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Field responses of predaceous arthropods to methyl salicylate: A meta-analysis and case study in cranberries

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A B S T R A C T
Methyl salicylate (MeSA) is a herbivore-induced plant volatile that has shown potential in attracting natural enemies. Here, we conducted a meta-analysis to evaluate the magnitude of natural enemy response to MeSA in the field, and tested its attractiveness to insect predators in commercial cranberry bogs. Eighteen experiments from 14 publications were used in the meta-analysis, resulting in 91 total observations. Of these, 41 documented significant attraction and the magnitude of this attraction response was not significantly different across predator and parasitoid taxa. Insect predators were monitored in cranberries using MeSA (PredaLure)-baited and unbaited yellow sticky traps. MeSA-baited traps caught greater numbers of adult hoverfly, Toxomerus marginatus, lady beetles, and green lacewings compared with unbaited traps. In another field experiment, predator abundance was monitored using traps placed near the MeSA lure (0 m), as well as at 2.5, 5, and 10 m away from the lure. Adult T. marginatus, the dominant predator species, showed a clear attraction to the point source but not to the other distances. In complementary studies we showed that MeSA emissions from PredaLures dropped quickly soon after deployment in the field but remained relatively high for over 4 weeks; flowering, but not vegetative, vines were a primary source of MeSA in cranberries; and, exposure to PredaLures triggered elevated MeSA emissions from vegetative vines. In conclusion, we find strong evidence that insect predators are broadly attracted to MeSA in agricultural fields, including cranberries; yet, whether this behavior can be manipulated to improve biological control needs further investigation.

1. Introduction

The use of agricultural practices to augment, conserve, and increase the efficacy of predatory arthropods is desirable in biological pest control programs (Barbosa, 1998; Jonsson et al., 2008). A novel approach to this is the manipulation of plant volatiles to attract predators of herbivores (Sabelis et al., 1999; Khan et al., 2008). Most plant species emit a unique blend of volatile organic compounds that can guide natural enemies of herbivores to food sources, i.e., nectar, pollen, and prey (Price et al., 1980; Vinson, 1981). These volatiles are constitutively emitted from various plant tissues, albeit mostly at low amounts; however, their emissions often increase in response to herbivore feeding (Vet and Dicke, 1992; Dicke and Vet, 1999; Turlings and Wäckers, 2004). Compared to constitutive volatiles, herbivore-induced plant volatiles (HIPV) offer a more detectable and reliable signal to natural enemies of herbivores because they are emitted in larger quantities and can provide information about the specific location of herbivores on plants (Vet and Dicke, 1992). In many instances the HIPV blend not only has greater amounts of volatiles than constitutively-emitted blends (i.e., quantitative differences), but also contains de novo-produced volatiles that are absent in the constitutive blend (i.e., qualitative differences) (Paré and Tumlinson, 1997).

In the last 30 years, numerous studies have demonstrated the attraction of predaceous arthropods to HIPV compounds both in the laboratory and field (Allison and Hare, 2009). Early studies showed that plants infested with spider mites attract the mites’ predators (Sabelis and van de Baan, 1983; Sabelis et al., 1984; Dicke, 1986). For example, several HIPV compounds attractive to the predatory mite Phytoseiulus persimilis Athias-Henriot are emitted from lima bean leaves damaged by its prey, the spider mite Tetranychus urticae Koch, compared with undamaged leaves (Dicke and Sabelis, 1989; Dicke et al., 1990). Four HIPVs: linalool, (E)-β-ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene, and methyl salicylate (MeSA), attracted females of P. persimilis in Y-tube olfactometer assays (Dicke et al., 1990; De Boer and Dicke, 2004). In a field situation, Drukker et al. (1995) found more predaceous anthonocoids near...
Attracted to this compound. Second, we tested for taxon-specific variation in levels of attraction to assess whether certain groups are more or less responsive than others. Specifically, we compared: (a) predaceous arthropods vs. parasitic hymenopterans; and (b) natural enemy groups (mostly at the family level, e.g., Coccinellidae, Syrphidae) known to attack herbivorous pests and frequently encountered in crop fields.

Published studies were gathered by searching the web of science database using the following keywords alone and in combination: methyl salicylate, MeSA, natural enemy, preda*, parasit*, biocontrol, biological control. We identified additional studies by thoroughly screening the cited works of all papers emerging from our initial search. To qualify for inclusion in the database and subsequent analysis, a study must have measured the abundance of at least one species or group of entomophagous arthropod in the presence and absence of MeSA. The response must have been measured using naturally-occurring organisms in the field and thus no laboratory studies were included. Furthermore, MeSA must have been isolated and manipulated as a pure compound emitted from a slow-release dispenser, not as a part of a blend consisting of multiple volatiles. Last, the studies must have reported means, a measure of variation (e.g., standard deviation, standard error), and sample sizes for both the control and treatment groups.

For each study, we calculated an effect size, Hedges' $d$, for each species or group of natural enemy sampled (see Rosenberg et al. (2000) for a discussion of various effect sizes and their calculations). The MeSA-free group was designated as the control and MeSA-exposed group as the treatment. As a result, positive effect sizes indicate an attraction response (i.e., more individuals caught in MeSA-exposed compared with MeSA-free traps), whereas negative effect sizes are a sign of repulsion. An effect size at or near zero would indicate that individuals display no preference, either for or against, MeSA. Effect sizes were calculated using seasonal means of arthropod abundance and we considered observations within a publication to be independent if they were derived from: (a) different species or taxa; (b) separate experiments; or (c) trials conducted over multiple years.

The statistical program MetaWin 2.0 was used to calculate effect sizes and conduct the analysis (Rosenberg et al., 2000). Resampling tests (999 iterations) were performed to generate cumulative effect sizes with 95% bootstrap confidence intervals. This approach is recommended to account for the violation of basic distributional assumptions that typically occurs with meta-analytical data (Adams et al., 1997). Given the heterogeneity among the studies, we analyzed the entire dataset using a model with no data structure to determine the grand mean effect size and confidence interval. This provides an overall assessment of the strength of natural enemy attraction to MeSA. Next, we used a mixed-effects categorical model to test for taxon-specific variation in response. As noted above, we compared, at a broad taxonomic level, predators vs. parasitic hymenopterans. However, we also evaluated the responses of eight key natural enemy groups, including: (i) Anthocoridae (O. tristicolor and Orius insidiosus (Say)), (ii) Braconidae + Ichneumonidae, (iii) Chalcidoidea (Encyrtidae, Mymaridae – Anagrus sp.), (iv) Coccinellidae (S. punctum picipes, C. septempunctata, Harmonia axyridis (Pallas), Propylea japonica (Thunberg)), (v) lacewings (Chrysopidae + Hemerobiidae), (vi) predaceous heteropterans (Lygaeidae + Miridae; mostly G. pallens and Deraeocoris brevis), and (vii) Syrphidae. Our sample size cut-off for analyzing individual natural enemy groups was five; if five or fewer observations existed in our dataset then we did not analyze this group. However, certain insect groups were merged (e.g., we combined Chrysopidae and Hemerobiidae to form a single lacewing category) in cases where sample sizes were five or less for any individual group. For each group a mean effect size ($d_+$) was calculated and attributed to this compound. To evaluate the magnitude of natural enemy responses to HIPVs in the field, we reviewed the published literature testing for attraction to synthetic MeSA deployed as lures. The goals of this analysis were twofold. First, we quantified the strength of the overall response to determine whether natural enemies are broadly


drawn from the literature testing for attraction to synthetic MeSA deployed as lures. The goals of this analysis were twofold. First, we quantified the strength of the overall response to determine whether natural enemies are broadly
reported with 95% bootstrap confidence intervals; effects are considered statistically significant if confidence intervals do not bracket zero. Between-group heterogeneity ($Q_0$) was tested against a chi-square distribution to determine if significant differences exist among groups.

To address the 'file-drawer problem' whereby non-significant effects are less likely to be published, we used Rosenthal's method ($z = 0.05$) to calculate a fail-safe value (Rosenberg et al., 2000). This estimates the number of non-significant observations that would need to be added to our dataset to change the outcome from significant to non-significant. In this case, our overall fail-safe value was calculated as 2621, indicating that our analyses are highly robust to the file-drawer effect.

2.2. Predator attraction to MeSA in cranberries

A field experiment was conducted in 2008 to test the effect of lures baited with MeSA on insect predator attraction in cranberries. The experiment was conducted in 15 commercial cranberry, V. macrocarpon cv. 'Early black', bogs (3–5 acre each) located in Chatsworth, New Jersey. A pair of yellow sticky traps (23 × 28 cm Un baited Pherocon AM; Trécé Inc., Adair, Oklahoma) was placed in each bog at least 100 m apart. One of the traps in each bog was baited with a MeSA lure (5 g load/lure; 90 d lure; average release rate ~35 mg/day over a 4 week period at 30 °C constant in the lab; PredaLure, AgBio Inc.), while the other trap had no lure (unbaited control). The study ran for a total of 8 weeks from peak bloom through fruit development (16 June–11 August). The position of treatments within each bog was assigned randomly. Traps were placed just above the canopy level and were collected, brought to the laboratory, and replaced weekly. MeSA lures were replaced after 4 weeks. No insecticides were applied to cranberry bogs during the course of the experiment. In the laboratory, numbers of adult insect predators on traps were counted under a stereomicroscope. All insect predators were identified to family and, when possible, to species. The principal predator families found were Syrphidae, Coccinellidae, and Chrysopidae. Other major insect groups were also identified and counted; these included species in the Tachinidae (parasitoids), Tabanidae, Muscidae, and Cicadellidae (crop pests).

For statistical analyses, predators were tested as a group for their response to MeSA with repeated-measures MANOVA. The model included block (bog), treatment (MeSA-baited vs. unbaited traps), time, and treatment × time. A significant MANOVA was followed by repeated-measures ANOVA for individual predator families. Data for all other insect families were also analyzed using repeated-measures ANOVA. After a significant ANOVA for treatment or treatment × time effects, comparisons between MeSA-baited versus unbaited traps for each week were done by paired t-tests. When needed, data were natural log-transformed before the analysis to achieve homogeneity of variance. Statistical analyses were performed using Minitab 16 (Minitab Inc., State College, Pennsylvania).

2.3. Predator attraction to MeSA over various distances

In 2009, a study was conducted to determine the effects of distance from MeSA lures on predator attraction. Here, we tested the hypothesis that the abundance of predators declines with distance from the lure. This study was conducted in ten cranberry, V. macrocarpon cv. 'Early black', bogs located in a commercial farm (Chatsworth, New Jersey). At each bog, a “set” of four yellow sticky traps was placed along a linear transect at 0, 2.5, 5, and 10 m from the center of the bog. In half of these bogs, one of the end traps (i.e., 0 m) was baited with a MeSA lure, whereas the rest of traps were left unbaited (no lure). All traps in the other five bogs had unbaited traps (controls). Treatments were randomly assigned to the bogs. Traps and MeSA lures were placed for 6 weeks, from 23 June until 6 August, and lures were replaced once on week 4. No insecticides were applied during the experiment. Traps were collected and replaced weekly and numbers of insect predators counted. Only Syrphidae, Coccinellidae, and Chrysopidae were counted in this study because these were the only predator families affected by MeSA in 2008 (see Section 3.2).

A "mixed effects" ANOVA was conducted for statistical analysis using the MIXED procedure of SAS (ver. 9.13, SAS Institute Inc., Cary, North Carolina), with treatment and distance as main variables and "set" in the model as a blocking variable. The distance by treatment interaction was further investigated using the SLICE option (SAS) to perform the test of simple main effect. For this experiment the SLICE gives us the pairwise comparisons of the control and treated traps. This is equivalent to performing single-degree-of-freedom contrasts to compare the control vs. treated at each distance separately, which is strongly justified by the highly significant treatment × distance interaction effect (in the case of hoverflies only; see Section 3.3).

2.4. Temporal release pattern of MeSA dispensers

This experiment investigated the pattern of MeSA emissions from commercial lures, and was conducted in a cranberry, V. macrocarpon cv. 'Early black', bog at the Rutgers Blueberry/Cranberry Center (Chatsworth, New Jersey). Four MeSA PredaLure dispensers and four dispensers without MeSA (blank controls) were hung above the cranberry canopy from 16 June until 11 August, 2008. Amounts of MeSA emissions for each dispenser were collected weekly. After measurements were taken, dispensers were hung back in the bog. To determine amounts of MeSA, individual dispensers were enclosed inside Magenta boxes (7.5 cm length × 7.5 cm width × 10 cm height; Magenta Corp., Chicago, Illinois). The lid of each of the boxes had two openings: one of the openings fitted an adsorbent trap (0.6 cm diameter × 7.6 cm long glass tube; Analytical Research Systems, Inc. Gainesville, FL), containing 30 mg of Super-Q (Alltech, Deerfield, Illinois), while the other opening served to clean the incoming air and fitted a Pasteur pipette containing 1.25 g of activated charcoal (Alltech). Volatiles were collected in Super-Q traps by pulling air from inside each Magenta box at a rate of ~2 ml min⁻¹, with the aid of a 12-volt vacuum pump (Sensidyne, Clearwater, Florida). Empty containers were sampled concurrently to control for container emissions (no detectable amount of MeSA was found in empty containers; data not shown). Samples were collected for 4 h (1100–1500 h) in the laboratory and analyzed as described below (Section 2.7).

2.5. Sources of MeSA emissions in cranberries

To determine possible sources of MeSA in cranberries, V. macrocarpon cv. ‘Early black’ and establish a baseline of MeSA emission to know how additional MeSA exposure affects emissions from cranberry leaves, we collected volatile emissions from vegetative (leaves only) and flowering (flowers and leaves) vines. Forty cranberry vines, with leaves and flowers (2–3 fully open flowers/terminal) or with only leaves, were cut at the base, placed in a glass vial containing water, and then placed inside a 4 L volatile collection chambers (Analytical Research Systems Inc.). (n = 4 replicates per plant tissue). As compared with an annual plant (Schmelz et al., 2001), excising stems at their base did not alter volatile emissions in a related perennial Vaccinium species (Rodriguez-Saona et al., 2011). Volatiles were collected in the greenhouse (20 ± 5 °C; 15:9 L:D), using a push–pull system (Rodriguez-Saona et al., 2009). Purified air entered the top of each chamber at a rate of 2 L min⁻¹, and volatiles were collected in Super-Q traps by pulling air from the
chambers at a rate of 1 L min⁻¹. Collections were conducted during daytime from 1000 to 1300 h, and identified and quantified via gas chromatograph (GC) (see Section 2.7).

### 2.6. Effects of MeSA-exposure on volatile emissions from cranberry leaves

Besides acting as a direct attractant, it is proposed that HIPVs can trigger a volatile response from plants that might be attractive to predators (Khan et al., 2008). To test for this, we exposed cranberry, _V. macrocarpon_ cv. ‘Early black’, plants to MeSA lures and then measured induced volatiles emissions. In these experiments, we used lures of varying strengths by placing three PredaLures in an environmental chamber (25 °C; 16:8 L:D) weekly for eight weeks starting on 15 February 2010 (i.e., _n_ = 3 replicates x 8 weeks, for a total of 24 lures). This procedure allowed us to age the lures at different rates, resulting in lures that were 1–8 weeks old before exposing them to plants. Each of the aged lures was placed separately in 17 cm diameter x 35 cm high Plexiglas chambers with a potted cranberry plant (containing ~40 vines) in the greenhouse for 24 h, starting at 0900 h. PredaLures were placed ~10 cm away from the plants. A similar number of plants ( _n_ = 3) was treated the same but not exposed to MeSA (controls). MeSA-exposed and control plants were then placed in volatile collection chambers, as described above, and collections were conducted from 1000 to 1300 h. After use, Plexiglas and volatile collection chambers were cleaned with tap water and 70% ethanol. Volatiles were identified and quantified by GC (as described in Section 2.7). After collections, all vines from plants were harvested, oven dried at 60 °C, and weighed. Emissions of volatiles from plants exposed to aged lures and controls were compared using ANOVA, followed by Tukey tests. Data ( _x_ ± 0.3) were natural log-transformed before analysis. If there was a significant effect of MeSA exposure as compared with controls, a Pearson correlation was then conducted to determine if individual volatile emissions correlate with PredaLure age.

### 2.7. Volatile analysis

Volatiles from Super-Q traps were eluted with 150 μL dichloromethane aided by a light stream of _N₂_. _n_-Octane (400 ng) was added to each sample as an internal standard. Quantification of volatiles was conducted on a Hewlett Packard 6890 Series Gas Chromatograph, equipped with a flamed ionization detector and an Agilent HP-1 column (10 m x 0.53 mm x 2.65 μm), and using helium as the carrier gas at a rate of 5 mL min⁻¹. The temperature program consisted of 40 °C for 1 min, followed by an increase to 180 °C at a rate of 14 °C/min, followed by a hold for 2 min at 180 °C, followed by an increase to 200 °C at a rate of 40 °C/min, followed by a hold for 2 min at 200 °C, then to 220 °C followed by a 5 min post-run hold. Compound quantification was based on comparison of peak areas with that of the internal standard. Cranberry volatiles were identified via coupled GC–mass spectrometry (MS) by comparison of spectral data from NIST library, retention indices, and retention times of commercially available compounds, as described in Rodriguez-Saona et al. (2009).

### 3. Results

#### 3.1. Meta-analysis

We identified 18 experiments reported from 14 publications, resulting in 91 total observations that tested the impact of MeSA on insect natural enemies in the field. We included the cranberry data in the present manuscript as one of the 14 studies. Overall, the effect of MeSA on natural enemy attraction was tested in nine different crops, involving at least 34 insect species in 21 families (Table 1). Approximately 24% of observations represented hymenopteran parasitoids, whereas the remaining 76% were predaceous insects. All studies used either PredaLure or vials of synthetic MeSA with cotton wicks as the lure, and sticky cards as the trap type. Of the 91 observations in our dataset, 41 documented significant attraction, 50 found non-significant effects, and none of the

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**Table 1**

A review of published studies testing the effect of synthetically deployed methyl salicylate (MeSA) on natural enemy attraction in crop fields.

<table>
<thead>
<tr>
<th>Ref</th>
<th>Crop</th>
<th>No. NEs sampled</th>
<th>Taxa that were significantly attracted to MeSA</th>
<th>Taxa that were not significantly attracted to MeSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hops</td>
<td>11 (Community)</td>
<td>Orius tristicolor, Stethorus punctum picipes, Deraeocoris brevis, Geocoris pallens, Syrphidae</td>
<td>Parasitic Hymenoptera, Anagrus sp., Coccinellidae (excluding S. punctum), Mmiridae (excluding D. brevis), Leptothrips mali</td>
</tr>
<tr>
<td>2</td>
<td>Hops</td>
<td>1 (Lacewing)</td>
<td>Stethorus punctum picipes, Geocoris pallens, Brachonidae, parasitic Hymenoptera, Chrysopa nigricor, Empididae, Hemerobius sp., Deraeocoris brevis, Sarcophagidae, Syrphidae</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Grape</td>
<td>16 (Community)</td>
<td>Microbryophytoptera, Orius tristicolor, Stethorus punctum picipes, Empididae, Geocoris pallens, Syrphidae</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Hops</td>
<td>11 (Community)</td>
<td></td>
<td>Bracoonidae, Anagrus daanei, Tachinidae, Orius tristicolor, Sarcophagidae, Syrphidae</td>
</tr>
<tr>
<td>5</td>
<td>Grape</td>
<td>2 (Chalcidoidea)</td>
<td>Metaphycus sp. (Encrytidae)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Grape</td>
<td>1 (Lacewing)</td>
<td>Chrysopa oculata</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Apple</td>
<td>3 (Lacewing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Strawberry</td>
<td>12 (Community)</td>
<td>Orius tristicolor, Chrysopa oculata, Chrysoperla porabunda</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Soybean</td>
<td>7 (Community)</td>
<td>Chrysopa oculata, Syrphidae</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Turnip</td>
<td>1 (Chalcidoidea)</td>
<td>Diadegma semialaun</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Cranberry</td>
<td>5 (Community)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Cherry</td>
<td>1 (Lacewing)</td>
<td>Chrysopa oculata, Coccinellidae, Syrphidae</td>
<td>Tachinidae, Anthocoridae</td>
</tr>
<tr>
<td>13</td>
<td>Cotton</td>
<td>11 (Community)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Soybean</td>
<td>4 (Community)</td>
<td>Coccinella septempunctata, Syrphidae</td>
<td></td>
</tr>
</tbody>
</table>

Ref = reference, NE = natural enemy; 1 = James, 2003a; 2 = James, 2003b; 3 = James and Price, 2004; 4 = James, 2005; 5 = James and Grasswitz, 2005; 6 = James, 2006; 7 = Jones et al., 2011; 8 = Lee, 2010; 9 = Mallinger et al., 2011; 10 = Ori et al., 2010; 11 = Rodriguez-Saona et al., this study; 12 = Töth et al., 2009; 13 = Yu et al., 2008; 14 = Zhu and Park, 2005.
observations resulted in repulsion. As a result, natural enemies as a whole (at least among those species that can be effectively sampled using sticky cards) were broadly attracted to MeSA in the field (Fig. 1A). The results were not different when we partitioned the dataset into predators vs. parasitoids (Fig. 1B; Qb = 0.085, df = 1, P = 0.711), indicating that the magnitude of response across these broad groups was comparable. When we further partitioned the dataset into eight natural enemy groups, of which lacewings, coccinellids, and anthocorids were the most commonly reported, we again found no difference in the magnitude of attraction across taxa (Fig. 1C; Qb = 5.356, df = 7, P = 0.617).

3.2. Predator attraction to MeSA in cranberries

Methyl salicylate (MeSA), time, and their interaction had a significant effect on adult insect predators (MANOVA for treatment: Wilks’ λ = 0.38, F = 72.28, P < 0.001; time: Wilks’ λ = 0.05, F = 34.09, P < 0.001; treatment × time: Wilks’ λ = 0.67, F = 3.13, P < 0.001; block: Wilks’ λ = 0.69, F = 1.24, P = 0.119). Traps baited with MeSA captured greater numbers of adult hoverflies (Diptera: Syrphidae), lady beetles (Coleoptera: Coccinellidae), and green lacewings (Neuroptera: Coccinellidae) (Table 2; Fig. 2). Interestingly, adult flight activity of all insect predators peaked during bloom (Fig. 2). Compared with unbaited traps, numbers of hoverflies were significantly higher on MeSA-baited traps in 7 out of 8 weeks sampled (Fig. 2A). On average, MeSA-baited traps captured ~4.5 times more hoverflies compared with unbaited traps. The most common hoverfly (>90% of all individuals found in our samples) on traps was identified as Toxomerus marginatus (Say). Similarly, MeSA-baited traps captured 1.8 and 7.6 times more lady beetles and lacewings, respectively, than unbaited traps. However, MeSA-baited traps captured higher numbers of these predators in only 2 out of 8 weeks sampled compared with unbaited traps (Fig. 2B and C), indicating that the attraction was not as consistent as with hoverflies. About 40, 35, 11, and 10% of lady beetles were identified as C. septempunctata L., H. axyridis, Hippodamia convergens Guérin-Méneville, and Coleomegilla maculata (De Geer), respectively. Other, less common (<5%), coccinellid species included Hippodamia parenthesis (Say), Cycloneda munda (Say), and Propylaea quaurodecimpunctata L. However, differences between MeSA-baited and unbaited traps were found only for C. septempunctata (ANOVA for treatment: F = 8.51, df = 1, P = 0.022; time: F = 29.6, df = 7, P < 0.001). There was no effect of MeSA on anthocorids (Table 2; Fig. 2D).

Among other insect feeding guilds, MeSA-baited traps captured significantly greater numbers of horse flies (Diptera: Tabanidae) and house flies (Diptera: Muscidae) (Table 2; Fig. 2E and F), and marginally (Table 2) increased numbers of parasitic tachinid flies (Diptera: Tachinidae) (Fig. 2G). Numbers of Cicadellidae were not affected by MeSA (Table 2; Fig. 2H). Two common cicadellid species were the sharp-nosed leafhopper, Scaphytopius malagensis Provancher, and the blunt-nosed leafhopper, Limotettix vaccinii (Van Duze).

3.3. Predator attraction to MeSA over various distances

There was a significant treatment × distance effect on numbers of adult hoverflies captured on traps (ANOVA for treatment: F = 0.71, df = 1, 16, P = 0.411; distance: F = 21.33, df = 3, 16, P < 0.001; treatment × distance: F = 9.7, df = 3, 16, P < 0.001), indicating that the attraction of hoverflies to MeSA was strongly influenced by distance. Number of hoverflies was 84% higher on traps placed adjacent to the MeSA lure (0 m) compared with unbaited traps (F = 25.92; df = 1, 16, P < 0.001) (Fig. 3). No differences were found on numbers of hoverflies between treatments when traps were placed 2.5, 5, or 10 m from the MeSA lure (all P values >0.05) (Fig. 3). Unlike 2008, we found no effect of treatment on numbers of lady beetles in 2009 (F = 0.72, df = 1, 16, P = 0.409). There were also no distance (F = 1.2, df = 3, 16, P = 0.342) or treatment × distance (F = 0.02, df = 3, 16, P = 0.995) effects on adult

### Table 2

Summary of repeated measures ANOVA for the effects of methyl salicylate (MeSA) on insect attraction in cranberries in 2008.

<table>
<thead>
<tr>
<th>Feeding Guilds Families</th>
<th>Treatment</th>
<th>Time</th>
<th>Treatment × Time</th>
<th>Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>df</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Predators</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syrphidae</td>
<td>275.12</td>
<td>1, 210</td>
<td>&lt;0.001</td>
<td>320.36</td>
</tr>
<tr>
<td>Coccinellidae</td>
<td>15.12</td>
<td>1, 210</td>
<td>&lt;0.001</td>
<td>12.64</td>
</tr>
<tr>
<td>Chrysopidae</td>
<td>25.45</td>
<td>1, 210</td>
<td>&lt;0.001</td>
<td>25.45</td>
</tr>
<tr>
<td>Anthocoridae</td>
<td>0.02</td>
<td>1, 182</td>
<td>0.877</td>
<td>93.33</td>
</tr>
<tr>
<td>Parasitoids</td>
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</tr>
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<td>Tachinidae</td>
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<td>1, 210</td>
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<td>5.17</td>
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<td>Herbivores</td>
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<td>1, 210</td>
<td>0.310</td>
<td>73.01</td>
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<tr>
<td>Others</td>
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<td>1, 210</td>
<td>&lt;0.001</td>
<td>25.02</td>
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<tr>
<td>Muscidae</td>
<td>6.83</td>
<td>1, 210</td>
<td>0.010</td>
<td>9.50</td>
</tr>
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</table>

Significant P values are in bold.

* Test for the effect of MeSA versus control.

* Test for the effect of week. Experiment ran for eight consecutive weeks, except for Anthocoridae where data for one week were lost.
lady beetles. Compared to 2008, C. septempunctata (45%), P. quatuordecimpunctata (26%), and C. maculata (20%) were the most abundant coccinellid species in 2009; while H. axyridis, H. convergens, H. parenthesis, and C. munda comprised less than 10% of all species. In 2009, adult lacewing counts on traps were too few to allow a meaningful statistical analysis.

3.4. Temporal release pattern of MeSA dispensers

In the field, emissions of MeSA from PredaLures decreased exponentially with time (Fig. 4). MeSA was released in high quantities from lures very quickly during the first week of deployment but after 2 weeks the release rate was stabilized. The amounts of MeSA in lures remained high even after four weeks of deployment (Fig. 4).

3.5. Sources of MeSA emissions in cranberries

We identified 36 volatile compounds emitted from flowering cranberries (Table 3). Eleven of these volatiles ((Z)-3-hexen-1-ol, hexanol, 2-heptanone, benzyl alcohol, phenyl acetaldehyde, aceto phenone, MeSA, hexyl butyrate, α-cubebene, copaene, and β-farnesene) were found in detectable amounts from flowering but not from vegetative vines (Table 3). These data indicate that flowers are the main source of MeSA in cranberries.

3.6. Effects of MeSA-exposure on volatile emissions from cranberry leaves

Exposure to aged PredaLures caused an increase in MeSA emissions from cranberry vegetative vines ($F = 17.84$, df = 8, 18, $P < 0.001$) (Fig. 5). However, this response was highly variable and not correlated with lure age (Pearson correlation = 0.263; $P = 0.529$), indicating that cranberry vines responded to PredaLure exposure regardless of its age. No other cranberry volatile compounds from vegetative vines were affected by PredaLure exposure compared with controls (all $P$ values $>0.05$).
Based on our review of the existing scientific literature on field responses to MeSA, we can conclude that natural enemies are indeed attracted to this volatile and, perhaps more importantly, the compound acts as a broad spectrum attractant. We found no systematic differences in the magnitude of response comparing predators vs. parasitoids (Fig. 1B) or across eight frequently encountered natural enemy groups (Fig. 1C). This supports its purported role as a universal lure for beneficial insects used in the biocontrol of agricultural pests (e.g., PredaLure). It also substantiates the outcomes from laboratory preference assays, which document that natural enemies frequently orient toward this volatile when offered a choice in foraging arenas (De Boer and Dicke, 2004, 2005; Ishiwari et al., 2007; Sasso et al., 2009; Ament et al., 2010; Shimoda, 2010).

The strength of attraction to MeSA in the field was quite strong. An effect size of 0.2 is considered small, 0.5 moderate, and 0.8 large (Cohen, 1988). The overall effect size for natural enemies as a group was 0.741. Although there were no statistical differences among taxa, hoverflies (Syrphidae) responded the strongest...
Notably, entomophagous dipterans (e.g., Tachinidae, Empididae) were the other group whose level of attraction compared with that of syrphids (d = 1.168), suggesting that chemotactic responses to MeSA may be phylogenetically conserved in the Diptera. Chalcidoidea wasps were the only taxonomic group whose overall effect was not significantly different from zero. This may simply be a consequence of low sample size (chalcidoidea had the fewest observations), or it may reflect the reality that the superfamily Chalcidoidea is an incredibly large and diverse group from which it is difficult to make broad generalizations.

Interestingly, the outcome of attraction appeared to be context-dependent across studies (see Table 1). In some cases this is likely due to the fact that most taxa were not identified to the species-level, especially among parasitic Hymenoptera. Thus, if two studies were working with different species assemblages in their fields, which is likely to often be the case, then inconsistencies can be driven by species-specific responses. Jones et al. (2011), for example, found that three congeneric species of lacewings (Chrysoperla plo-rabunda, C. nigricornis, and C. oculata) responded differently to the same HIPV blends in apple orchards. In other cases, crop background may partially drive this variation if MeSA ‘pulls’ differently for the same HIPV blends in apple orchards. In other cases, crop background may partially drive this variation if MeSA ‘pulls’ differently when embedded in a different plant matrix. We also found evidence for differential response by the same species in the same crop. James (2003a) found attraction of O. tristicolor to MeSA in hogs, but O. tristicolor was not attracted in Jones (2005) despite occurring in the same crop, in the same region, by the same researcher. Clearly more work is needed to identify when and why context-dependent responses occur before HIPVs can be called upon as a reliable pest management tool.

In cranberries, hoverflies, lady beetles (Coccinellidae), and green lacewings (Chrysopidae) were attracted to MeSA-baited traps in 2008. These results are consistent with previous findings in hops (e.g., James, 2003a, 2005), grapes (e.g., James and Price, 2004), and soybeans (e.g., Mallinger et al., 2011) that show similar attraction of hoverflies to MeSA. T. marginatus is a hoverfly commonly found in cranberries (Voss, 1996), and was the dominant predator species in this study. Adult syrphid flies frequently visit flowers to feed on pollen and nectar (Cowgill et al., 1992; Carreck and Williams, 2002). Thus, not surprisingly, their flight activity in cranberries peaked during bloom (Fig. 2). During our studies, we noticed that T. marginatus adults usually hover around cranberry flowers (C.R.S., personal observation), which prompted us to examine volatile emissions from flowering cranberries. It is likely that odors emitted from cranberry flowers attract hoverflies differentially to the same HIPV blends in apple orchards. In fact, MeSA is a common floral volatile (Schiestl, 2010), and was a component of the cranberry floral blend (Table 3), suggesting that this is an adaptive response of hoverflies for food location. Other families of flies (Diptera) were also attracted to MeSA, including Tabanidae, Muscidae, and Tachinidae in cranberries (Table 2), supporting the outcome from our meta-analytical review that dipterans are notably attracted to this compound. It is likely that some of these flies use MeSA as a generalized cue to locate flowers for a source of nectar (e.g., Kniepert, 1980; Pont, 1993; Stireman et al., 2006).

Besides being emitted from flowers, MeSA is a HIPV often induced in plants after herbivore feeding, particularly by phloem-sucking herbivores such as aphids (Staut et al., 2010) and cell-content feeders like mites (Dicke et al., 1980). MeSA is the volatile derivative of the phytohormone salicylic acid (SA), a product of the SA-dependent defense pathway that protects plants against certain pathogens and insects (Stout et al., 2006). The SA pathway is often induced by phloem-sucking and cell-content plant feeders but not by chewing herbivores (Walleng, 2000). However, aphids and mites are not pests commonly present in cranberries in the northeast (Averill and Sylvia, 1998). Larvae of T. marginatus are reportedly shown to feed on cranberry tipworm, Dasineura oxyccocana (Diptera: Cecidomyiidae) in cranberries (Voss, 1996). Thus, it is possible that tipworm infestation induces MeSA emissions in cranberries and that this induced volatile response may attract female hoverflies to infested sites for oviposition. In the case of hoverflies, where adults can act as pollinators (Larson et al., 2001) and the larvae are predators, plants will benefit through increased emissions of both floral volatiles and HIPVs; this will likely minimize any conflicts on the regulation of volatiles released by plants for pollinator attraction and herbivore defense through predator attraction (Kessler and Halitschke, 2009).

Although we have yet to show that herbivore damage induces MeSA emissions in cranberries, the fact that exposure to PredaLure increased MeSA emissions from vegetative vines suggests that synthetic MeSA is signaling neighboring plants to produce this compound. Alternatively, MeSA could have been adsorbed and re-released (passive emission). For instance, Choh et al. (2004) showed that uninfested Lima beans exposed to volatiles from T. urticae-infested leaves emit a similar blend of volatiles to those from infested leaves; however, treating uninfested leaves with a protein-synthesis inhibitor prior to exposure to volatiles from infested leaves prevented this volatile response, concluding that the response is passive.

Contrary to our expectations, we did not see a decline in MeSA emissions from exposed cranberry plants as the age of PredaLures increased (Fig. 5), despite the fact that MeSA emissions from PredaLures declined slowly after a week of deployment (Fig. 4). This suggests that there is a limit in MeSA production or adsorption in cranberries such that higher amounts of MeSA did not result in greater MeSA emissions from exposed plants. Although commonly proposed as a possible mechanism of natural enemy attraction to MeSA (e.g. James et al., 2005; Khan et al., 2008), to our knowledge this is the first study to document that exposure to PredaLures increases MeSA emissions from plants. Our exposure studies were conducted under controlled greenhouse conditions and will need verification in the field. However, this outcome has important implications for understanding the mechanisms of MeSA attraction, opening the possibility that crop plants play an active role in recruiting predators from fields in which lures are deployed.

The response of hoverflies to MeSA was highly localized, i.e., MeSA attracted hoverflies close to the source of emissions. There was no difference in hoverfly captures between MeSA-baited and unbaited traps at a distance of ≥2.5 m. Similarly, Mallinger et al. (2011) found no differences in natural enemy abundance between traps in MeSA-treated and untreated plots at distances of 1.5 m from the lure. Lee (2010) also showed attraction of laccoides to the MeSA point source, but not 5 and 10 m away from it. Spatial limitations of natural enemy attraction at or near to the site of MeSA release can potentially disrupt their efficacy if prey are absent within the attracted area by wasting their foraging time. The risk is also high for predators to associate the lack of prey with the presence of MeSA and consequently learn to avoid it in future encounters, also interfering with the enemies’ foraging behavior. This, however, does not mean that hoverflies do not pick up the scent from distances far away from the lures because the actual path of the flies was not determined. Simply, it means that once hoverflies detect the MeSA in the environment they most likely will follow the plume to the source of emission.

While some studies have reported attraction of lady beetles (e.g., James and Price, 2004; James, 2005; Zhu and Park, 2005; Yu et al., 2008) and lacewings (e.g., James, 2003b; James and Price, 2004; Lee, 2010; Jones et al., 2011; Mallinger et al., 2011) to MeSA, others have reported a lack of attraction (Lee, 2010). This variation has been found even within a single crop. For example, Lee (2010) showed variation in attraction of lacewings to MeSA from year to year. This suggests that attraction of these predators to MeSA is inconsistent and specific to a crop system. Similarly, in our study lady beetles and lacewings were attracted to PredaLures in 2008,
but not in 2009. Possible explanations include variable population sizes of these natural enemies among years with some years being nearly absent, differences in weather conditions and other factors that may cause the predators to remain inactive, and availability of alternative food such that the predators’ response to MeSA lures might be dependent on prey abundance. Anthocoridae is another family of predators commonly attracted to MeSA (e.g., James and Price, 2004; Yu et al., 2008; Lee, 2010), but this was not the case in our system. Most likely some species within predator families are more attracted to MeSA than others, which might explain these inconsistencies. These differences among agro-ecosystems highlight how unpredictable the response of insect predators can be to MeSA temporally and spatially.

Although our studies provide new insights on the response of insect predators to MeSA, it remains unclear how MeSA might function as an attractant for predators in cranberry bogs, and how MeSA emitters might be optimally deployed in this or any other crop system. Puente et al. (2008) concluded that the utility of herbivore-induced plant volatiles would be greatest when only a small proportion of plants were attacked by herbivores and were emitting volatiles. As a result, the greater the proportion of plants that were attacked, the smaller was the benefit of herbivore-induced volatiles to attract natural enemies to any individual plant. In the present study, flowers are a major source of MeSA in cranberry bogs, and if plants are flowering at largely the same time, then the value of adding artificial MeSA emitters to attract predators might be minimal. Similarly, placing numerous emitters within a bog or a field may only result in attracting potential predators to lures in the absence of prey rather than to prey-infested plants. This would be especially true if the emitters released MeSA at rates far greater than plants (compare Fig. 4 with Table 3). Widespread deployment of such lures may lead to saturation of the area with MeSA odors; thereby, potentially disrupting the abilities of predators to precisely locate host-infested and MeSA-emitting plants much like widespread deployment of sex pheromones disrupts mate location and reproduction in pest species (Cardé, 2007).

An ecological cost of using HIPVs to manipulate natural enemies is their potential attraction of crop pests. For example, Dickens (2006) showed attraction of adult Colorado potato beetles, Leptinotarsa decemlineata (Say), to a blend of plant volatiles consisting of (Z)-3-hexenyl acetate, linalool, and MeSA. In cranberries, bluntnosed leafhoppers (Cicadellidae) transmit a phytoplasma that causes false blossom disease (Beckwith and Hutton, 1929). Leafhoppers were, however, not attracted to MeSA and did not find an ecological cost for the use of PredaLures in cranberries. Lee (2010) also found no response of Cicadellidae to MeSA lures. In conclusion, PredaLure has the potential to attract insect predators in cranberries, in particular T. marginatus adults that were highly and reliably attracted to MeSA in this study. Not many studies have determined the impact of using HIPVs to attract predators on pest suppression (e.g., Mallinger et al., 2011), and this is the direction we will take in future research. Attraction of predators to point source emissions of MeSA is, however, somewhat troublesome as discussed above, and further studies need to investigate if this localized attraction can still provide benefits to plants. Moving forward, additional studies are clearly needed for other HIPVs. While certain volatiles show promise (e.g., 2-phenylethanol, (Z)-3-hexen-1-ol), too few studies have tested their impact in the field to compare with MeSA. Additionally, certain natural enemy taxa were notably absent from the insect community sampled in the current group of studies reviewed from our meta-analysis (Table 1). For example, the entire ground-foraging guild of predators, e.g., spiders, carabids, has not been tested (but see Lee, 2010). The same is true for some foliar-foraging predators (e.g., Nabidae, Pentatomidae) that are known to be important in pest suppression. It is likely that sticky cards are simply ineffective at trapping these taxa and alternative sampling techniques need to be developed. Future studies in cranberries will investigate the attraction of parasitic wasps (Hymenoptera) and ground predators to MeSA using various sampling techniques.

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