



Semiochemical lures reduce emigration and enhance pest control services in open-field predator augmentation



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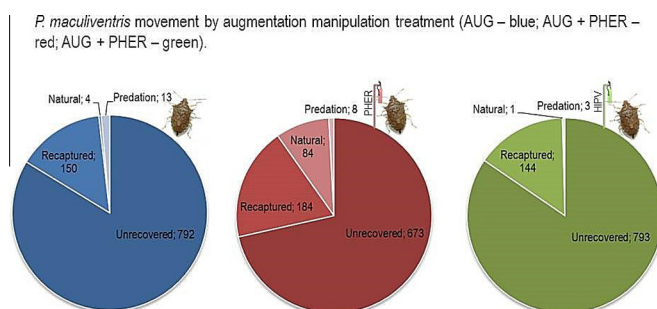
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HIGHLIGHTS

- Retention rates of augmented *P. maculiventris* were increased in optimal weather.
- Optimal weather + aggregation pheromone further reduced *P. maculiventris* emigration.
- Semiochemical lures elicited point-source attraction of predators.
- Augmented and wild *P. maculiventris* contributed to pest control services.
- Parasitoids (Diptera and Hymenoptera) were attracted to both pheromone and HIPVs.

GRAPHICAL ABSTRACT



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ABSTRACT

Augmentation biocontrol is a commercially viable pest management tactic in enclosed glasshouse environments, but is far less effective in open-field agriculture where newly released enemies rapidly disperse from release sites. We tested the potential for behavior-modifying semiochemicals to increase the retention of mass released predatory stink bugs, *Podisus maculiventris* Say (Hemiptera: Pentatomidae), for enhanced consumption of hornworm caterpillars, *Manduca sexta* L. (Lepidoptera: Sphingidae). To do so, we used controlled-release dispensers to emit the herbivore-induced plant volatile, methyl salicylate (MeSA), or *P. maculiventris* aggregation pheromone from tomato field plots. Overall, we recaptured ca. 17% of released individuals after 36 h. This rate, however, was significantly affected by weather (12% vs. 22% recapture in rainy vs. dry weeks, respectively) and semiochemical deployment, but only under optimal weather conditions (19% vs. 26% recapture in control vs. pheromone plots, respectively, during dry weeks). Further, we detected behavioral responses of wild *P. maculiventris* to semiochemical treatment with 94% of all captured adults (=84 of 89 total) found in pheromone plots. Only 24 of 567 (4%) captured stink bugs tested positive for immunomarking, suggesting that hornworm predation occurred but at a low frequency. Importantly, we documented that sentinel caterpillar prey were depleted by predators at a higher rate in stink bug augmented plots on tomato plants occurring near (<3 m from) the MeSA and pheromone lures. These data empirically demonstrate that both semiochemicals are capable of increasing pest consumption via attraction of *P. maculiventris*. Future work should focus on mechanisms of lure attraction and the long-term consequences of predator development in fields with elevated semiochemical emissions.

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1. Introduction

Augmentation biological control (hereafter, “augmentation”) is the practice of mass releasing natural enemies in a pest-infested crop, aimed at obtaining more effective control than that provided by naturally-occurring predators and parasitoids. While this technique has potential application in a wide range of agricultural systems, it has primarily been employed in greenhouses for the management of horticultural pests such as whiteflies, thrips, and mites (Gerling et al., 2001; Gillespie, 1989; van Lenteren et al., 1997; van Lenteren and Woets, 1988). Implementation in open-field agriculture is far less prevalent and frequently unsuccessful when tested. For example, only 15% of experimental field studies effectively reduced pest abundance to target densities, whereas 64% failed (Collier and Van Steenwyk, 2004). Importantly, dispersal of augmented beneficials beyond the targeted area was noted as a central factor underlying the failure of these release programs. Heimpel and Asplen (2011) also highlighted excessive dispersal as a key mechanism driving the failure of inundative biocontrol releases. For example, only 9 lady beetles (*Hippodamia convergens* (Guérin-Méneville)) were recaptured 24 h after releasing 7125 adults into wheat, corn, and alfalfa fields, yielding a recapture rate of merely 0.1% (Kieckhefer and Olsen, 1974). Later attempts released an astounding 2,250,000 individuals and after just 4 days not a single released predator was detected in the field (Kieckhefer and Olsen, 1974). With such poor retention, far more enemies must be purchased than are actually needed to account for loss due to emigration, making augmentation cost ineffective as a pest management tool for the vast majority of crops.

Restricting natural enemy movement can reduce emigration, thereby enhancing the per-capita impact of released individuals on prey. Prior work has mostly emphasized techniques that physically manipulate wings to discourage or prevent flight (e.g., clipping, artificial selection, identification of natural flightless strains), which have proven quite effective (Ferran et al., 1998; Ignoffo et al., 1977; Lommen et al., 2008, 2013; Seko et al., 2008) but also come with ecological and/or physiological costs. Namely, flightless predators have shown reduced survival and fecundity compared with flight-capable individuals (Seko and Miura, 2009). Integrating behavior-modifying semiochemicals with predator or parasitoid releases, however, may decrease the dispersal of augmented beneficials without the associated costs of flightlessness. Two promising candidates include herbivore-induced plant volatiles (HIPVs) and aggregation pheromones.

HIPVs are chemicals released from plants after herbivore feeding damage that are often used by higher trophic level arthropods to locate their prey (Kessler and Baldwin, 2001; Thaler, 1999; Turlings et al., 1990). Recent reviews have stressed the utility of HIPV manipulations in modern biocontrol research (Kaplan, 2012; Khan et al., 2008; Rodriguez-Saona et al., 2012; Turlings and Ton, 2006). However, these perspectives and the existing empirical work exclusively focus on applications of HIPVs as attractants in conservation biocontrol to increase recruitment of naturally-occurring enemies into crop fields (James, 2003; James and Price, 2004; Mallinger et al., 2011; Rodriguez-Saona et al., 2011). HIPVs could serve an analogous role in augmentation by acting as arrestants, decreasing emigration from release sites. To date, no study has tested this hypothesis.

Aggregation pheromones are compounds emitted by male insects that are attractive to conspecifics, including both sexes and multiple life stages (i.e., adults + immatures) (Matthews and Matthews 2010). In pest management, aggregation pheromones are used in attraction-annihilation whereby insecticide-baited pheromone traps are used to lure and kill pests (Lanier, 1990). In comparison, relatively few aggregation pheromones have been identified for beneficial arthropods. The only case in which a natural

enemy aggregation pheromone has been used in biocontrol came after the identification and synthesis of the aggregation pheromone from the spined soldier bug, *Podisus maculiventris* (Say) (Aldrich et al., 1984). Two companion studies used synthetic pheromone in dispensers bordering the release plot to facilitate the movement of augmented nymphs from their hatching site into pest-infested potato fields (Aldrich and Cantelo, 1999; Sant’Ana et al., 1997). Thus, similar to the mechanisms by which HIPVs function, aggregation pheromones may be useful in increasing natural enemy retention time in an area, as well as recruiting natural populations from adjacent habitats.

We tested the hypothesis that HIPVs and aggregation pheromone reduce emigration of augmented predators from field plots and increase pest consumption in a crop-pest-predator system consisting of tomato, hornworm caterpillars (*Manduca sexta* L.), and the predaceous stink bug, *P. maculiventris*.

2. Materials and methods

2.1. Study system

The tobacco hornworm, *M. sexta*, is a specialist herbivore on solanaceous plants and a common defoliating pest of tomato throughout the United States with tendencies to outbreak in the Northeast and Northern Midwest (Foster and Flood, 2005). Hornworms used in this project derived from a laboratory colony maintained in West Lafayette, Indiana and were reared as neonates on artificial diet until use in field trials (see Section 2.5).

The stink bug, *P. maculiventris*, is a native generalist predator, predominantly consuming lepidopteran and coleopteran larvae, which feeds by piercing prey and sucking their internal fluids via its rostrum. It has shown promise as a biocontrol agent for inundative releases (Biever and Chauvin, 1992a,b; Evans, 1982; Hough-Goldstein, 1998) and is currently the only commercially available predaceous stink bug in North America (Rincon-Vitova Insectaries, Ventura, CA). Adult *P. maculiventris* used in our experiment were maintained in a laboratory colony established with insects from Rincon-Vitova Insectaries and supplemented yearly with field caught individuals from local populations. The colony was maintained at 16:8 LD at 26 °C with bean and tomato plants for water and *ad libitum* mealworms, *Tenebrio molitor* L., as prey.

2.2. Experimental design

Sixteen 100 m² field plots, each containing ca. 100 tomato plants (5 rows × 20 plants/row) with >75 m inter-plot spacing, were established during the summer of 2012 on the Meigs Farm at Throckmorton Purdue Agricultural Center (Lafayette, IN, USA). Processing tomato seedlings (RG-611; Red Gold Inc., Elwood, IN, USA) were transplanted from mist-houses into plastic-covered, raised beds in late May. All plots received drip irrigation, fertilizer, and herbicide applications as needed to manage weeds, but insecticides were not used.

Plots were assigned to one of four augmentation/semiochemical treatments in a randomized complete block design, each block replicated four times: (i) *P. maculiventris* release + aggregation pheromone [AUG + PHER]; (ii) *P. maculiventris* release + HIPV [AUG + HIPV]; (iii) *P. maculiventris* release + no semiochemicals [AUG]; and (iv) No *P. maculiventris* release + no semiochemicals [CTRL].

2.3. Semiochemical treatments

The *P. maculiventris* aggregation pheromone was formulated with the three primary components in the following ratio: 7.6% (E)-2-hexenal, 0.4% benzyl alcohol and 92% α -terpineol

(Sigma–Aldrich, St. Louis, MO, USA). The pheromone was released from 5 ml polyethylene screw-cap vials (Wheaton Science Products, Millville, NJ, USA). Two holes (2 mm diameter) were drilled in each vial, which allowed for a string to hold the vial on a bamboo stake, as well as to facilitate pheromone diffusion. Each vial contained 4 mL of the pheromone mixture and was replaced every two weeks for the duration of the experiment (release rate = ca. 75 mg/day). Two release devices were placed at plant terminal height within the outside rows at opposite corners of each plot assigned to pheromone enhancement (Fig. 1) during the first week of July, one week prior to the first predator release. Empty vials on stakes but without pheromone were added as structural controls in both CTRL and AUG treatment plots. For the HIPV application we used 40d Predalure (AgBio Inc., Westminster, CO), a commercially available methyl salicylate (MeSA) lure that is attractive to numerous natural enemy taxa (James and Price, 2004; Rodriguez-Saona et al., 2011).

2.4. Augmentation

Predator releases were conducted in one replicate block (although only 3 of the plots received supplemental predators, CTRL did not) each week from July through September (12 total weeks). Prior to each release, 300 mixed-age adult *P. maculiventris* (50:50 M:F sex ratio) were removed from the laboratory colony. Each individual was marked on its pronotum using fine-tipped nontoxic paint markers (Treehouse Studio, Oklahoma City, OK, USA) to identify their release plot (1–16) and date (1–12). A marking system employing two different colored dots allowed for unique identification of all 36 cohorts (3 plots/week × 12 weeks) released throughout the season. Adults were placed in individual containers with water and one *M. sexta* neonate larva 48 h prior to release. Approximately 1 h before release, we combined adults of a cohort into a large plastic container. All releases were conducted in late afternoon (ca. 18:00) when temperatures were declining to avoid heat-related mortality. Each container was

placed in the center of the assigned plot and the top was removed, allowing free movement of the augmented *P. maculiventris* into the plot. Thirty-six hours later, each plot was visually searched for 10 min, at which time all encountered adult *P. maculiventris* (i.e., augmented or naturally occurring) were collected, identified, recorded, and stored at -80°C for future analysis in the predation assay.

2.5. Predation assay

To assess the impact of our semiochemical treatments on hornworm predation, we employed two different analysis techniques. First, we created prey reservoirs on 10 plants per plot, chosen with stratified randomization (see Fig. 1) on the day prior to *P. maculiventris* augmentation. On each plant, a large tomato leaf was labeled with field tape and ten 1st and 2nd instar *M. sexta* larvae were distributed across the leaflets. Prey reservoirs were recounted 48 h later, immediately following *P. maculiventris* recapture, and the number of caterpillars missing used as a proxy for predation pressure. Based on previous experience with this system, early instar hornworm caterpillars are relatively sedentary and do not move between leaves or plants. Further, individuals rarely drop from plants, except in extreme weather. Thus, missing hornworms were assumed to have been killed by predators. Daily predation rate was calculated per plant and averaged across the 10 reservoirs to create a plot-level average.

In addition to changes in prey density, we used immunomarking to track actual field predation. Immunomarks are vertebrate immunoglobulin proteins that can be used as a biochemical tracer to directly quantify predation events (see Hagler, 2006, 2011; Hagler and Durand, 1994). We recently developed an immunomarking protocol using the tomato–*M. sexta*–*P. maculiventris* system through which we determined that caterpillars reared on rabbit IgG-infused artificial diet retained their mark for at least 7 days after being transferred to unmarked tomato leaves (Kelly et al., 2012). More importantly, the mark transfers from caterpillar to stink bug during predation events and remains detectable in predators for ca. 48 h. Thus, we marked all caterpillars deployed in the aforementioned prey reservoirs with rabbit IgG prior to field releases. Before hatching, *M. sexta* eggs were placed in petri dishes on 1.0 mg/mL rabbit IgG-enriched artificial diet following methods in Kelly et al. (2012) (rabbit IgG: Equitech-Bio Inc., Kerrville, TX, USA; hornworm diet: Southland Products Inc., Lake Village, AR, USA). Neonates were reared on enhanced diet for ca. 3 days to ensure adequate mark uptake and then transferred to the field for placement as prey reservoirs as noted above. Recaptured *P. maculiventris* were later assayed using ELISA for presence of the rabbit IgG mark indicating predation on *M. sexta* larvae (see Section 2.6).

2.6. ELISA procedure

Each predator sample was ground in 500 μL of tris-buffer saline (TBS) and assayed by the rabbit IgG ELISA described by Hagler (1997) and Kelly et al. (2012). 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 standard deviations above the pooled mean OD value of all negative controls, as recommended by Sivakoff et al. (2011). Mean negative ELISA value and standard deviation were calculated across all plates.

2.7. Scouting

Weekly scouting was conducted in all 16 plots to collect information about naturally-occurring herbivores and natural enemies. Plants were visually surveyed for insect presence with a focus on study-specific pests (e.g., Lepidoptera, Pentatomidae), as well as other

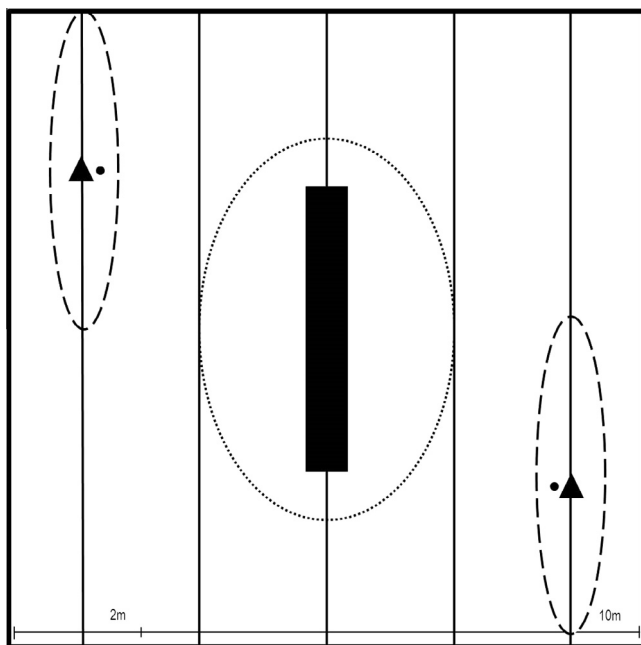


Fig. 1. Schematic of experimental plot. Black lines delineate tomato rows (20 plants/row). Triangles = placement of semiochemical treatments. Circles = yellow sticky cards. Rectangle = location of weekly augmentation release containers. Prey reservoirs for monitoring caterpillar predation were placed on plants either “NEAR” (large dash) or “FAR” from lures (dotted).

important tomato herbivores (e.g., Thripidae, Aleyrodidae, Aphididae) and affiliated natural enemies (e.g., Anthorcoridae, Tachinidae, *Cotesia congregata*, other parasitic Hymenoptera). Scouting involved visual plant surveys for a total of 10 min per plot. In addition, 2 yellow sticky cards were placed in opposite corners of each plot, between the outer rows directly adjacent to the semiochemical lures at mid-plant height, and exchanged every two weeks to monitor populations.

2.8. Statistical analyses

For mark-release-recapture data, ANOVA was used to analyze the proportion of *P. maculiventris* recaptured with both treatment and rainfall as categorical predictor variables. Proportion data were arcsine square-root transformed to normalize the distribution. Rainfall was treated as a categorical rather than continuous variable because precipitation was virtually non-existent for the entire week or extremely high with no intermediate values across the summer. On-site weather station data were used to separate augmentation trials by rainfall into two groups: (i) those that had little to no rain in 36 h post-release and <1 inch total for the week (hereafter, 'DRY') vs. (ii) those that had rainfall at some point during the 36 h post-release period and a weekly total >1 inch (hereafter, 'WET'). Neither plot nor block had an effect on recapture and were thus removed from both this model and all subsequent analyses.

For naturally-occurring *P. maculiventris*, numbers were relatively low, with only 89 total insects collected. Therefore, data were summed per plot over the season and analyzed with ANOVA using square root + 0.5 transformed counts as the dependent factor. One difference in our analysis of released versus naturally-occurring *P. maculiventris*: for released individuals, we only analyzed data for the 3 treatments that were used to test augmentation (i.e., not the CTRL), whereas data from all 4 treatments were used for wild individuals.

For hornworm predation assays, the average number of *M. sexta* larvae consumed per day (i.e., the number missing compared with starting density) per plot was square root transformed and analyzed with ANOVA. Initially, only one value per plot (i.e., the overall average of 10 plants) was used for predation analysis; however, due to the likelihood that semiochemicals may be effective on a relatively small (e.g., <5 m) scale we also analyzed the data according to plant distance from lures. For this, subsets of data based on the plot location (Fig. 1; FAR = > 2.5 m from a lure, NEAR = < 2.5 m from a lure) were analyzed to test for treatment effect according to different sampling regimes (FAR or NEAR from lures). The transfer of protein mark to *P. maculiventris* was also analyzed with ANOVA on the proportion of individuals per plot testing positive for predation over the season.

The impact of semiochemical treatment on arthropod communities was tested using individual ANOVAs on the square root + 0.5 transformed plot sums for all arthropod species that had seasonal totals ≥ 50 individuals.

All analyses were completed using Statistica (vers. 11; StatSoft, Tulsa, OK, USA).

3. Results

3.1. Augmentation recapture

Semiochemical treatment, overall, did not affect the proportion of marked *P. maculiventris* recaptured (Table 1; $F_{2,24} = 1.54$, $p = 0.2381$); however, low rainfall significantly increased retention ($F_{1,24} = 19.82$, $p = 0.0002$). Releases in DRY weeks had 78% higher recapture rates than WET, regardless of semiochemical treatment ($\bar{X}_{\text{DRY}} = 0.218$; $\bar{X}_{\text{WET}} = 0.122$). Although there was no significant interaction between treatment and rainfall ($F_{2,24} = 0.81$,

$p = 0.4558$), planned comparisons indicate AUG + PHER baited plots improved retention under DRY conditions (Fig. 2; $F_{1,24} = 4.54$, $p = 0.0436$).

Semiochemical treatment, however, increased the number of wild *P. maculiventris* collected (Table 1; $F_{3,12} = 38.55$, $p < 0.0001$). Planned comparisons confirmed the hypothesis that more natural *P. maculiventris* were recovered in aggregation pheromone lure baited plots ($F_{1,12} = 114.25$, $p < 0.0001$), with 94% of all individuals collected over the summer occurring in the AUG + PHER treatment (Fig. 3).

3.2. Predation on prey reservoirs

Semiochemical treatment alone had a marginal effect on predation of hornworm caterpillars when tested using all caterpillars in the plot (Fig. 4a; $F_{3,44} = 2.11$, $p = 0.1120$). When data were restricted to either samples taken "near" or "far" from lures, however, we found strong effects of plot-level treatment. Namely, caterpillars far from the lures at the center of release sites experienced higher predation in the semiochemical-free AUG treatment (Fig. 4b; $F_{3,44} = 6.97$, $p = 0.0006$), whereas the opposite was the case when analyzing data using caterpillars near lures (Fig. 4c; $F_{3,44} = 7.19$, $p = 0.0005$).

3.3. Protein mark transfer to *P. maculiventris*

Of 567 *P. maculiventris* captured, including both released and wild individuals, only 24 (4.2%) tested positive for rabbit IgG, indicating recent predation on *M. sexta* larvae (Table 1). No treatment effect was observed for the proportion of predators testing positive per plot, although this effect was marginally significant ($F_{2,9} = 3.12$, $p = 0.0942$).

3.4. Arthropod communities

No effects of semiochemical treatment were found on the abundance of any herbivore, omnivore or predator (Table 2). However, we detected significant effects on tachinid flies (Fig. 5a; $F_{3,12} = 7.42$, $p = 0.0045$) and parasitic Hymenoptera (Fig. 5b; $F_{3,12} = 4.28$, $p = 0.0284$). Both parasitoids were more abundant in MeSA- and pheromone-baited plots (Hymenoptera [$F_{1,12} = 12.73$, $p = 0.0039$]; Tachinidae [$F_{1,12} = 20.25$, $p = 0.0007$]).

We only collected 11 total non-*Podisus* Pentatomidae (0 in CTRL, 2 in AUG, 6 in AUG + PHER and 3 in AUG + HIPV). Wild caught *M. sexta* were included in the Lepidoptera category; however, only 3 individuals were collected (1 in CTRL, 0 in AUG, 0 in AUG + PHER and 2 in AUG + HIPV). We also recovered 9 *C. congregata* (Hymenoptera: Braconidae) parasitized *M. sexta* caterpillars (1 in CTRL, 3 in AUG, 0 in AUG + PHER and 5 in AUG + HIPV). Although these insect groups are ecologically pertinent to the study system, they were not statistically analyzed because of low counts.

4. Discussion

This work demonstrates the important role semiochemicals play in mediating natural enemy behavior in cases of augmentation, most notably when optimal weather conditions are exploited. The uniformly low recapture rates across all treatments in WET compared with DRY weather suggest that abiotic factors should be a primary consideration for timing releases. Little to no rain during and immediately following augmentation resulted in retention rates 78% higher than adverse conditions. This outcome corresponds with Collier and Van Steenwyk's (2004) review, which highlighted 'unfavorable environment' as the most commonly cited ecological factor limiting the success of augmentation among

Table 1
Raw data for augmented, recaptured and naturally occurring *P. maculiventris* by plot treatment. Data show number released, number recaptured, proportion recaptured, number of wild individuals, number of individuals testing positive for hornworm consumption ('predation events'), and proportion of recaptured individuals testing positive for hornworm predation.

Treatment	No. released	No. recaptured	Proportion recaptured	No. wild	Predation events	Proportion marked
AUG	946	150	0.159	4	13	0.084
AUG + PHER	941	184	0.196	84	8	0.030
AUG + HIPV	938	144	0.154	1	3	0.021
Total	2825	478	0.170	89	24	0.042

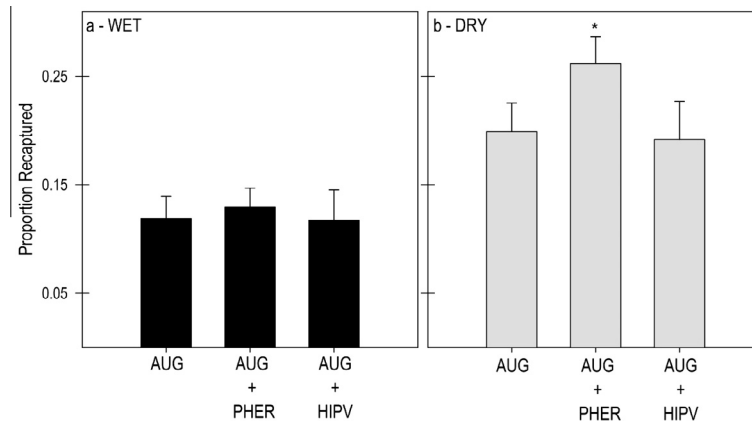


Fig. 2. Proportion (mean + SE) of released *P. maculiventris* recaptured 36 h post release by treatment in WET (a) and DRY (b) weeks. Gray bars show data from DRY weeks with <1 inch weekly total rain and black bars show WET with >1 inch. Only AUG + PHER had a significantly higher recapture rate between DRY and WET conditions. Asterisk indicates significant difference from the control.

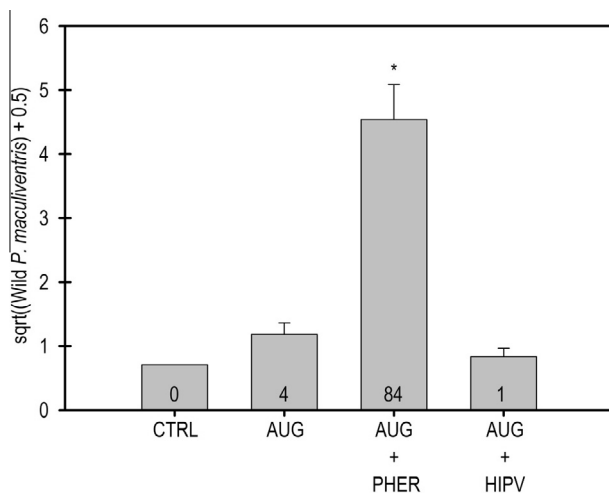


Fig. 3. Number of naturally collected *P. maculiventris* per plot (mean + SE) across treatments. Asterisk indicates significant difference from the control. Numbers inside gray bars are raw data for total number summed over the season.

published field studies. Importantly, our work differs from these previous accounts in two respects. First, previous studies defined 'unfavorable environment' as hot and/or dry conditions. In our study, temperature variation played no role in the likelihood of stink bug recapture (data not shown) and unlike previous studies precipitation was opposite from the current trend – excessive rainfall, rather than lack of rain, reduced recapture rates. Second, most of the cited study systems affected by abiotic factors deployed predaceous mites. This taxonomic difference is notable because mites are small arthropods that are perhaps more likely to be adversely affected by weather, whereas predaceous stink bugs represent one of the largest and most robust predators commercially

available. The unusual weather patterns during the summer of 2011 in the Midwestern U.S., which was marked by severe drought punctuated by brief periods of intense rainfall, may be partially responsible for this effect. It remains unclear whether excessive rain elicited emigration of stink bugs from plots or increased mortality rates. However, it was not uncommon to encounter dead *P. maculiventris* from augmented cohorts, particularly around the plant base where plastic beds were torn and/or pitted.

When weather conditions were DRY, higher recapture rates in the AUG + PHER plots indicates that deployment of aggregation pheromone-baited lures decreased *P. maculiventris* emigration. A comparable phenomenon was reported in two published field studies where strategic placement of pheromone-baited lures mediated *P. maculiventris* nymph dispersal (Aldrich and Cantelo, 1999; Sant'Ana et al., 1997). Both studies only augmented nymphs, reducing the likelihood for loss due to emigration because they cannot fly from the release site. Conversely, the broad-spectrum HIPV, MeSA, did not affect *P. maculiventris* recapture rates. This may be due to a lack of prior learning experience with the compound. Generalist predators are known to require associative learning (i.e., pairing of stimulus with food reward) for developing attraction to HIPVs, including MeSA (Allison and Hare, 2009; Drukker et al., 2000; Glinwood et al., 2011), and insectary-derived individuals lack this association. A pre-release training regime that consisted of volatile + food reward exposure is worth considering for future efforts that integrate HIPVs with augmentation biocontrol. However, the fact that wild stink bugs also selectively responded to pheromone and not HIPVs (Fig. 2) suggests that MeSA may simply be a poor candidate for attracting *P. maculiventris*. To our knowledge, no published studies have documented pentatomids responding to MeSA in the field.

Field predation assays revealed several key patterns. First, although many stink bugs emigrate from semiochemical-free plots, some fraction clearly remain at the release site and feed on prey (compare CTRL vs. AUG in Fig. 4a), suggesting that augmentation

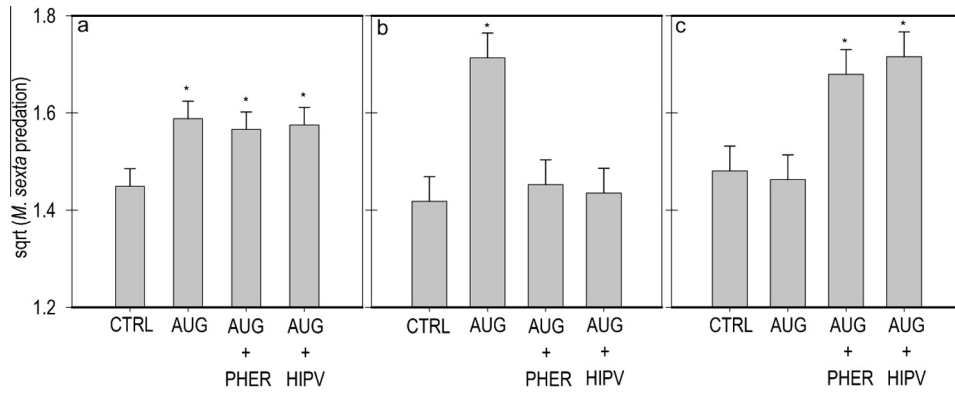


Fig. 4. Number of *M. sexta* consumed per day (mean + SE) by overall treatment (a) and treatment × location within plot (b – FAR from the semiochemical lure, c – NEAR the semiochemical lure). Asterisks indicate significant difference from the control.

Table 2

Individual ANOVA results for the effect of semiochemical treatment on the tomato arthropod community. Asterisks indicate significant effect of treatment on abundance.

Arthropod group	$F_{3,12}$	p
<i>Herbivore</i>		
Aleyrodidae	1.53	0.2528
Aphididae	1.35	0.3035
Cicadellidae	0.64	0.5746
Lepidoptera	1.21	0.3489
Thripidae	0.91	0.4665
<i>Omnivore</i>		
Berytidae	0.19	0.9019
<i>Predator</i>		
Total Complex	1.06	0.4033
<i>Parasitoid</i>		
Hymenoptera*	4.28	0.0284
Tachinidae*	7.42	0.0045

alone provides some baseline pest suppression value. Interestingly, this effect only occurred within a few meters of the release location and not at the outer edges of the plot (compare CTRL vs. AUG in Fig. 4b vs. 4c). Thus, the few retained *P. maculiventris* display a natural tendency to remain within several meters of their release. Second, MeSA and pheromone were quite effective at ‘pulling’ stink bugs from the central release site to increase predation near lures on the outer edges of plots (compare CTRL vs. AUG + PHER and AUG + HIPV in Fig. 4b vs. 4c). These combined data provide evidence that semiochemicals manipulate *P. maculiventris* behavior to enhance pest consumption. This enhancement, however, came with a cost; namely, pulling predators to plot edges reduced their impact on caterpillars in the plot center. Recent work has warned of this phenomenon (the ‘robbing Peter to pay Paul’ effect) whereby semiochemical-mediated movement of natural enemies initiates downstream effects, manifested as aggravated pest outbreaks in adjacent areas ‘robbed’ of their consumers (Braasch and Kaplan, 2012; Jones et al., 2011). Unlike our mark-release-recapture evaluation, the predation assay data suggest that MeSA worked as well as pheromone in manipulating stink bug behavior. It would be interesting for future studies to also test the combined rather than singular impacts of HIPVs and pheromone. Recent work screening semiochemical blends for *Chrysopa* sp. lacewing attraction in apple orchards demonstrated strong synergism between HIPVs (MeSA) and aggregation pheromone (iridodial) when presented together (Jones et al., 2011).

While protein marking confirmed that *P. maculiventris* indeed consumes hornworms in the field, the rate was quite low (4.2%), limiting our ability to draw conclusions from this technique. The

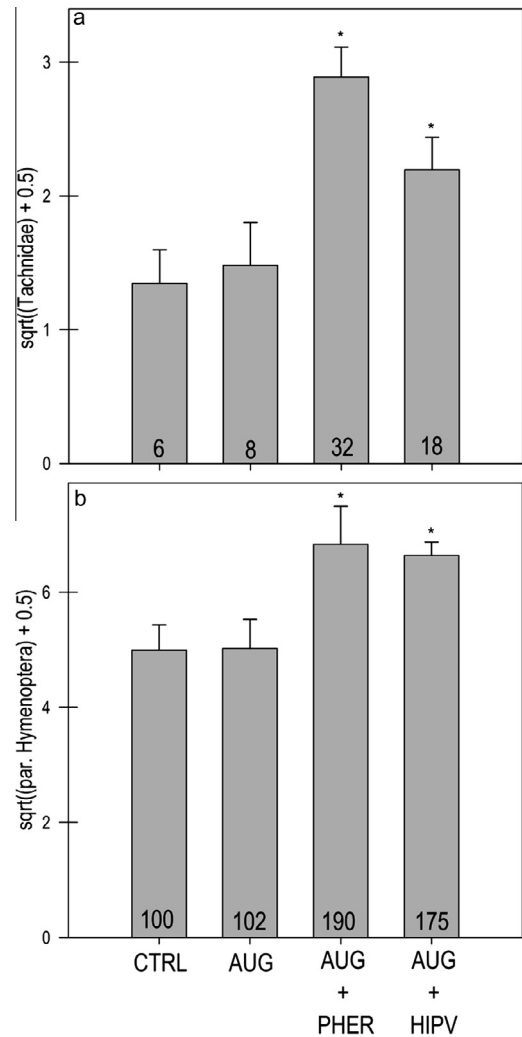


Fig. 5. Seasonal counts (mean + SE) of Tachnidae (a) and Hymenoptera (b) by semiochemical treatment. Numbers inside gray bars indicate raw data for season totals.

impact of semiochemicals on the proportion testing positive for immunoglobulin was marginally significant ($p = 0.0942$), and data trends suggest that deploying pheromone or MeSA may in fact reduce prey capture efficiency (AUG = 8.4%, AUG + PHER = 3.0%, HIPV = 2.1%). This is especially the case for HIPVs, which have been suggested to reduce per-capita predator foraging efficiency when

deployed as synthetic lures due to chemically masking natural HIP-Vs emitted from pest-damaged crops (Kaplan, 2012).

Notably, semiochemical treatment had no impact on herbivores, providing evidence that lures do not elicit non-target effects by attracting pests. Both MeSA and pheromone attracted parasitic wasps and flies, but not predators. Methodological biases may be partially responsible for this divergence. Parasitoids are small and only sampled via yellow sticky cards, resulting in much higher seasonal counts compared with larger, ground-dwelling predators such as spiders and carabid beetles that are visually scouted at a much lower frequency. Though previous research shows that HIP-Vs such as MeSA attract a broad suite of natural enemies (Braasch et al., 2012; Rodriguez-Saona et al., 2011), species-specific pheromones are considered selectively attractive, making the parasitoid response to stink bug pheromone puzzling. This association is likely the result of stink bug parasites eavesdropping on their host's pheromone signals, as has been shown in other parasitoid-host systems (Wertheim et al., 2003, 2005; Wyatt, 2004). Attraction to *P. maculiventris* pheromone has been previously observed within Tachinidae by the specialist, *Hemyda aurata* (Robineau-Desvoidy), and the Pentatomidae generalist, *Euclytia flava* (Townsend), as well as within Hymenoptera by the generalist egg parasitoid *Telenomus* sp. (Aldrich et al., 1984). Although insects from sticky cards were only identified to the family level, *Telenomus* sp. and *H. aurata* were collected from vials containing the aggregation pheromone lure. Additionally, *Ceratina* sp. (Hymenoptera: Apidae) were collected from vials mid-summer, likely due to an overlap in components of the pheromone blend and their floral resources. Analogous patterns of tachinid attraction to pentatomid aggregation pheromone have been reported for the consperse stink bug, *Euschistus conspersus* (Uhler), an orchard pest in the Pacific Northwest (Krupke and Brunner, 2003). Together, these data point to the potential negative consequences of using aggregation pheromone, if elevated parasitoid attraction counteracts the benefits associated with retaining more *P. maculiventris*. Future experimental studies would benefit from assessing the inter-generational consequences of semiochemical enhancements to determine whether the short-term benefits also result in a net improvement in pest control over longer time scales.

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