were combined). For the beetles collected in the first trial in 1995, a *z*-statistic proportions test (SigmaStat, Ver. 2.03, SPSS Inc., Chicago, IL, USA) was conducted for each possible pairwise combination of *H. convergens* cohorts (e.g., rabbit marked, chicken marked, and unmarked) to determine if there were significant differences in the proportion of individuals containing whitefly antigens. Yate's correction for continuity was applied to each *z*-statistic calculation (Glantz, 1997).

3. Results

3.1. 1995 release trials

3.1.1. First release trial. A total of 90 000 beetles in cotton and 45 000 beetles in cantaloupe were marked and released during each of the four trials conducted in 1995 and 1996. Only 323 marked (0.24% of the total released) beetles were recaptured from both crops during the first release trial in 1995. The recapture rate of marked beetles was much lower after 8 and 15 days than after 3 days (figure 2A).

Half (49.7%) of the beetles recaptured in cotton 3 days after the first inundative beetle release originated from the cotton release site, 13.5% recaptured originated from the cantaloupe release site, and 36.8% were native to the study site (unmarked). Most (84.2%) of the beetles recaptured in cantaloupe 3 days after release originated from the cantaloupe release, 5.0% recaptured originated from the cotton release, and 10.8% were native to the study site. The net movement of marked beetles was greater from cantaloupe to cotton ($n = 25$) than from cotton to cantaloupe ($n = 6$). The proportion of beetles containing whitefly prey remains was higher for the released beetles than the native beetles (figure 3A).

Most of the beetles (45.9%) captured in cotton 8 days after the first inundative beetle release were native to the study site, 38.5% originated from the cotton release site, and 15.6% originated from the cantaloupe release site. Very few beetles ($n = 8$) were captured in cantaloupe 8 days after the inundative release. Of these, six were native and two were originally released in cantaloupe. The only movement of marked beetles between crops was from cantaloupe to cotton ($n = 17$). The proportion of individuals containing whitefly prey remains was higher for the released beetles than the native beetles (figure 3B).

The majority of beetles (62.1%) captured in the cotton fields 15 days after the first inundative beetle release were native to the study site and 37.9% originated from the cotton release site. None of the beetles recaptured in cotton originated from the cantaloupe release site. Most of the beetles (64.9%) recaptured in cantaloupe 15 days after the inundative release were native to the study site, 21.6% originated from the cantaloupe release site, and 13.5% originated from the cotton release site. The only movement of beetles between crops was from cotton to cantaloupe ($n = 5$). The proportion of individuals containing whitefly prey remains was higher for the released beetles than the native beetles (figure 3C).

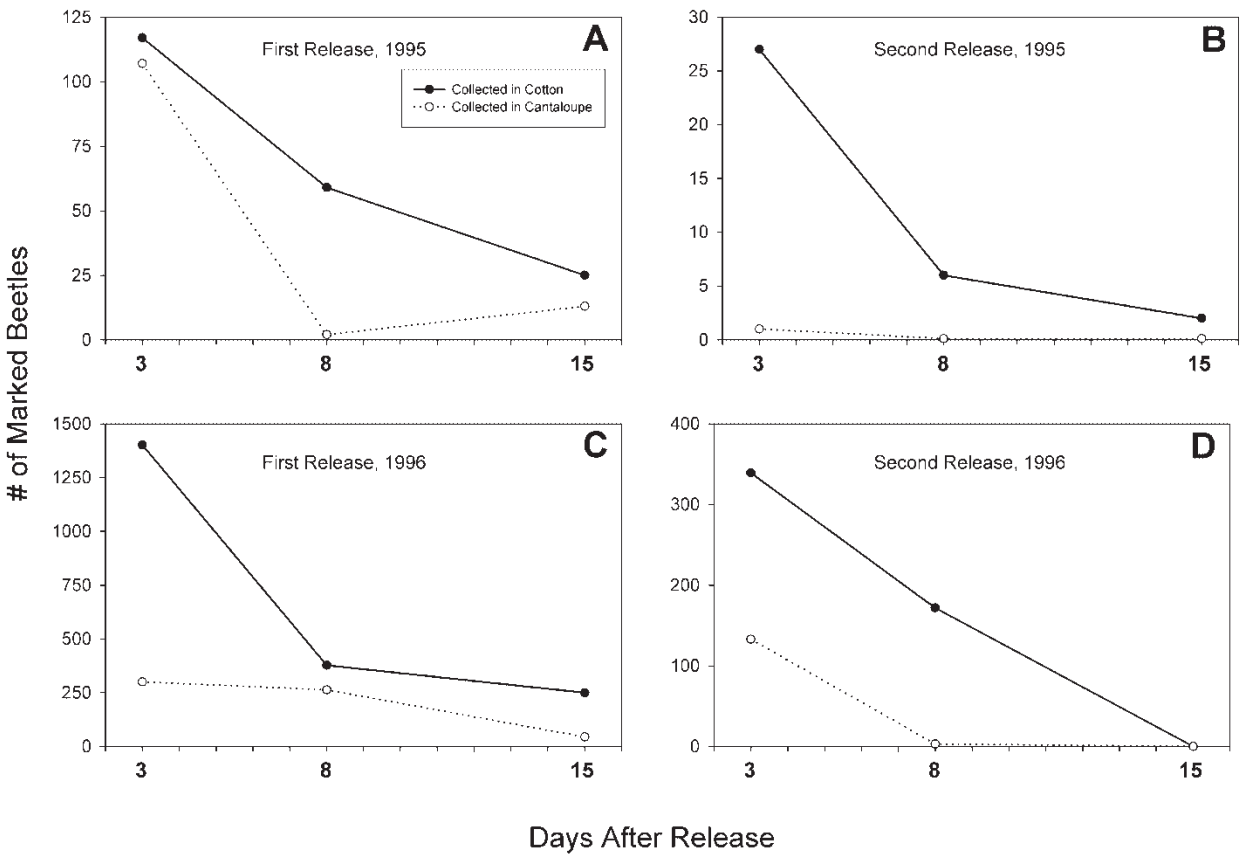

Figure 2. Total number of marked Hippodamia convergens *collected in cotton and cantaloupe 3, 8, and 15 days after release.*

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3.1.2. Second release trial. Only 36 marked beetles (< 0.1% of the population released) were recaptured from both crops during the second release trial. Of these, only one beetle containing a rabbit IgG mark was recaptured in the cantaloupe (on day 3). The number of beetles recaptured in cotton decreased over time (figure 2B).

The majority (73.5%) of beetles recaptured in cotton 3 days after the second inundative beetle release originated from the cotton release site, 5.9% originated from the cantaloupe release site, and 20.6% were native to the study site. Only seven beetles were recaptured in cantaloupe 3 days after the inundative release, and all but one was native to the study site. The

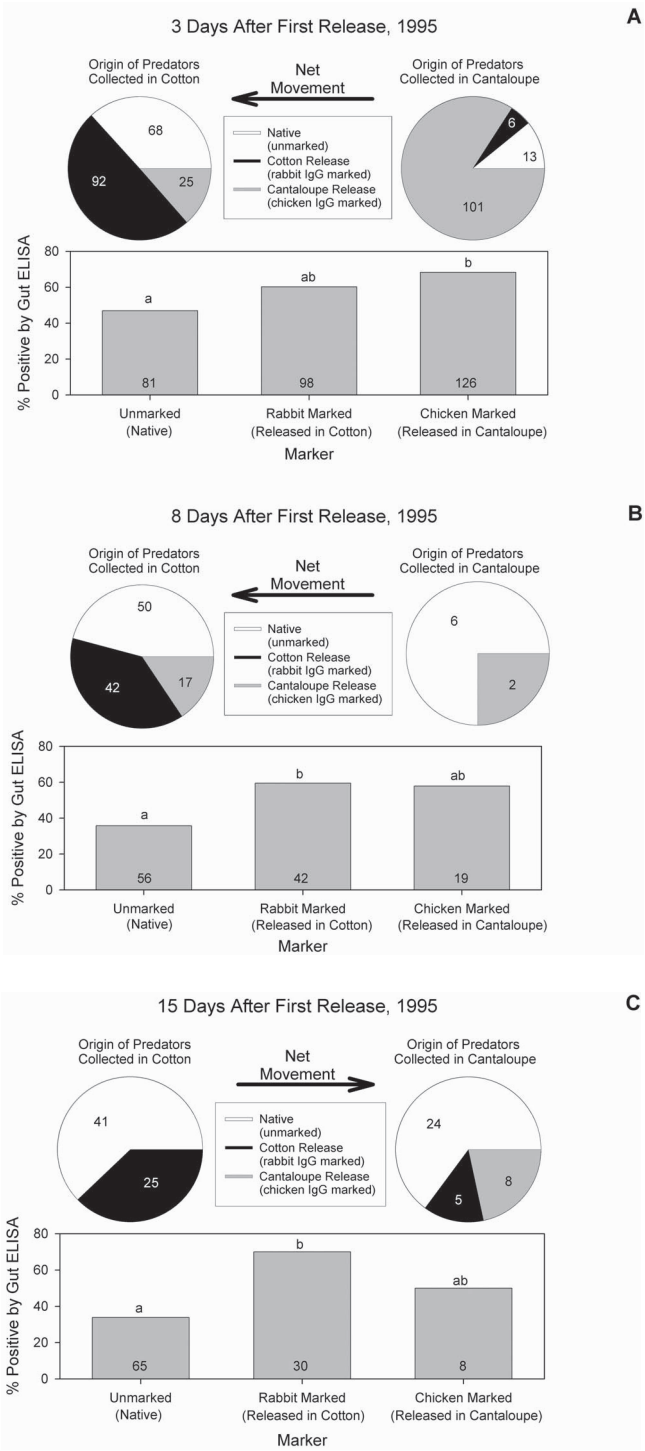

Figure 3. Origin of Hippodamia convergens *collected in cotton and cantaloupe 3, 8, and 15 days after the first initial release in 1995 (pie charts). The number within each slice of the pie charts is the number of beetles collected. The bar charts show the percentage of unmarked (native), rabbit IgG marked (released in cotton), and chicken IgG marked (released in cantaloupe) beetles scoring positive by ELISA for the presence of whitefly antigens. The number inside each bar is the number of beetles assayed by ELISA. Percentages with the same letter above the bar are not significantly different (z-test for proportions, p < 0.01).*

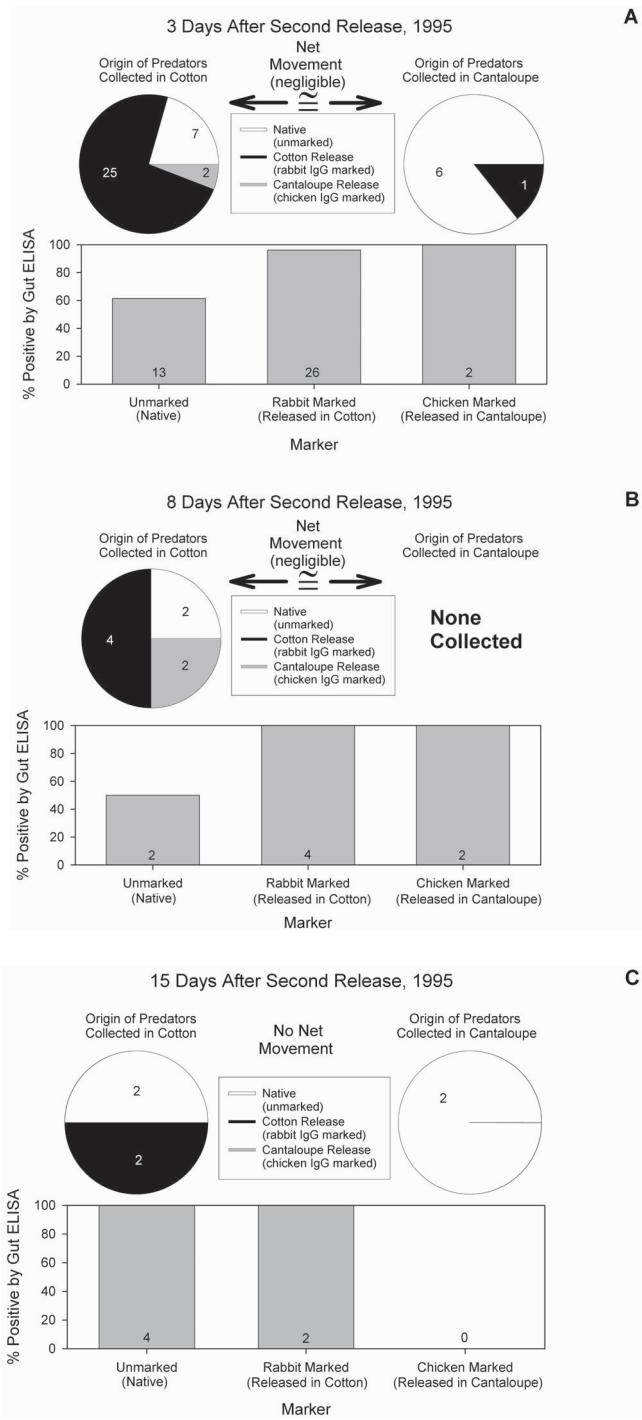

Figure 4. Origin of Hippodamia convergens *collected in cotton and cantaloupe 3, 8, and 15 days after the second initial release in 1995 (pie charts). The number within each slice of the pie charts is the number of beetles collected. The bar charts show the percentage of unmarked (native), rabbit IgG marked (released in cotton), and chicken IgG marked (released in cantaloupe) beetles scoring positive by ELISA for the presence of whitefly antigens. The number inside each bar is the number of beetles assayed by ELISA. The percentage of positive responses by ELISA were not analysed for statistical differences because of the small sample sizes.*

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proportion of individuals containing whitefly prey remains was higher for the released beetles than the native beetles (figure 4A). Only eight and six marked and unmarked beetles were collected from both crops at 8 and 15 days after the second inundative release, respectively (figures 4B and C). The small sample sizes make it difficult to draw conclusions about net movement and feeding activity of the beetles.

3.2. 1996 release trials

3.2.1. First release trial. A total of 2636 marked (2.0% of the total released) beetles were recaptured from both crops during the first release trial in 1996. The number of marked beetles recaptured in each crop decreased substantially each sampling date after their release (figure 2C).

The majority of beetles (89.5%) captured in cotton 3 days after the first inundative release originated from the cotton release site, 3.4% originated from the cantaloupe release site, and 7.1% were native to the study site. Most (65.4%) of the beetles captured in cantaloupe 3 days after the inundative beetle release were native to the study site, 20.9% originated from the cantaloupe release site, and 13.7% originated from the cotton release site (figure 5A). The net movement of marked beetles was greater from cotton to cantaloupe ($n = 119$) than from cantaloupe to cotton ($n = 51$).

The majority of beetles (63.7%) captured in cotton 8 days after the first inundative beetle release originated from the cotton release site, 6.9% originated from the cantaloupe release site, and 29.3% were native to the study site. Most (62.7%) of the beetles captured in cantaloupe 8 days after the inundative beetle release were native to the study site, 12.9% originated from the cantaloupe release site, and 24.4% originated from cotton release site (figure 5B). The net movement of marked beetles was greater from cotton to cantaloupe ($n = 172$) than from cantaloupe to cotton ($n = 37$).

The majority of beetles (63.2%) captured in cotton 15 days after the inundative release were native to the study site, 24.7% were from the cotton release site, and 12.1% were from the cantaloupe release site. Most (71.2%) of the beetles captured in cantaloupe 15 days after the inundative release were native to the study site, 13.7% were from the cantaloupe release site, and 15.1% were from cotton release site (figure 5C). The net movement of marked beetles was greater from cantaloupe to cotton ($n = 82$) than from cotton to cantaloupe ($n = 22$).

3.2.2. Second release trial. A total of 647 marked (0.5% of the total released) beetles were recaptured from both crops during the second release trial in 1996. Only four beetles were found in the cantaloupe 8 days after release and none were found 15 days after release in cantaloupe or cotton (figure 2D).

The majority of beetles (73.7%) captured in cotton 3 days after the inundative release originated from the cotton release site, 2.5% originated from the cantaloupe release site, and 23.8% were native to the study site. Most (55.6%) of the beetles captured in cantaloupe 3 days after inundative release originated from the cantaloupe release site, 3.6% originated from the cotton release site, and 40.9% were native to the study site (figure 6A). The net movement of marked beetles between the two crops was about the same.

Most (45.7%) of the beetles captured in cotton 8 days after the inundative release originated from the cotton release site, 13.8% originated from the cantaloupe release site, and 40.5% were native to the study site. Only four beetles were collected in cantaloupe 8 days after the inundative release. Of these, one originated from the cantaloupe release site, two originated from the cotton release site, and one was native to the study site (figure 6B). The net movement of marked beetles was much higher from cantaloupe to cotton ($n = 40$) than from cotton to cantaloupe ($n = 1$).

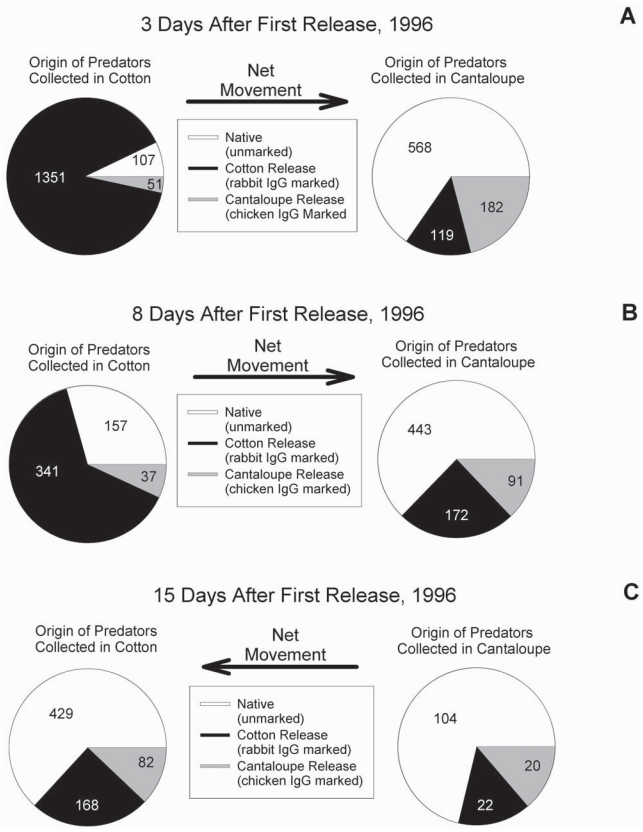

Figure 5. Origin of Hippodamia convergens *collected in cotton and cantaloupe 3, 8, and 15 days after the first initial release in 1996. The number within each slice of the pie charts is the number of beetles collected.*

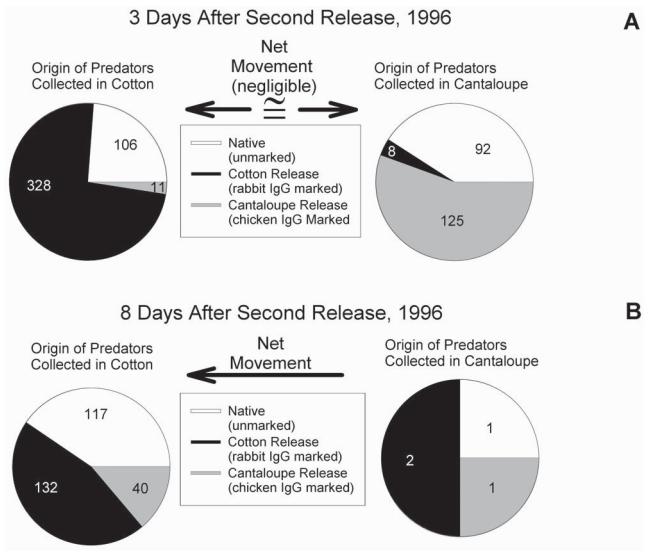

Figure 6. Origin of Hippodamia convergens *collected in cotton and cantaloupe 3, 8, and 15 days after the second initial release in 1996. The number within each slice of the pie charts is the number of beetles collected.*

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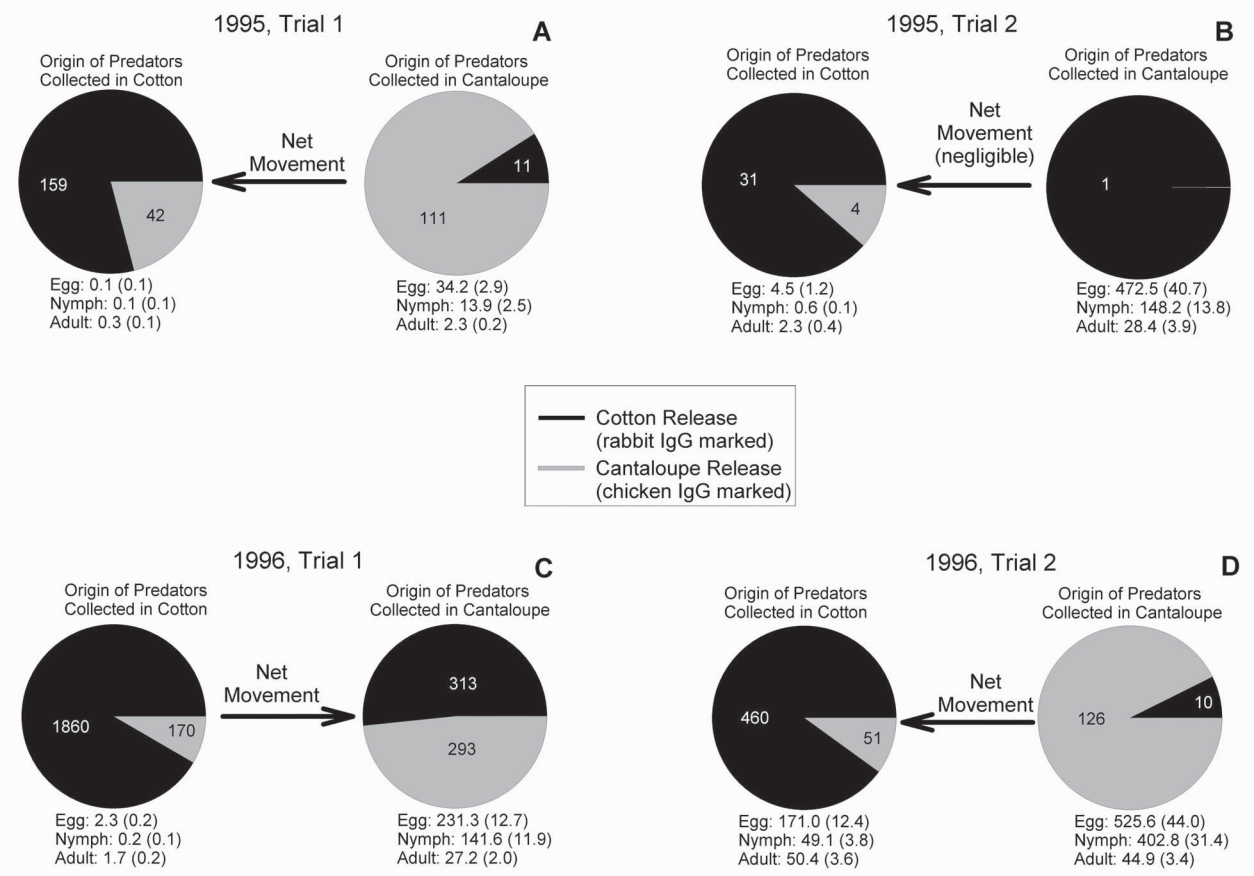

Figure 7. Origin of marked Hippodamia convergens *collected in cotton and cantaloupe over the three sampling dates of each release trial in 1995 and 1996. The number within each slice of the pie charts is the number of beetles collected. The numbers below each pie chart are the estimated mean ($\pm$ SE) densities of whitefly eggs, nymphs, and adults in each crop.*

3.3. Net movement during each release trial

The population densities of all whitefly life-stages and the net movement of marked beetles for each of the four release trials (i.e., the three sampling dates of each trial were combined) are shown in figure 7. Whitefly populations were much higher in the cantaloupe. In three of the four trials, the net movement of beetles was greater from cantaloupe to cotton than cotton to cantaloupe.

4. Discussion

Understanding the movement and feeding patterns of both native natural enemies and their augmented counterparts is of paramount importance for developing viable and trustworthy conservation and inundative biological control programs. It is critical that we have reliable methods for marking natural enemies so we can monitor the movement of large inundative releases of both predators and parasitoids. The use of vertebrate proteins for marking natural enemies is a promising alternative to many conventional marking procedures (Hagler and Jackson, 2001). Both rabbit IgG and chicken IgG were retained longer on *H. convergens* than DayGlo^{TM} dust in field cages (Hagler, 1997a) and rabbit IgG is retained well on parasitoids in laboratory and field studies (Hagler, 1997b; Hagler and Jackson, 1998; Hagler *et al.*, 2002). Antibodies used in the ELISAs are protein-specific: anti-rabbit IgG does not cross react with chicken IgG and *vice versa* (Hagler, 1997a). This facilitates the marking of different cohorts of individuals using different proteins. Here we report for the first time the application of multiple protein marks for labeling insects in a field study. The use of multiple protein marks allowed us to distinguish released beetles from their native counterparts and to monitor the intercrop movement of beetles released into separate crops.

Despite the fact that several studies have deemed *H. convergens* to be an ineffective inundative biological control agent (see Hagen, 1962; Hodek, 1967; Obrycki and Kring, 1998), we chose it for this study because it is the only predator species commercially available in large enough numbers at an affordable cost to conduct a meaningful study. Their poor performance is usually attributed to the fact that they do not remain long at their target site because of their instinct to disperse (Hagen, 1962; Kieckhefer and Olsen, 1974; Rankin and Rankin, 1980; Davis and Kirkland, 1982; Flint *et al.*, 1995; Dreistadt and Flint, 1996; Obrycki and Kring, 1998). Ideally, mass-released predators should remain at their targeted site for an extended period of time. Our results concur with the findings of others regarding their propensity to disperse after release. Over the course of this 2 year study we only recaptured 3642 of the $\approx$540 000 adult *H. convergens* we released ($\approx$0.7% of the population released). Of these, 2427, 883, and 332 were recaptured 3, 8, and 15 days after release, respectively. The steady decline in recovery rates of marked beetles with each subsequent sampling date after release support the findings of other dispersal studies showing that *H. convergens* typically leave their release sites after 2 to 4 days (Kieckhefer and Olson, 1974; Davis and Kirkland, 1982; Driestadt and Flint, 1996).

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Overall, we collected 5987 marked and unmarked beetles. The distribution of the 2345 native (unmarked) beetles was similar between the two crops with 53.6% coming from the cantaloupe and 46.4% coming from cotton. A total of 2845 beetles was recaptured that contained the rabbit IgG mark. Of these, 82.2% remained at the cotton release site and 11.8% moved from the cotton to the cantaloupe. Of the 797 beetles recaptured containing the chicken IgG mark, 66.5% remained in the cantaloupe and 33.5% moved from the cantaloupe to the cotton. The net movement of beetles between crops was highly variable. The trend was that more beetles moved from cantaloupe to cotton than cotton to cantaloupe. This was in spite of the fact that there were more whiteflies in the cantaloupe (figure 7). However, the cantaloupe was undergoing rapid senescence during the summer season. It is plausible that the dying cantaloupe was not a suitable habitat for the beetles. Legaspi *et al*. (1997) and Hagler *et al*. (2002) also suggested that a contributing factor to whitefly predator and parasitoid migration from crops was the decline in the quality of the host plant.

The protein-marking procedure and the protein-specific ELISAs described here were originally developed as a spin-off of an established predator gut content ELISA (Hagler *et al*., 1992a,b) to distinguish released predators from native ones, while simultaneously analyzing predator gut contents by a pest-specific ELISA. The whitefly-specific ELISA has been used previously to qualitatively identify key native predators of silverleaf whitefly in cotton (Hagler and Naranjo, 1994a,b). Here we used it to compare the feeding activity of commercially purchased beetles with their native counterparts. We hypothesized that commercially purchased beetles would not feed as readily on whitefly as their desert-adapted counterparts. Prior to their release, commercially purchased beetles were collected from their cool overwintering site in Oregon (Sherman, 1938; Hagen, 1962; Bennett and Lee, 1989; Roach and Thomas, 1991), shipped to Arizona, and stored for several days in a refrigerator. Then, without any preconditioning, they were released into one of the harshest desert agroecosystems in the world where daily high temperatures typically exceed 43°C. Unexpectedly, the released beetles always had a higher proportion of individuals scoring positive for whitefly remains than their desert-adapted counterparts. Moreover, the proportion of released beetles containing whitefly remains was about the same whether they were collected in cotton or cantaloupe. Perhaps the commercially purchased beetles were starved at the time of their release and they fed more readily on the predominate prey (i.e., whiteflies) than there temporally-adapted counterparts.

Even though *H. convergens* is not an ideal candidate for inundative biological control because of it tendency to disperse from targeted release sites (Obrycki and Kring, 1998), its commercial availability presented us with a valuable opportunity to validate the ELISA procedures under realistic field conditions. Recent advances in predator rearing technology may make it possible in the near future to apply these techniques to evaluate promising predaceous biological control agents that have been reared in captivity on artificial diets or on host prey (Cohen, 1992; Grundy *et al*., 2000). Furthermore, the multiple marking technique described here can be applied to minute parasitoids or insect pests to study various aspects of insect dispersal (Hagler and Miller, 2002; Hagler *et al*., 2002).

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