INTRODUCTION

Semiochemicals produced by herbivores and their host plants could play a major role in locating and recognizing herbivorous prey by foraging carnivorous arthropods (Lewis and Martin, 1990; Godfray, 1994). This has triggered considerable interest in the use of such semiochemicals for manipulating carnivores as biological control agents (Letourneau and Altieri, 1999). Herbivore-induced plant volatiles (HIPVs) emitted from plants in response to infestation by pest herbivores may be of special interest in biological control (Dicke et al., 1990). In fact, there are several reports of HIPVs that attract carnivorous natural enemies such as predatory mites, insect predators, and parasitoid wasps (Dicke et al., 1998; Dicke, 1999).

Some compounds in HIPVs are also produced by leaves treated with jasmonic acid (JA), a compound that activates defense signaling pathways against herbivores and pathogens in plants (Boland et al., 1995; Dicke et al., 1999; Gols et al., 1999; Ozawa et al., 2000). JA treatment is expected to make plants less susceptible to herbivores (Thaler et al., 1996; Baldwin, 1998) and more attractive to carnivores such as predatory mites (Dicke et al., 1999; Gols et al., 1999) and parasitoid wasps (Thaler, 1999).

Recently, we demonstrated that methyl salicylate (MeSA), a derivative of salicylic acid (SA) that activates defense signaling pathways against pathogens in plants (Boland et al., 1995; Dicke et al., 1999; Gols et al., 1999; Ozawa et al., 2000). MeSA treatment is expected to make plants less susceptible to herbivores (Thaler et al., 1996; Baldwin, 1998) and more attractive to carnivores such as predatory mites (Dicke et al., 1999; Gols et al., 1999) and parasitoid wasps (Thaler, 1999).

The responses of two insect predators of spider mites, Scolothrips takahashii (Thysanoptera: Thripidae) and Oligota kashmirica benefica (Coleoptera: Staphylinidae), to volatile compounds from leaves treated with aqueous jasmonic acid (JA) and/or gaseous methyl salicylate (MeSA) in an olfactometer were examined. Adult females of O. kashmirica benefica exhibited a significant preference for JA+MeSA-treated leaves when compared with uninfested leaves. In contrast, adult females of S. takahashii significantly preferred MeSA- and JA+MeSA-treated leaves to uninfested leaves. Neither predator showed any preference for JA-treated leaves when compared with uninfested leaves. The results argue in favor of manipulating the behavior of natural enemies of herbivores as a method of biological control against herbivorous pests in agroecosystems. This is the first report to suggest that MeSA can be a useful tool for enhancing the effectiveness of carnivorous natural enemies of spider mites.

Key words: Oligota kashmirica benefica, Scolothrips takahashii, lima bean, jasmonic acid, methyl salicylate

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from lima bean leaves induced by the two-spotted spider mites *Tetranychus urticae* than volatiles induced by JA treatment alone (Ozawa et al., 2000). In contrast, volatile compounds from JA-treated lima bean leaves are more similar to those from leaves infested with the common armyworm caterpillars *Mythimna separata*, than those from leaves treated with MeSA and JA+MeSA (Ozawa et al., 2000). However, whether the attractiveness of JA and/or SA induced volatiles to carnivores coincides with that of their resemblance to herbivore-induced volatiles remains to be investigated. In the present study, two predatory insect species, *Scolothrips takahashii* (Thysanoptera: Thripidae) and *Oligota kashmirica benefica* (Coleoptera: Staphylinidae), were used in an effort to demonstrate that volatile compounds induced by MeSA treatment and/or JA+MeSA treatment are more attractive to carnivorous natural enemies of *T. urticae* than those induced by JA treatment in lima bean leaves.

*S. takahashii* and *O. kashmirica benefica* are specialized insect predators and important natural enemies of pest spider mites, such as *T. urticae*, *T. kanzawai* and *Panonychus citri*, in Japanese orchards such as Japanese pear and Satsuma mandarin orchards (Shimoda and Ashihara, 1996; Shimoda et al., 1997a, b). Those predators do not prey on *M. separata* larvae. Both predators frequently migrate between orchard trees and plants within and/or around the orchard (e.g., groundcover, weeds, or windbreaks) to exploit abundant prey (Shimoda and Takabayashi, 2001a). We previously demonstrated that adult females of *S. takahashii* (Shimoda et al., 1997a) and *O. kashmirica benefica* (Shimoda and Takabayashi, 2001b) were attracted to HIPVs from *T. urticae*-infested lima bean leaves under both laboratory and field conditions. However, little is known about the olfactory responses to volatile compounds induced by chemicals such as JA and MeSA. The objective of this study was to examine responses of the two above mentioned predators to odors from plants treated with JA, MeSA, or JA+MeSA.

**MATERIALS AND METHODS**

**Insect rearing.** *S. takahashii* were collected from leguminous arrowroot plants *Pueraria lobata* (Wild.) infested with *Tetanychus* spp. spider mites in Kyoto City, Japan, in 1998. *O. kashmirica benefica* were collected from copperleaf plants *Acalypha australis* infested with *T. kanzawai* and *T. phaselus* at Kyoto University in 1998. Both predators were reared on *T. urticae* on lima bean plants (*Phaseolus lunatus* cv. Sieva) for 1 to 3 generations under laboratory conditions (25±2°C, 60±10% relative humidity and L16:D8 light cycles). Both fertilized and starved predators (adult females 7–30 d old post-emergence) were used for the bioassays. To obtain starved predators, approximately 20 adults of each predator species were introduced into a sealed plastic case (10 cm in diameter, 4.5 cm in depth) containing a small piece of moist filter paper, for 24 h prior to experimentation.

*M. separata* were obtained from a culture reared at the National Institute of Sericultural and Entomological Sciences in Tsukuba, Ibaraki, Japan, in 1990. They were reared on the artificial diet (Insecta LF; Nihon Nousan Kogyo Ltd., Yokohama, Japan) in a climate room (25±2°C, 60±10% relative humidity and L16:D8 light cycles).

**Plants.** Lima bean plants were used for rearing the spider mites and the predators. The plants were cultivated individually in clay pots (8 cm in diameter, 7 cm in depth) in a greenhouse (25±3°C, 60±10% relative humidity and L16:D8 light cycles). Young primary leaves from 2- to 3-week-old plants were used for the experiments.

**Preparation of odor sources.** Five different types of odor sources were prepared in this study.

1. Mite-infested leaves. Five detached primary leaves with petioles were placed separately in a glass vial (15 ml) filled with distilled water. A piece of lima bean leaf heavily infested with *T. urticae* was placed on each of the uninfested leaves under both laboratory and field conditions. However, little is known about the olfactory responses to volatile compounds induced by chemicals such as JA and MeSA. The objective of this study was to examine responses of the two above mentioned predators to odors from plants treated with JA, MeSA, or JA+MeSA.
each placed in a glass vial (15 ml) filled with distilled water. The vials were placed in a glass bottle (2 l). Approximately 300 *M. separata* larvae (1st to 2nd stadium) were then placed on the leaves. After 2 d, five leaves with a damaged area of 5–10% per leaf were selected. Following the removal of the caterpillars and their associated products (e.g., feces), the leaves were transferred to clean glass vials (15 ml) filled with distilled water. The vials were placed in a sealed glass bottle (2 l), and the leaves were kept in the bottle for 22 h before being used as an odor source. Other conditions and procedures were the same as those described above (1).

(3) JA-treated leaves. Five detached primary leaves with petioles were kept separately in 5 plastic tubes (1.5 ml) with 1 ml of aqueous jasmonic acid (1 mM) for approximately 2 h under laboratory conditions (25°C, 60±10% relative humidity and L16:D8 light cycles) to allow them to take up all of the solution. The leaves were then separately transferred to five glass vials (15 ml) filled with distilled water. The opening of each vial was sealed with Parafilm® (American National Can, Chicago, IL, USA). The treated leaves were placed together in a sealed glass bottle (2 l) and then kept for approximately 22 h under laboratory conditions. Other conditions and procedures were the same as described above (1).

(4) MeSA-treated leaves. Gaseous MeSA was prepared by adding approximately 2.5 ml MeSA (Wako Chemicals) to a sealed glass bottle with two openings. The bottle was kept at 27.5±2.5°C for several hours, allowing the air in the bottle to become saturated with gaseous MeSA. The concentration of gaseous MeSA in the bottle was approximately 30 ppb, as determined in our preliminary investigation (Ozawa et al., 2000). Five detached primary leaves with petioles were placed separately in five glass vials filled with distilled water in a glass bottle. The opening of each vial was sealed with Parafilm® to prevent gaseous MeSA from dissolving into the water in the vials. A total of 2 l of air saturated with gaseous MeSA was collected with a glass syringe (200 ml) from the prepared bottle and immediately injected into the glass bottle. The glass bottle was then sealed and kept for 21 h under laboratory conditions. We then opened the bottle for 1 h. Other conditions and procedures were the same as described above (1).

(5) JA+MeSA-treated leaves. Five detached primary leaves with petioles were exposed to aqueous JA for approximately 2 h under laboratory conditions. The treated leaves were placed together in a sealed glass bottle and exposed to gaseous MeSA for 21 h under laboratory conditions. We then opened the bottle for 1 h. Other conditions and procedures were the same as described above (1).

(6) Clean leaves. Five detached primary leaves with petioles were kept for approximately 24 h in a sealed glass bottle (2 l). Other conditions and procedures were the same as described above (1).

**Bioassay.** The following five types of experiments were conducted. Experiment 1: Mite-infested leaves versus clean leaves. Experiment 2: Caterpillar-infested leaves versus clean leaves. Experiment 3: JA-treated leaves versus clean leaves. Experiment 4: MeSA-treated leaves versus clean leaves. Experiment 5: JA+MeSA-treated leaves versus clean leaves. In each experiment, five treated and five untreated lima bean leaves were used as sample and control odor sources, respectively.

A Y-tube olfactometer (Takabayashi and Dicke, 1992) was used in each experiment. The airflow through each olfactometer arm was determined with a flow meter and was found to be 2.5 l/min. Adult females of each predator species were individually introduced at the start point on a Y-shaped iron wire, which was positioned at the center of the glass tube. The behavior of each predator was observed at 5-min intervals. The observation was terminated when a predator reached the far end (finish line) of one of the arms. Although *O. kashmirica benefica* often flew within the glass tube, the observation was continued unless they flew before passing through the junction on the Y-shaped wire. Predators that did not reach the finish line (called no choice) during 5 min of observation were excluded from statistical analysis. A different iron wire was used in every bioassay. The connections of odor source containers (conical flask: 3.8 l) to the arms were replaced after every five bioassays. In every experiment, a total of 80–100 predators were tested over 4 d.

**Data analysis.** The results of each experiment were analyzed for significance by a chi-square test (Sokal and Rohlf, 1998). The null hypothesis was that predators exhibited a 50 : 50 distribution over the two odor sources. In addition, the results from
the three chemical treatments on predator species were analyzed for significance by contingency table tests (d.f. = 2 and \( p = 0.05 \)). When there was a significant effect of chemical treatment on the degree of predator attraction, each individual test was performed by contingency table tests, using the Bonferroni approach (d.f. = 1 and \( p = 0.05/3 = 0.016 \)).

RESULTS

*O. kashmirica benefica* exhibited a significant preference for mite-infested leaves over clean leaves (chi-square test; chi-square = 10.646, d.f. = 1 and \( p < 0.001 \); Fig. 1). However, there was no significant difference between the number of *O. kashmirica benefica* attracted to caterpillar-infested leaves and those attracted to clean leaves (chi-square test; chi-square = 1.209, d.f. = 1 and \( p = 0.272 \)). The predators did not discriminate between JA-treated leaves and clean leaves (chi-square test; chi-square = 0.821, d.f. = 1 and \( p = 0.365 \)). Similar results were obtained with insects offered MeSA treated leaves and clean leaves (chi-square test; chi-square = 0.127, d.f. = 1 and \( p = 0.722 \)). However, the predators were significantly more attracted to JA + MeSA-treated leaves than to clean leaves (chi-square test; chi-square = 3.968, d.f. = 1 and \( p < 0.05 \)). The results in the three chemical treatments were not significantly different (contingency table test; chi-square = 1.183, d.f. = 2 and \( p = 0.554 \)), indicating that there was no significant effect of chemical treatment on the degree of predator attraction.

*S. takahashii* exhibited a significant preference for mite-infested leaves over clean leaves (chi-square test; chi-square = 13.535, d.f. = 1 and \( p < 0.001 \); Fig. 2); however there was no significant difference between the number of *S. takahashii* attracted to caterpillar-infested leaves and those attracted to clean leaves (chi-square test; chi-square = 0.051, d.f. = 1 and \( p = 0.821 \)). The predators did not discriminate between JA-treated leaves and clean leaves (chi-square test; chi-square = 0.011, d.f. = 1 and \( p = 0.915 \)). However, the predators were significantly more attracted to MeSA-treated leaves than to clean leaves (chi-square test; chi-square = 4.427, d.f. = 1 and \( p < 0.05 \)). They also preferred JA + MeSA-treated leaves over clean leaves (chi-square test; chi-square = 15.124, d.f. = 1 and \( p < 0.001 \)). The results in the chemical treatments were significantly different (contingency

![Fig. 1.](image-url)
indicating that there was a significant effect of chemical treatment on the degree of predator attraction. A significant difference was also observed between JA-treated leaves and JA+MeSA-treated leaves (contingency table test; chi-square = 8.607, d.f. = 1 and p < 0.01).

DISCUSSION

This study showed the importance of the blend of volatile compounds induced by either herbivores or chemicals in attracting predators to herbivore-infested plants. In a Y-tube olfactometer, adult females of *S. takahashii* (Shimoda et al., 1997a) and *O. kashmirica benefica* (Shimoda and Takabayashi, 2001b) were attracted to HIPVs from lima bean leaves infested with *T. urticae*. The present study provides further evidence that each predator species is attracted to *T. urticae*-infested lima bean leaves. We have also shown that neither predator species was attracted to volatiles from lima bean leaves mechanically damaged by rubbing with carborundum on a wet cotton wool pad (Shimoda et al., 1997a; Shimoda and Takabayashi, 2001b).

Thus, the volatiles emitted from lima bean leaves infested with *T. urticae* would provide specific information on the presence of their prey for the two predator species. This is supported by evidence that neither predator species was attracted to lima bean leaves infested with the non-prey caterpillar *M. separata*, suggesting that both predator species discriminate between prey-infested and non-prey-infested plants by olfaction.

To compare the similarity of induced volatiles emitted from JA- and/or MeSA-treated leaves and those from herbivore-infested leaves, Ozawa et al. (2000) excluded MeSA in the blend of volatiles, as they were unable to conclude whether MeSA found in the headspace of MeSA-treated leaves was induced or adsorbed. By contrast, in this study, which focused on the similarity of the total blend of volatiles emitted from the treated leaves and those from infested leaves, we re-calculated the similarity of the blends including MeSA (calculation method, see Ozawa et al., 2000). The headspace that was both qualitatively and quantitatively most similar to the headspace of *T. urticae* infested leaves was that from JA+MeSA treated leaves, while the least similar was that from JA-treated leaves; chi-square = 8.659, d.f. = 2 and p < 0.05), indicating that there was a significant effect of chemical treatment on the degree of predator attraction. A significant difference was also observed between JA-treated leaves and JA+MeSA-treated leaves (contingency table test; chi-square = 8.607, d.f. = 1 and p < 0.01).
JA-treated leaves. The degree of attraction of JA-treated leaves and MeSA-treated leaves, but not to blends that are similar to those induced by JA treatment had no significant effect on the degree of attraction of the predators, the results indicate that the predators responded only to plant volatiles induced with chemical treatments that are most similar to those induced by T. urticae.

Both of the two insect predators were specialized predators of spider mites including the genus Tetranychus (Shimoda and Ashihara, 1996; Shimoda et al., 1997a, b), and both were attracted to T. urticae-infested lima bean leaves in this study. However, the olfactory responses of the two specialist insect predators to chemical-induced plant volatiles were somewhat different: S. takahashii were attracted to MeSA-treated leaves, whereas O. kashmirica benefica were not. The specificity in the response of predators to the specific blend of volatiles informing the presence of prey-infested leaves differed according to the predator species.

Most studies in the context of biological pest control have focused on carnivore attraction to JA-treated plants both in the laboratory and in the field (Dicke et al., 1999; Gols et al., 1999; Thaler, 1999). Although the treatment of plants with MeSA has been thought to be effective against plant pathogens (Durner et al., 1997), the effect of MeSA treatment to carnivorous natural enemies of herbivore pests has not yet been reported. Our results further suggest that MeSA can be a useful tool for enhancing the effectiveness of carnivorous natural enemies of spider mites. Further studies should be aimed at investigating the effects of the MeSA- and SA+JA-induced volatile compounds on predator attraction under field conditions.

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REFERENCES


Table 1. The mean distance in cluster analysis between a blend of volatiles from lima bean leaves treated with aqueous jasmonic acid (JA) and/or gaseous methyl salicylate (MeSA) and that from lima bean leaves infested with Tetranychus urticae or Mythimna separata.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T. urticae</th>
<th>M. separata</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA</td>
<td>0.88±0.04 c</td>
<td>0.19±0.03 a</td>
</tr>
<tr>
<td>MeSA</td>
<td>0.41±0.13 b</td>
<td>1.17±0.01 c</td>
</tr>
<tr>
<td>JA+MeSA</td>
<td>0.12±0.01 a</td>
<td>0.80±0.04 b</td>
</tr>
</tbody>
</table>

The distance was calculated using the ion intensity of each compound for each sample. Values were mean±SE of the distances between three individual samples of a chemical treatment and those of an herbivore. Means in the same column followed by a different letter were significantly different according to the Bonferroni approach (d.f.=1 and p=0.05/3=0.016), followed by the Kruskal-Wallis test (d.f.=2 and p=0.05). When the mean distance between two volatile blends is small, these blends are qualitatively and quantitatively similar, and vice versa. For further details, see text.