Employing Immunomarkers to Track Dispersal and Trophic Relationships of a Piercing-Sucking Predator, *Podisus maculiventris* (Hemiptera: Pentatomidae)

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Employing Immunomarkers to Track Dispersal and Trophic Relationships of a Piercing-Sucking Predator, *Podisus maculiventris* (Hemiptera: Pentatomidae)

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ABSTRACT Immunoproteins are markers that are useful for monitoring dispersal and/or pest consumption, but current application techniques are less effective for the large guild of piercing-sucking predators important in biocontrol. We quantified the use of protein immunomarks in tracking emigration of spined soldier bug, *Podisus maculiventris* Say (Hemiptera: Pentatomidae) and predation on the hornworm caterpillar, *Manduca sexta* L. (Lepidoptera: Sphingidae). An external protein mark was topically applied to adult *P. maculiventris* to assess persistence under field conditions for >2 wk. Internal marks were incorporated into the artificial diet of *M. sexta* to test retention of the internal mark in the prey and uptake of the mark by predators. External marks remained detectable in 100% of individuals after 3 d and >50% still tested positive at 12 d after application in the field. Internal diet-based marking was also effective in tracking feeding by *P. maculiventris* on *M. sexta*, especially using rabbit IgG that was far more persistent than chicken IgY. Nearly 90% of stink bugs fed caterpillars previously reared on protein-enriched diet retained their mark for 24 h. Surprisingly, diet concentration and time reared on diet had comparatively little impact on mark retention. Development on unmarked tomato leaves clearly diluted the initial diet mark, but plant-reared individuals that were marked were still successfully detected in 35 and 20% of the predators.

KEY WORDS biological control, protein marking, ELISA, augmentation, predation monitoring

Accurately detecting and monitoring predation events are valuable for understanding the role secondary consumers play in biological control (Symondson et al. 2002). Although in some cases this can be accomplished through direct visual observation in the field, this approach is time consuming, inefficient, and impractical for small inconspicuous insects whose normal foraging behavior may be altered by human presence (Hagler and Naranjo 1996). Postmortem gut content analysis allows researchers to detect predation events without lengthy observation periods; however, gut dissections are unacceptable for key feeding guilds such as piercing-sucking predators that liquefy their ingested prey (Luck et al. 1988, Cohen 1995).

The advent of molecular techniques, namely, enzyme linked immunosorbant assay (ELISA), and polymerase chain reaction (PCR), have modernized predation monitoring by allowing the detection of prey-specific proteins or DNA, respectively (Sheppard and Harwood 2005, Gariepy et al. 2007). While the relative merits of ELISA versus PCR have been debated, a recent methodological comparison concluded that ELISA can process samples 15-fold more rapidly at only 7% the cost of PCR (Fournier et al. 2008). This cost differential is further exacerbated by eliminating the most expensive step of ELISA—the development of species-specific monoclonal antibodies, and, instead marking prey with vertebrate immunoglobulins such as rabbit IgG or chicken IgY (Hagler and Durand 1994; Hagler 2006, 2011; Mansfield et al. 2008). These 'immunomarks' can be applied to pest and/or predator either externally (e.g., spraying onto the cuticle) or internally via integration into their diet, providing a reliable tracer (Hagler and Jackson 2001).

Although immunomarking has primarily been used for mass-marking pests and natural enemies to track dispersal, it can also be used to mark prey for predation studies. For example, Hagler (2006) reared fourth instar pink bollworm larvae, *Pectinophora gossypiella* (Saunders), on IgG-enriched artificial diet for 24 h, after which an individual larva was fed to an earwig, *Labidura riparia* (Pallas). After a subsequent 48 h starvation period, 100% of the earwigs tested positive for the presence of IgG. Earlier work, however, suggests that the success rate for this technique may depend on predator feeding guild (i.e., chewing vs. piercing-sucking mouthparts). Specifically, Hagler and Durand (1994) assessed the efficiency of IgG transfer from externally sprayed insect prey (moth eggs and whitefly adults) to two chewing predators

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(*Hippodamia convergens* (Guerin) and *Collops vittatus* (Say)) vs. two piercing-sucking predators (*Geocoris punctipes* (Say) and *Orius tristicolor* (White)). Nearly 100% of the chewers tested positive for IgG 1 hr after prey consumption compared with only 29.5% for the piercing-sucking species. Thus, immunomarking can be highly effective in documenting pest consumption, but underestimates natural enemy impact by failing to adequately capture the full diversity of feeding styles in predator communities.

The poor success rate of trophic transfer for stylet-bearing predators may, in part, be because of the methodology associated with pest marking. In the aforementioned study, insects were marked by spraying IgG onto prey, allowing them to dry, and then offering them to consumers. Given that hemipteran predators like *G. punctipes* and *O. tristicolor* pierce through the cuticle of their prey (where the applied IgG is concentrated) and feed internally, it is not surprising that few individuals acquire the mark. An alternative approach is to integrate immunomarks into diet, allowing for a potentially more persistent tracer that is expressed throughout the prey rather than simply on the surface of the cuticle. Two recent studies documented the greater success of IgG transfer to *G. punctipes* and *O. tristicolor* when prey were internally marked (Hagler 2006, 2011); however, in these studies prey were simultaneously labeled internally and externally and thus internal marking was not isolated.

While diet-based immunomarking shows great promise as a novel tool for tracking predator-pest interactions in the field, the methodology still needs to be refined for use in any predator-prey system. Specifically, we identified the following four questions. First, which immunomark, rabbit IgG or chicken IgY, is most effective? Second, what concentration (i.e., protein per volume diet) leads to the strongest and most persistent mark? Third, does exposure time to each protein (i.e., number of days reared on diet) affect the efficiency of mark transfer? Last, and most importantly, how long does the mark persist when caterpillars are transferred from enriched diet to an unmarked plant? This last question is crucial for the technique to be applied in realistic field settings. Ideally, caterpillars would initially be reared on diet in the lab and subsequently released into field plots where the predator community is sampled. We predict that mark retention will decrease with time off-diet as leaf tissue dilutes the strength of the initial signal.

Here we examine the practicality of using the immunomarking procedure to study dispersal and prey selection of the spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). Both adults and nymphs are highly polyphagous, broadly consuming lepidopteran and coleopteran larvae, but they have also been reported feeding on insects from the Ephemeroptera, Orthoptera, Heteroptera, Diptera, and Hymenoptera (McPherson 1980). It is currently the only predaceous stink bug in North America sold commercially, and has shown promise in controlling crop pests such as Colorado potato beetle (Evans 1982, Hough–Goldstein 1998, Aldrich and Cantelo 1999). However, *P. maculiventris* is also extremely mobile and thus an ideal target species to develop immunomarking techniques for evaluating postaugmentation dispersal and pest consumption in the field.

Materials and Methods

Predator. Adult *P. maculiventris* were purchased from Rincon–Vitova Insectaries (Ventura, CA). Upon arrival they were reared in mesh cages placed in a greenhouse (a photoperiod of 16:8 [L:D] h; 26°C). The cages contained an ad libitum supply of mealworms, *Tenebrio molitor* L., and tomato plants for food.

Prey. Tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae), eggs were purchased from Carolina Biological Supply (Burlington, NC; Item #143880). Eggs were placed on paper towels until neonate emergence, after which larvae were immediately used in experiments.

Experiment 1: External Predator Mark. The persistence of an externally applied immunomark on *P. maculiventris* was determined to evaluate the utility of this approach in tracking its dispersal from release sites. Approximately 150 individuals were placed in a 5 liters styrofoam cooler and sprayed with 25 ml of 1.0 mg/ml rabbit IgG (≥95% reagent grade; Sigma–Aldrich, St. Louis, MO) solution. Mosquito netting was secured on top of the cooler and individuals were allowed to air dry for 3 h before field exposure. Fifty adults were released in each of three 1 m^3 mesh cages containing 12 tomato plants. Cages were placed in an open agricultural field surrounded by tobacco, soybean, and melons. For the duration of the experiment (June–July 2011), the average temperature and rainfall were 24.3°C and 18.3 cm, respectively, with morning relative humidity at 80–90%. Approximately 15 *T. molitor* and 10 *M. sexta* larvae were placed in each cage every 3 d to provide food for the predators. Mark persistence was assessed by collecting 19–25 individual predators at 0, 3, 6, 9, 12, and 18 d postrelease. Stink bugs were stored individually in 1.5 ml microtubes at −80°C until ELISA analysis (see below). A group of 20 unsprayed individuals, reared on *T. molitor*, *M. sexta*, and tomato plants, serving as negative controls were also collected, frozen, and assayed by ELISA.

Experiment 2: Internal Prey Mark. To test the factors affecting immunomark persistence for *M. sexta* reared on protein-enriched diet, we manipulated the following four factors in a fully crossed factorial design experiment: 1) mark type (rabbit IgG vs. chicken IgY), 2) protein concentration of the diet (high vs. low), 3) time reared on protein-enriched diet (1, 2, or 3 d), and 4) time off the protein-enriched diet (0, 1, 2, 3, 5, or 7 d). In this experiment, we only assessed the effects of these factors on the persistence of the protein marks in the prey and not the transfer of the marks to the predator (see experiment 3).

Neonate larvae were reared on hornworm artificial diet (Southland Products Inc., Lake Village, AR) that contained either 0.5 or 1.5 mg/ml of rabbit IgG or chicken IgY (99% pure; Innovative Research, Novi,

MI). Diet concentrations were selected to represent a range of exposure levels based on data reported from previous studies using immunoglobulin proteins in artificial diet (Hagler and Miller 2002, Hagler 2006). The IgG or IgY was added to the diet mixture when it cooled to 50°C, which is considered cool enough to avoid denaturing the proteins, but above the temperature at which diet congeals. Exposure time on the protein-enriched diets was based on the development time of *M. sexta*, which require approximately 3 d before molting into their second instar. Thus, we compared neonates along their full range of daily exposure in the first instar. Last, time off the protein-enriched artificial diets was included to test the potential loss of the mark as a result of caterpillars feeding on their natural food source (i.e., an unmarked tomato plant). To do so, we transferred larvae from protein enriched diets to petri dishes containing tomato leaves for the allotted time, after which samples of ≈20 larvae were removed and frozen at −80°C until ELISA analysis.

Larvae fed for 2 d on standard diet without protein enhancements were used as negative controls. In addition, before the start of the experiment, the artificial diet (specifically, soybean flour, wheat germ, or proprietary mixes) was tested using the ELISA protocols described below to confirm that the ingredients do not cross-react with the protein-specific ELISAs.

Experiment 3: Internal Prey-Predator Mark Transfer. Transfer of the protein mark from an internally marked *M. sexta* to *P. maculiventris* via a predation event was evaluated to determine if this procedure can be used to monitor predation in the field. Neonate *M. sexta* were fed for 3 d on 1.0 mg/ml rabbit IgG or chicken IgY enhanced diets, after which larvae were individually placed in petri dishes containing one adult *P. maculiventris* that had been starved for 48 h. Predators were allowed to feed continuously on the protein-marked larva for 1 h, after which individuals were collected either immediately or held for 1, 2, or 4 d with water but no prey in a petri dish ($n = 20$ predators per time interval). All samples were stored at −80°C until ELISA analysis. Negative controls consisted of predators that were fed an unmarked *M. sexta* larva.

A second trial was performed using *M. sexta* reared following the aforementioned protocol on either rabbit IgG or chicken IgY enhanced diet for 3 d, but then fed for an additional 3 d on tomato leaves. After tomato exposure, larvae were fed to *P. maculiventris* as above and all hornworm-fed predators were collected and frozen immediately after feeding.

ELISA Procedure. Each predator sample was ground in 500 μl of tris-buffer saline (TBS) and assayed by the rabbit IgG and chicken IgG ELISAs described by Hagler (1997). The optical densities (OD) were measured using a microplate reader set at 650 nm, 10 min after substrate addition. A positive test was indicated by an OD reading 6 SDs above the pooled mean OD value of the negative controls as per recommendations from Sivakoff et al. (2011). Mean negative ELISA value and standard deviation were calculated across all plates for each specific experiment (e.g., all *M. sexta* negatives on rabbit tested plates, all *M. sexta* negatives on chicken tested plates).

Table 1. Treatments, replications, and percent individuals testing positive of externally marked *P. maculiventris* in experiment 1

Days after marking	*n*	% positive
0	25	100
3	25	100
6	22	82
9	22	59
12	21	57
18	19	31

Data Analysis. For all three experiments, data were statistically analyzed using Proc GLIMMIX in SAS, version 9.2 (SAS Institute, Cary, NC) with ELISA optical densities as the continuous response variable and assuming an exponential data distribution. In experiments 1–3 we used the following predictor variables, respectively: experiment 1, time; experiment 2, mark-type (chicken vs. rabbit), protein concentration (high vs. low), time reared on protein-enriched diet, and time reared on unmarked tomato leaves; and experiment 3, mark-type and time after feeding. For multi-factor analyses (experiments 2 and 3) we first analyzed the highest-order interaction term between predictor variables and sequentially removed nonsignificant interactions from the final model.

Results

Experiment 1: External Predator Mark. In total, 134 *P. maculiventris* were assayed by the rabbit IgG-specific ELISA. The mark was detected in 100% of marked individuals after 3 d and >57% of the individuals 12 d after application (Table 1; time effect: $F_{1,132} = 75.37$, $P < 0.001$). Overall, the unmarked negative control *P. maculiventris* yielded an average ELISA OD value of only 0.051 ± 0.012 (SD). The duration of external mark retention was determined to be 15.81 d (Fig. 1).

Experiment 2: Internal Prey Mark. In total, 1,328 ($n = 590$ rabbit IgG; $n = 738$ chicken IgY) *M. sexta* larvae were tested by either the rabbit IgG- or chicken IgY-specific ELISA (statistical outcome summarized

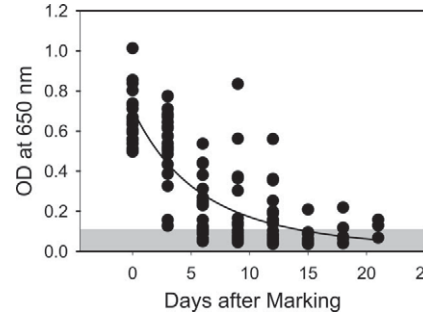

Fig. 1. Distribution of optical densities (OD) at 650 nm of *Podisus maculiventris* externally marked with rabbit IgG solution. The gray area shows the ELISA positive control threshold value (e.g., the mean negative control ELISA value 6 + SD).

Table 2. Outcome for statistical analysis in experiment 2 that evaluated the effects of four experimentally manipulated factors on caterpillar mark retention

Effect	*F*	*P*
Mark type (MT)	4.31	0.0382
Concentration	8.35	0.0039
Days on diet (DD)	0.46	0.4988
Days on tomato (DT)	119.09	<0.0001
MT × DT	17.31	<0.0001
DD × DT	8.75	0.0031
MT × DD × DT	4.14	0.0421

Only significant ($P < 0.05$) interaction terms are reported. For all effects, the numerator and denominator degrees of frequency are 1 and 1,326 respectively.

in Table 2). Overall, protein type had a large impact on mark persistence with rabbit IgG demonstrating far greater longevity compared with chicken IgY (Figs. 2 and 3). In most cases, 100% of caterpillars still tested positive for rabbit IgG after 7 d of developing on unmarked tomato, whereas only 24.8% tested positive for chicken IgY over the same time period. This pattern was evident when comparing ELISA absorbance values over time, which displayed a near instantaneous decline for chicken IgY after being placed on tomato compared with rabbit IgG values that were far more persistent. As a result, we found a highly significant statistical interaction between protein type and time on tomato.

As expected, time reared on tomato was a significant main effect in the overall statistical model, indicating that absorbance values declined with time. However, a significant interaction between time on tomato and days on diet was driven by ELISA values remaining more steady when reared for longer on diet (i.e., compare the relatively steep regression slopes in Figs. 2A and 2B with Figs. 2E and 2 F where optical density remained more steady, particularly for rabbit IgG). Last, the main effect of diet concentration was significant because of higher overall ELISA readings from caterpillars reared on high concentration diet. For example, regardless of time on diet and/or tomato, 100% of the caterpillars marked with the higher concentration of rabbit IgG tested positive, whereas several of the low concentration IgG treatments had cohorts that were <100% marked (Fig. 3). Unmarked *M. sexta* yielded OD values of 0.037 ± 0.002 and 0.037 ± 0.003 when assayed using anti-rabbit IgG and anti-chicken IgY ELISAs, respectively.

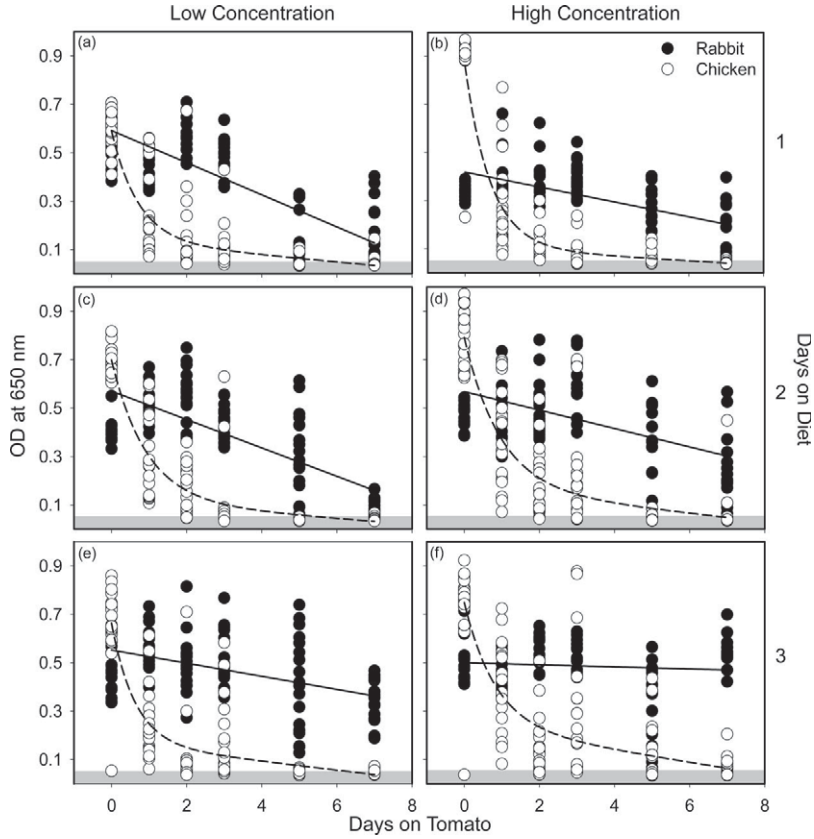

Fig. 2. Distribution of OD at 650 nm of *Manduca sexta* internally marked with rabbit (solid circles) or chicken (open circles) protein, incorporated at both low (a, c, e) and high (b, d, f) concentrations for either 1 (a + b), 2 (c + d), or 3 (e + f) days and subsequently reared on tomato leaves for 0–7 d. The gray area shows the ELISA positive control threshold value (e.g., the mean negative control ELISA value + 6 SD).

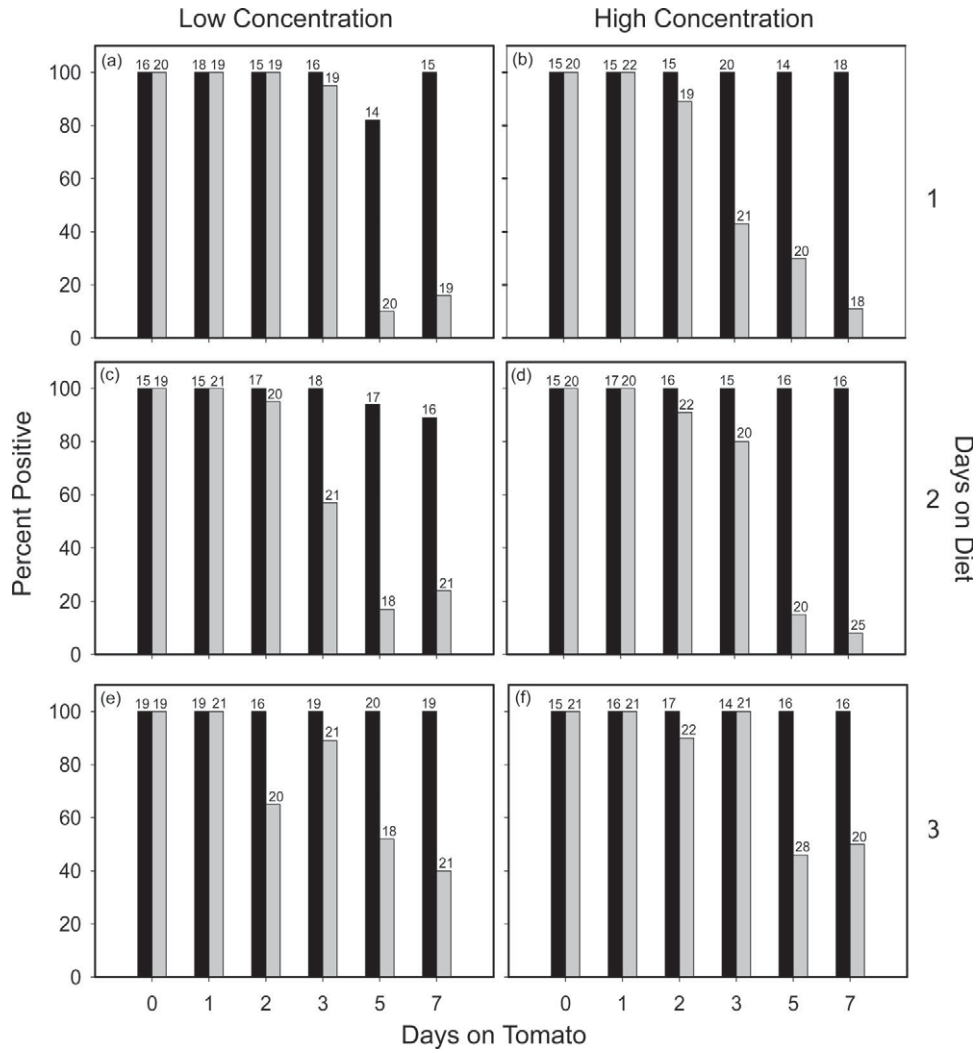

Fig. 3. Percentage of *Manduca sexta* testing positive after internally marked with rabbit (black bar) or chicken (gray bar) protein, incorporated at both low (a, c, e) and high (b, d, f) concentrations for either 1 (a + b), 2 (c + d), or 3 (e + f) days and subsequently reared on tomato leaves for 0–7 d. Values above bars indicate the number of larvae tested per group.

Experiment 3: Internal Prey-Predator Mark Transfer. In total, 196 *P. maculiventris* were analyzed by ELISA after consuming a rabbit IgG- or chicken IgY-marked prey item. As in the previous experiment, rabbit IgG was a more effective marker than chicken IgY (Fig. 4; time effect: $F_{1,152} = 47.97$, $P < 0.0001$; mark type effect: $F_{1,152} = 0.24$, $P = 0.6215$; time × mark type interaction: $F_{1,152} = 5.14$, $P = 0.0248$). This was due, however, to a statistical interaction between time after feeding and protein type. As in experiment 2, rabbit IgG was simply a more persistent mark. Four days after feeding on a marked prey item, nearly one-third of stink bugs in the rabbit IgG treatment tested positive compared with only 5% in the chicken IgY treatment, a six-fold difference (Table 3). The negative control *P. maculiventris* yielded OD values of 0.043 ± 0.006 and 0.049 ± 0.003 when assayed using anti-rabbit IgG and anti-chicken IgY ELISAs, respectively.

In our second trial, which allowed marked caterpillars to develop on unmarked tomato leaves for 3 d before being offered to stink bugs, rabbit IgG was again more persistent with 35% of predators testing positive for mark retention compared with 20% of chicken IgY-fed individuals. The average OD for *P. maculiventris* fed rabbit IgG marked larvae was 0.11 ± 0.037 compared with 0.075 ± 0.009 those fed the chicken IgY marked larvae.

Discussion

Our work has identified immunomarking techniques that can be used to monitor both predator dispersal and prey consumption involving a key biocontrol agent, *P. maculiventris*, and common vegetable pest, *M. sexta*, as its prey. Field data on external mark degradation suggest that rabbit IgG is effective for

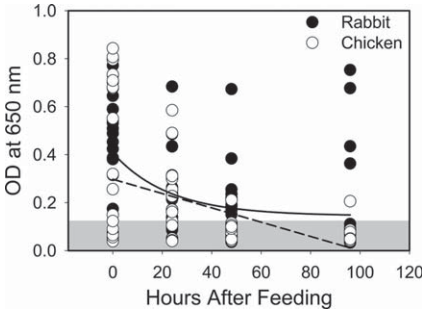

Fig. 4. Distribution of OD at 650 nm of *Podisus maculiventris* after feeding on a *M. sexta* larvae marked with rabbit (solid circles) or chicken (open circles) protein and being held for 0, 24, 48, and 96 h before analysis. The gray area shows the ELISA positive control threshold value (e.g., the mean negative control ELISA value + 6 SD).

monitoring *P. maculiventris* movement from release sites, especially during the early stages of postrelease evaluation. Within the first week of field exposure, >80% of recaptured individuals tested positive, 50–60% retained their mark during the second week, and 30–40% by the third week. Thus, like virtually all external tracers, its efficacy decreases over time. This retention time, however, is lower than for other natural enemies previously tested. Hagler (1997), for example, found that nearly 100% of *Hippodamia convergens* (Guerin) retained an external IgG mark in the field for 2 wk with fewer positives (60–90%) reported during the third and fourth weeks. We speculate that the more persistent mark exhibited for *H. convergens* is because of interspecific differences in cuticular hydrocarbons affecting protein adhesion and/or differences in abiotic factors (e.g., rain or humidity) across the two study sites. For example, relative humidity levels were <10% at the Phoenix, AZ site (J. R. Hagler, personal observation), whereas average relative humidity during this study was 80–90%.

Our work further demonstrates that internally marked prey can be detected in the gut of this piercing-sucking predator. Compared with Hagler and Durand (1994), who documented 29.5% transfer efficiency from externally labeled prey to two heteropteran predators, our estimated rate (80%) over the same time period was nearly three-times higher. In fact, a greater percentage of *P. maculiventris* retained their mark 4-d after feeding on internally marked *M. sexta* than predators fed externally sprayed herbivores and tested immediately (i.e., no time delay) in the aforementioned study. Moreover, we suspect that our data may underestimate the success rate of this technique because predators were only allowed to feed on caterpillars for 1 hr. Although this was useful for experimentally standardizing exposure level across treatments, during actual predation events in the field individuals will likely continue feeding beyond this time point. An additional advantage of internally marking prey is that the damaging stages for most pests are nymphs and larvae such as caterpillars, which shed their cuticle with each molt. In these cases, the pest would also shed the mark if externally applied, unlike diet integration. Thus, we suspect that external spraying is more effective for eggs and/or adults than immature stages that undergo molting between instars. Alternatively, double-marking (i.e., simultaneously using both internal and external marks) may be the most reliable for field studies as was the case with *G. punctipes* and *O. tristicolor* (Hagler 2011).

Table 3. Treatments, replications, and percent individuals testing positive of *P. maculiventris* after predation on an internally marked *M. sexta* larva in experiment 3

Treatment	Hours after feeding	*n*	% positive
Rabbit	0	20	80
	24	18	89
	48	20	65
	96	20	30
	Tomato	20	35
Chicken	0	20	70
	24	18	67
	48	20	10
	96	20	5
	Tomato	20	20

Of the factors we tested to improve the quantitative (ELISA OD values) and qualitative (percent positive) immunomarking performance, mark type was clearly the most important with rabbit IgG superior to chicken IgY. This matches the outcome from previous comparisons. For example, 100% of *H. convergens* retained rabbit IgG as an external mark to monitor dispersal after 14 d of field exposure, whereas 100% of chicken IgY sprayed individuals retained their mark for only 2 d postrelease after which progressively fewer beetles tested positive (Hagler 1997). Interestingly, immunoglobulin concentration and number of days caterpillars were reared on enriched diet had comparatively minor impacts on the qualitative performance of this technique. Although higher IgG concentration and longer feeding of caterpillars on diet yielded higher ELISA results, the lower IgG concentration coupled with the shorter feeding period also yielded satisfactory results. The lower concentration and shorter exposure period may be beneficial for two reasons: 1) IgG and IgY are relatively expensive proteins and these data suggest their usage can be minimized without sacrificing sensitivity of the ELISAs, and 2) only rearing caterpillars for 24 h before release is more amenable to mass production of sentinel prey.

Rearing caterpillars on tomato leaves clearly reduced the degree to which immunomarks successfully transfer to consumers. For example, 80 and 70% of the *P. maculiventris* tested positive for rabbit IgG and chicken IgY, respectively, when tested immediately after consuming a marked caterpillar, whereas only 35 and 20% tested positive after a marked caterpillar developed on tomato for 3-d. This is surprising because rearing caterpillars on tomato for 3-d had no ostensible impact on IgG concentration when measured directly in the prey. This emphasizes the need to evaluate mark persistence postconsumption instead of inferring mark transfer based on concentration in the prey's body. Many predators selectively feed on

specific prey parts (Cohen 1995), and thus quantifying mark strength in the whole prey may overestimate (or underestimate, depending on predator feeding behavior and where immunomarks are localized) the persistence of the signal.

For future studies, we intend on using both mark types to evaluate the role of behavior-modifying semiochemicals on *P. maculiventris* emigration and focal prey consumption in the field. Based on the data collected here, a field study would be most reliable if predators were recaptured within 24 h of a predation event, to prevent compromising the accuracy of the gut content assay results (e.g., false negatives). Because the vast majority of existing work has fine-tuned this approach in laboratory settings, future studies would benefit from attempting to apply immunomarks in open-field agriculture to monitor biocontrol by naturally occurring arthropods.

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References Cited

Aldrich, J. R., and W. W. Cantelo. 1999. Suppression of Colorado potato beetle infestation by pheromone-mediated augmentation of the predatory spined soldier bug, Podisus maculiventris (Say) (Heteroptera: Pentatomidae). Agric. Forest Entomol. 1: 209–217.

Cohen, A. C. 1995. Extra-oral digestion in predaceous terrestrial arthropods. Annu. Rev. Entomol. 40: 85–103.

Evans, E. W. 1982. Timing of reproduction by predatory stinkbugs (Hemiptera : Pentatomidae): Patterns and consequences for a generalist and a specialist. Ecology 63: 147–158.

Fournier, V., J. R. Hagler, K. Daane, J. de Leon, and R Groves. 2008. Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera : Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. Oecologia 157: 629–640.

Gariepy, T. D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. J. Appl. Entomol. 131: 225–240.

Hagler, J. R. 1997. Field retention of a novel mark-release-recapture method. Environ. Entomol. 26: 1079–1086.

Hagler, J. R. 2006. Foraging behavior and prey interactions by a guild of predators on various lifestages of *Bemisia tabaci*. Ann. Appl. Biol. 149: 153–165.

Hagler, J. R. 2011. An immunological approach to quantify consumption of protein-tagged *Lygus hesperus* by the entire cotton predator assemblage. Biol. Control. 58: 337–345.

Hagler, J. R., and C. M. Durand. 1994. A new method for immunologically marking prey and its use in predation studies. Entomophaga 39: 257–265.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. Annu. Rev. Entomol. 46: 511–543.

Hagler, J. R., and S. E. Naranjo. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study, pp. 383–399. *In* W.O.C. Symondson and J. E. Liddell (eds.), Ecology of Agricultural Pests: Biochemical Approaches. Chapman & Hall, London, United Kingdom.

Hough–Goldstein, J. A. 1998. Use of predatory Pentatomids in integrated pest management of the Colorado potato beetle (Coleoptera : Chrysomelidae), pp. 209–223. *In* M. Coll and J. R. Ruberson (eds.), Predatory Heteroptera: Their Ecology and Use in Biological Control. Entomological Society of America, Lanham, MD.

Luck, R. F., B. M. Shepard, and P. E. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Annu. Rev. Entomol. 33: 367–391.

Mansfield, S., J. R. Hagler, and M.E.A. Whitehouse. 2008. A comparative study on the efficacy of a pest-specific and prey-marking enzyme-linked immunosorbent assay for detection of predation. Entomol. Exp. Appl. 127: 199–206.

McPherson, J. E. 1980. A list of the prey species of *Podisus maculiventris* (Hemiptera, Pentatomidae). Gt. Lakes Entomol. 13: 17–24.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19: 751–762.

Sivakoff, F. S., J. A. Rosenheim, and J. R. Hagler. 2011. Threshold choice and the analysis of protein marking data in long-distance dispersal studies. Methods Ecol. Evol. 2: 77–85.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11: 627–641.

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