Synthetic Herbivore-Induced Plant Volatiles as Field Attractants for Beneficial Insects

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ABSTRACT

Evidence for field attraction by beneficial insects to synthetic herbivore-induced plant volatiles (HIPVs) is presented. Three synthetic HIPVs (methyl salicylate, (Z)-3-hexenyl acetate, (E)-4,8-dimethyl-1,3,7-nonatriene) were evaluated in a Washington state hop yard during April–October 2002 for attractiveness to beneficial insects. The predatory mirid, Deraeocoris brevis (Uhler), and the anthocorid, Orius tristicolor (White), were attracted to sticky cards baited with (E)-3-hexenyl acetate, while the geocorid, Geocoris pallens Stal., and hover flies (Syrphidae) were attracted to methyl salicylate-baited cards. The coccinellid, Stethorus punctum picipes (Casey), was attracted to both HIPVs in July and September. The (E)-4,8-dimethyl-1,3,7-nonatriene did not attract any beneficial insects. Lygus hesperus Knight, Leptothrips mali (Fitch), Anagrus spp., other Miridae, Coccinellidae, and parasitic Hymenoptera were not attracted to the three HIPVs tested. The possible exploitation of HIPVs in enhancing spring populations of beneficial insects and conservation biological control in cropping systems is discussed.

KEYWORDS

herbivore-induced plant volatiles, natural enemies, attraction, conservation biological control, synomone

SOME PLANTS RESPOND TO herbivore damage by producing volatiles that attract natural enemies of the herbivores responsible for the damage (Dicke et al. 1990a, Turlings et al. 1990, Vet and Dicke 1992, Stowe et al. 1995, Takabayashi and Dicke 1996). The qualitative and quantitative characteristics of herbivore-induced plant volatiles (HIPVs) can vary according to the herbivore involved, the plant species, and even genotype (Turlings et al. 1990, Takabayashi and Dicke 1994). The phenolic compound, methyl salicylate, has been identified in the HIPV blends from at least 10 plant species, including limabeand damaged by spider mites, Tetanychus urticae Koch (Dicke et al. 1990a, Ozawa et al. 2000a), cucumber (T. urticae) (Agrawal et al. 2002), cabbage (caterpillars, Pieris spp) (Geervliet et al. 1997), pear (Psyllidae) (Scutareanu et al. 1997), hops (hop aphid, Phorodon humuli Schrank) (Campbell et al. 1993), bird cherry (bird cherry oat aphid, Rhopalosipham padi [L.]) (Ginwood and Petterson 2000), Ninkovic et al. (2003), potato (Colorado potato beetle, Leptinotarsa decemlineata Say) (Bolter et al. 1997), Nicotiana attenuata (caterpillars, leaf bugs, flea beetles) (Kessler and Baldwin 2001), and Lotus japonicus (T. urticae) (Ozawa et al. 2000b). Tobacco plants inoculated with tobacco mosaic virus also produced methyl salicylate (Shulaev et al. 1997). Two other common HIPVs identified from a range of arthropod-damaged plants (e.g., corn, cabbage, beans, pear, cotton) are the terpene, (E)-4,8-dimethyl-1,3,7-nonatriene, and the ester, (Z)-3-hexenyl acetate (Dicke et al. 1990a, Rose et al. 1998, Pare and Tumlinson 1996, Dicke 1999).

Laboratory studies have demonstrated methyl salicylate to be attractive to the predatory mite, Phytoseius persimilis Athias-Henriot (Dicke and Sabelis 1988; Dicke et al. 1990a, b, Ozawa et al. 2000b), and the predatory bug, Anthocoris nemoralis (Fabricius) (Drukker et al. 2000). In contrast, olfactometer studies showed methyl salicylate to be repellent to the aphid pests, Aphis fabae Scop. and Sitobion avenae (Hardie et al. 1994, Petterson et al. 1994). Similarly, field experiments showed methyl salicylate reduced trap catches of the aphid, P. humuli Schrank, during spring colonization of hop yards (Losel et al. 1996). Ninkovic et al. (2003) demonstrated that methyl salicylate significantly delayed establishment and reduced infestation of the bird cherry oat aphid (R. padi) in oats treated with a pellet formulation of the semiochemical. James (2003), in hop yard experiments in Washington state, showed synthetic methyl salicylate to be an attractant for the green lacewing, Chrysopa nigricornis Burmeister.

The (Z)-3-hexenyl acetate, a green leaf volatile emitted by many plants after herbivore or artificial mechanical damage, is attractive to a number of natural enemies, including the aphid parasitoid, Aphidius ervi Haliday (Du et al. 1998). However, another Aphidius parasitoid (Aphidius sp.) did not show a...
strong electroantennogram (EAG) response to (Z)-3-hexenyl acetate in a study by Han and Chen (2002). The coccinellid, Coccinella septempunctata L., and to a lesser extent the chrysopid, Chrysopa sinica Tjeder, showed stronger EAG responses (Han and Chen 2002). Dicke et al. (1990b) and Scutareanu et al. (1997) showed that (Z)-3-hexenyl acetate did not attract the predatory mite, P. persimilis, nor the predatory bug, A. nemoralis. The (E)-4,8-dimethyl-1,3,7-nonatriene is a common HIPV resulting from spider mite feeding and is attractive to P. persimilis (Dicke et al. 1990a, b).

Most of the research to date on HIPVs and response by natural enemies has been conducted under laboratory conditions (Hunter 2002). If these semiochemicals are to realize their potential in applied entomology, then more studies on HIPVs and natural enemy responses in the field environment need to be conducted. In Washington hop yards, effective conservation biological control of mites and aphids appears to depend on spring recruitment of winged predators (James et al. 2003). The use of synthetic HIPVs to enhance the recruitment of beneficial insects in early season hops might provide a useful pest management tool. This study provides field data on the attractiveness of three synthetic HIPVs, methyl salicylate, (Z)-3-hexenyl acetate, and (E)-4,8-dimethyl-1,3,7-nonatriene, to beneficial insects in a Washington state hop yard.

Materials and Methods

A trapping experiment was conducted in an un-sprayed 1-ha hop yard at Washington State University-Prosser from 11 April to 24 October 2002 to determine whether three synthetic HIPVs were attractive to beneficial arthropods. Yellow sticky cards (23 × 18 cm; Trece, Salinas, CA) baited with 2-ml glass vials (containing 1 ml of candidate HIPV solutions or left unbaited) were tied to wooden poles ~2 m above the ground (poles in hop yards are used to carry wires for supporting hop plants). The glass vials were 3.5 cm in length and 1 cm wide, with a 5-mm internal diameter of the opening. HIPVs tested were neat solutions of methyl salicylate (99%; Sigma Aldrich, St. Louis, MO), (Z)-3-hexenyl acetate (98%; Sigma-Aldrich, St Louis, MO), and 10 mg of (E)-4,8-dimethyl-1,3,7-nonatriene in 2 ml hexane (synthesized by R. J. Bartelt, United States Department of Agriculture, Peoria, IL). The (E)-4,8-dimethyl-1,3,7-nonatriene was replaced weekly; the other two chemicals were replaced fortnightly. The release rates for methyl salicylate and (Z)-3-hexenyl acetate were similar (~0.5 ml/fortnight), while 1 ml of (E)-4,8-dimethyl-1,3,7-nonatriene evaporated within 4–6 d. Bait vials were taped (black tape, minimizing exposure to direct sunlight) vertically to the lower edge of the cards, which were placed in a 3 × 4 grid with at least 10 m between the randomized treatments. All treatments were replicated three times, and the treatment area occupied about half of the 1-ha hop yard. Sticky cards were replaced weekly and examined under a stereomicroscope for beneficial arthropods. Eleven groups or species of predators or parasitoids were sufficiently numerous to allow assessment of attraction: Deraeocoris brevis (Uhler) (Hemiptera: Miridae), Geocoris pallens Stal. (Hemiptera: Coccinellidae), Orius tristicolor (White) (Hemiptera: Anthocoridae), Leptothrips mali (Fitch) (Thysanoptera: Thripidae), Anagrus spp. (Hymenoptera: Mymaridae), Miridae (excluding D. brevis and L. hesperus), Syrphidae, Coccinellidae (excluding S. punctum pipicis), and Hymenoptera (parasitic families including Ichneumonidae, Scelionidae, Encyrtidae, and Mymaridae [excluding Anagrus spp.]). Miridae were considered to be beneficial arthropods in this study because most species (even L. hesperus) have at least some carnivorous behavior (Wheeler 2001). Coccinellidae were comprised mostly of the three aphidophagous species, Coccinella transversoguttata Faldermann, Hippodamia convergens Guerin-Meneville, and Harmonia axyridis Pallas. Anagrus spp were evaluated separately because of our experience with this genus. Voucher specimens of the species mentioned in this work are held at Washington State University-Prosser. Data were log (x + 1) transformed to equalize variance and analyzed.
using analysis of variance (ANOVA) and Fisher least significant difference procedure.

**Results**

The predatory mirid, *D. brevis*, and the anthocorid, *O. tristicolor*, were trapped in significantly higher numbers on (Z)-3-hexenyl acetate-baited cards than on unbaited, methyl salicylate, or (E)-4,8-dimethyl-1,3,7-nonatriene-baited cards (*D. brevis, F* = 3.36; df = 3, 68; *P* = 0.03; *O. tristicolor, F* = 4.20; df = 3, 68; *P* = 0.0088) (Table 1). The difference for *D. brevis* was most pronounced in August when populations were largest (Figs. 1 and 2). For both species, attraction to (Z)-3-hexenyl acetate did not occur when populations were small. The geocorid predator, *G. pallens*, and...
hover flies (Syrphidae) were significantly attracted to methyl salicylate-baited cards compared with unbaited, (Z)-3-hexenyl acetate or (E)-4,8-dimethyl-1,3,7-nonatriene-baited cards (G. pallens, $F = 3.71$; df = 3, 68; $P = 0.0156$; Syrphidae, $F = 16.1$; df = 3, 68; $P = 0.0000$) (Table 1). Once again, attraction was most pronounced when populations were large, during June–August for G. pallens and in July, September, and October for syrphids (Figs. 1 and 2). No attempt was made to identify syrphid species. Although S. punctum picipes was not significantly attracted to any of the HIPVs when analyzed over the entire trapping period (Table 1), significantly greater numbers of this coccinellid were caught on methyl salicylate and (Z)-3-hexenyl acetate-baited cards in July and September (July, $F = 13.2$; df = 3, 12; $P = 0.0004$; September, $F = 3.37$; df = 3, 12; $P = 0.05$) (Fig. 1). No attraction to the HIPVs was detected for L. hesperus, L. mali, Anagrus spp., other Miridae, other Coccinellidae, or parasitic Hymenoptera ($P > 0.05$) (Table 1). No attraction by any insect species or group was noted for (E)-4,8-dimethyl-1,3,7-nonatriene.

Discussion

Hunter (2002) in a review of plant volatile-natural enemy interactions, makes the point that although laboratory evidence for plants manipulating the foraging of natural enemies by volatile emission is now overwhelming, very few studies have been published showing natural enemy responses to plant volatiles under field conditions. In addition, the few outdoor studies that have been published are often correlative (higher densities of predators around pest-infested plants than uninfested plants) (e.g., Drukker et al. 1995, Shimoda et al. 1997), or involve inducing HIPVs from plants (without other predator-associated cues) and measuring responses by natural enemies (e.g., Thaler 1999, Ockroy et al. 2001). Field attraction of beneficial insect species to synthetic HIPVs was shown in studies by Flint et al. (1979), Zhu et al. (1999), and more recently by James (2003).

This study showed that methyl salicylate is an attractant for the bigeyed bug, G. pallens, and hover flies, and (Z)-3-hexenyl acetate is an attractant for the mirid, D. brevis, and the minute pirate bug, O. tristicolor. In addition, both chemicals appeared to have some attractiveness to themite-eating ladybeetle, S. punctum picipes.

Previously, methyl salicylate was demonstrated to be attractive to the predatory mite, P. persimilis, the pirate bug, A. nemoralis, and the green lacewing, C. nigricornis (Dicke et al. 1990a, b; Ozawa et al. 2000b; James 2003). Demonstration of field attraction of species of predatory true bugs, beetles, flies, and lacewings to these two HIPVs suggests that synthetic HIPV formulations might be used in integrated pest man-
agement programs to aid conservation biological control.

The simple sticky card/HIPV bait method used in this study was sufficient to identify the differences in attraction reported in this work. However, in most cases in which attraction occurred, it was most evident when the attracted species occurred in reasonably large numbers in the hop yard. Improvements to methodology that tie volatile release/dispersion more closely to the sticky trapping surface might improve detection of attractiveness at lower population levels. Volatile plumes from vials positioned at the bottom edge of sticky cards tied vertically to poles may be less successful in directing attracted insects to the sticky area than, for instance, vials positioned in the center of horizontally oriented sticky cards, as is characteristic of δ-type insect-monitoring traps. Other types of bait/trap systems could also be used in field studies on HIPVs (e.g., James 2003). The question of dosage was not addressed in this study, and it is possible that the high level of volatilization (far in excess of naturally occurring HIPVs) may have been repellent to some natural enemies. For example, Whitman and Eller (1992) showed that some parasitic Hymenoptera are capable of orienting to very low doses of green leaf volatiles and are repelled by moderate doses. Dose response of beneficials to HIPVs needs to be addressed in future studies.

The results reported in this work are a small, but significant first step indicating the potential of HIPVs as field attractants for beneficial arthropods. Other HIPVs remain to be field tested, and the current list of HIPVs may expand in the future as other studies examine different arthropods on different plants. Exploitation of HIPVs as attractants for beneficial arthropods in crop systems may require slow-release dispensation of synthetic volatiles over the crop area, or at least on the perimeter. Retention of natural enemies within the crop could also be influenced by synthetic HIPVs, but to some extent may be better facilitated by pest presence and naturally occurring HIPVs.

This research was undertaken in an effort to increase early season recruitment of winged beneficial arthropods to hop yards in Washington state. Conservation biological control, based on recruitment and maintenance of a community of natural enemies, has been shown to aid in the management of the principal pests of hop, two-spotted spider mite, T. urticae, and hop aphid, P. humuli, in Washington (James et al. 2003). A key component in the success of this strategy is spring colonization of hop yards by winged, generalist predators of mites and aphids such as coccinellids, anthocorids, geocorids, and neuropterans. A robust fauna of these natural enemies in spring effectively contains pest hot spots preventing their dispersal throughout the crop. The current study suggests that deployment of methyl salicylate and (Z)-3-hexenyl acetate dispensers in spring hops may enhance recruitment of key natural enemies such as O. tristicolor, C. pallens, and D. brevis. Methyl salicylate was shown by Losel et al. (1996) to reduce trap catches of P. humuli in a hop yard (numbers of beneficial insects were not recorded). These authors concluded that methyl salicylate was repellent to P. humuli; thus, the use of this semiochemical in hop yards may serve two benefits to pest management: enhanced recruitment of beneficial insects and repellence of the pest, P. humuli.

Early season recruitment of beneficial insects is a key to better biological control in many crop systems. Synthetic HIPVs, if they work on a crop scale, could improve biologically based pest management in a range of crops.

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