Role of host plant volatile in the host-finding behavior of the strawberry leaf beetle, *Galerucella vittaticollis* Baly (Coleoptera: Chrysomelidae)

Masatoshi Hori,* Kazuya Ohuchi and Kazuhiro Matsuda

Laboratory of Insect Science and Bioregulation, Graduate School of Agricultural Science, Tohoku University; Sendai 981–8555, Japan

(Received 5 September 2005; Accepted 2 March 2006)

---

**Abstract**

The strawberry leaf beetle, *Galerucella vittaticollis* Baly, is an oligophagous insect that feeds on strawberry and polygonaceous plants. Behavioral responses of *G. vittaticollis* to host and non-host plant volatiles were investigated. Beetles were attracted to the odors of their host, rosaceous plant, *Fragaria ananassa* Duchn., and polygonaceous plants, *Rumex obtusifolius* L., *Fagopyrum esculentum* Moench, *Polygonum thunbergii* Sieb. et Zucc., *P. cuspidatum* Sieb. et Zucc. and *P. blumei* Meisn. They were not attracted to non-host plants, *Raphanus sativus* L. (Brassicaceae), *Lycium chinense* Mill. (Solanaceae), *Artemisia princeps* Pampan. (Compositae) and *Triticum aestivum* L. (Gramineae). The main component of the headspace of all host plants tested was one of the green leaf volatiles, cis-3-hexenyl acetate. This compound was detected also in the non-host plants except *T. aestivum*. However, its relative content in *A. princeps* or *L. chinense* was different from that in host plants. The headspace of *R. sativus* contained one of the isothiocyanates, characteristic components of brassicaceous plants, in addition to cis-3-hexenyl acetate. The attractancy of cis-3-hexenyl acetate and two other typical green leaf volatiles, cis-3-hexen-1-ol and trans-2-hexenal, to the beetles was examined. The beetles were significantly attracted to only cis-3-hexenyl acetate. The findings suggest that *G. vittaticollis* uses cis-3-hexenyl acetate as an olfactory cue to find the host plants, strawberry and polygonaceous plants.

**Key words:** *Galerucella vittaticollis*; host plant; olfactory response; attractancy; cis-3-hexenyl acetate

---

INTRODUCTION

Although the strawberry leaf beetle, *Galerucella vittaticollis* Baly, is one of the pests of the strawberry plant, *Fragaria ananassa* Duchn. (Rosaceae), it also feeds on the leaves of polygonaceous plants such as the *Fagopyrum esculentum* Moench, *Rumex obtusifolius* L. (Matsuda and Matsumoto, 1974). Organic acids such as oxalic, malic, tartaric and citric acid, and quercetin glycosides are characteristic components of polygonaceous plants. The beetles are stimulated to feed by quercetin glycosides such as quercitrin, rutin (Matsuda, 1976), avicularin, hyperoside and isoquercitrin (Ohta et al., 1998), but are not stimulated by the organic acids mentioned above (Matsuda and Matsumoto, 1975). Adati and Matsuda (1993) also reported that some leaf surface wax components of *F. ananassa* acted as feeding stimulants that play an important role in host selection after reaching the plants. However, the host finding mechanism that allows the beetles to reach the plants remains unclear.

It has been proven that many insect species use host plant odors as olfactory cues in finding hosts (Visser, 1986). Regarding the behavioral responses of Chrysomelidae to host plant odors, it is known that *Leptinotarsa decemlineata* (Say) are attracted to a specific combination of green leaf volatiles, *trans*-2-hexenal, *cis*-3-hexenyl acetate, *cis*-3-hexenol and *trans*-2-hexenal, from potato (Bernays and Chapman, 1994). Isothiocyanates, components of brassica volatile, attract *Phyllotreta cruciferae* (Goeze) and *P. striolata* (Fabricius) (Feeny et al., 1970).

In this study, we investigated the behavioral responses of *G. vittaticollis* to their host and non-host plant volatiles and typical green leaf volatiles in order to clarify the role of the volatiles as olfactory cues.
cues in their host-finding behavior.

MATERIALS AND METHODS

Insects. G. vittaticollis were collected at the experimental field of Tohoku University and reared for successive generations in a constant-temperature room at 24±1°C under a photoregime of 16L:8D. The beetles were reared on leaves of R. obtusifolius collected from the experimental field. Adult females were used for all bioassays except for the behavioral response to R. obtusifolius. For the behavioral response to R. obtusifolius, both male and female adults were tested.

Plants. Leaves of F. ananassa, F. esculentum and T. aestivum were taken from potted plants. Leaves of R. sativus were taken from cultivated plants. Leaves of R. obtusifolius, P. cuspidatum, P. blumei, L. chinense and A. princeps were collected from the experimental field of Tohoku University. Leaves of P. thunbergii were gathered from the botanical garden of Tohoku University.

Chemicals. cis-3-Hexenyl acetate (purity: 98%), cis-3-hexen-1-ol (98%) and trans-2-hexenal (98%) were obtained from Wako Pure Chemical Ind. Ltd. (Osaka, Japan).

Bioassays with olfactometer. Behavioral responses of G. vittaticollis were investigated with a glass Y-tube olfactometer (Fig. 1). Using an air pump, an airflow (700 ml/min) was introduced into each arm of the olfactometer through activated charcoal, a humidifier and each odor-source container. In this way, two well-separated laminar airflows were generated in the olfactometer. The adult beetles that emerged were starved for 24 h and used for the tests. The beetles were individually introduced into the olfactometer. The beetle in the olfactometer chose one side of the two arms by chemotaxis. The observation ended when the beetle reached the finish line of one of the olfactometer arms, with a maximum observation duration of 10 min per beetle. If the beetle did not reach the finish line within this time period, the trial was terminated and the beetle was replaced (about 30% of the test beetles were replaced). The treatment and control arms were exchanged after half of the replications had been accomplished. Experiments were conducted in a constant-temperature room under lighted condition and at 25±1°C. Forty-six replications using different beetles were carried out for each sample.

Behavioral responses of the beetles were estimated by the excess proportion index (EPI) according to the following formula:

\[ EPI = \frac{nt - nc}{nt + nc} = 2PT - 1, \]

\[ PT = \frac{nt}{nt + nc}, \]

- EPI>0: attractancy;
- EPI=0: no preference;
- EPI<0: repellency,

where, nt and nc represent the total number of beetles that chose the treatment and the control arms, respectively, and PT represents the proportion of beetles that chose the treatment arm (Sakuma and Fukami, 1985). The 95% fiducial limits of PT were calculated from critical values of the variance ratio F, and then transformed into those of EPI. When the lower limit of the 95% fiducial limit was above 0, it was concluded that the beetles were significantly attracted.

To examine the responses to plant odors, fresh leaves (5 g) of a test plant and a sheet of filter paper...
Host Finding Behavior of *G. vittaticollis*

---

(Advantec, No. 1, diameter 90 mm) moistened with distilled water (1 ml) were placed in the treatment-side of the container, while three sheets of filter paper similarly moistened with distilled water (1 ml) were placed in the control-side of the container.

To investigate the responses to green leaf volatiles, a piece of filter paper (Advantec, No. 1, 10×20 mm) treated with 10 μl liquid paraffin (Wako Pure Chemical Ind. Ltd., Osaka, Japan; density: 0.825–0.850 g/ml at 20°C) solution of each compound was placed in the treatment-side of the container. As a control, a piece of filter paper treated with a 10 μl liquid paraffin was placed in the control-side of the container.

**Chemical analysis.** Headspace components of the test plants were extracted with solid-phase micro extraction (SPME) and identified by comparison of GC retention times and mass spectra to those of the standard chemicals.

1) SPME: Fresh leaves (5 g) of the test plants were placed into a sample vial (40 ml) with a Mininert® valve. The headspace of the plant leaves in the vial was extracted with SPME (100 μm polydimethylsiloxane, Supelco, Pennsylvania, USA) for 1 h. The headspace of the plant leaves was extracted at ca. 5°C, because headspace was efficiently extracted at this temperature in a preliminary experiment.

2) Gas chromatography-mass spectrometry (GC-MS): A Hitachi G-5000M gas chromatograph equipped with a DB-5 column (30 m×0.25 mm id, 0.25 μm film thickness, J&W) and a Hitachi M-7200 mass selective detector were used. The mass data were analyzed by a GC/3DQMS system (Hitachi). Helium was used as the carrier gas at a column head pressure of 100 kPa. GC was set for splitless injection (splitter opened after 1 min). The temperature program of the column oven was isotherm 5 min at 35°C, 4°C/min gradient to 200°C, isotherm 16 min at 200°C, 10°C/min gradient to 300°C, and isotherm 5 min at 300°C. The injector and detector temperature were 220°C and 150°C, respectively.

**RESULTS**

Olfactometer tests revealed that *G. vittaticollis* females were significantly attracted to the odors of their host plants, *F. ananassa, R. obtusifolius, F. esculentum, P. cuspidatum, P. thunbergii* and *P. blumei* (Fig. 2). Both male and female adults were attracted to the odor of *R. obtusifolius*. Non-host plants, *R. sativus, L. chinense, A. princeps* and *T.*

---

**Fig. 2.** Behavioral responses of *Galerucella vittaticollis* to the host and non-host plant odors in olfactometer tests. $EPI=(nt-nc)/(nt+nc)$; $nt, nc$: total number of beetles that chose the treatment and control arms, respectively. Error bars indicate 95% fiducial limits of $EPI$. Adult females were used for the tests except for the test of *R. obtusifolius*. For *R. obtusifolius*, both male and female adults were tested. Forty-six replications were carried out for each plant.
cis-3-Hexenyl acetate was also detected in the headspace of non-host plants except for *T. aestivum*. However, it was a minor component and its relative content was only 1% in the headspace of *A. princeps*. Although cis-3-hexenyl acetate was one of the main components in *L. chinense*, its relative content was about 30% and the other main component (relative content: about 30%), which was undetectable in host plants, was detected at the retention time of 29.43 min. In *R. sativus*, only cis-3-hexenyl acetate was a major component (relative content: 89%) and the other components were minor; same as in the host plants. However, about 6% 4-(methylthio)-3-butenyl isothiocyanate, which was one of the characteristic components of brassicaceous plants, isothiocyanates, was contained in the headspace of non-host plants except for *P. cuspidatum*. cis-3-Hexenyl acetate significantly attracted the beetles at concentrations of 0.01, 0.03 and 0.05%, showing EPI values of 0.57, 0.48 and 0.35, respectively, while it did not attract at concentrations of 0.005 and 0.1% (Fig. 3). Among all the concentrations tested, 0.01% showed the highest attractancy. Attractancy was reduced with increasing concentrations above 0.01%. cis-3-Hexenyl acetate is a typical green leaf volatile. Other typical green leaf volatiles, cis-3-hexen-1-ol and trans-2-hexenal, failed to show significant attractancy to the beetles.
at any concentration tested, although the volatiles tended to attract them at a concentration of 0.03%, showing EPI values of 0.30 and 0.26, respectively.

**DISCUSSION**

This study revealed that *G. vittaticollis* utilized one of the green leaf volatiles, *cis*-3-hexenyl acetate, as an olfactory cue for finding host plants. The attractancy of green leaf volatiles is also known in other insect species. *Melolontha melolontha* are attracted to *cis*-3-hexen-1-ol (Reinecke et al., 2002). *Anoplophora glabripennis* are attracted to *cis*-3-hexen-1-ol, one of the leaf volatiles from the host tree, *Acer negund* (Li et al., 2003). Hopkins and Young (1990) reported that blends of *cis*-3-hexenyl acetate, *cis*-3-hexen-1-ol, *trans*-2-hexenal and 1-penten-3-ol volatilized from host plants played an important role as olfactory cues for orientation of *Melanoplus sanguinipes*. *cis*-3-Hexenyl acetate enhances the attractancy of *P. xylostella* to the pheromone (Reddy and Guerrero, 2000). *Melolontha hippocastani* are also attracted to *cis*-3-hexen-1-ol, and the attractancy is enhanced by the pheromone (Ruther and Hilker, 2003). As mentioned above, some insect species use the green leaf volatiles as olfactory cues for orientation to their host plants and to their mates.

In this study, *G. vittaticollis* were strongly attracted to *cis*-3-hexenyl acetate, a characteristic component of the host plants of this insect, but not to the other two green leaf volatiles. The beetles were not attracted to *T. aestivum*, whose headspace did not contain *cis*-3-hexenyl acetate. Although *cis*-3-hexenyl acetate is a general volatile of green plants, its relative content differs among plant species. Actually, relative contents of *cis*-3-hexenyl acetate in the headspace of *L. chinense*, *A. princeps* and *T. aestivum* were different from those of the host plants. The composition ratio of *cis*-3-hexenyl acetate may be important for attractancy to *G. vittaticollis*. Although the headspaces of *A. princeps* and *L. chinense* contained *cis*-3-hexenyl acetate, its relative contents were 1% and 30%, respectively. It is assumed that *G. vittaticollis* were not attracted to *A. princeps* because *cis*-3-hexenyl acetate was not a characteristic component of this plant. The headspace of *L. chinense* contained a different main component than *cis*-3-hexenyl acetate. No attractancy of *L. chinense* may be caused by the difference of composition ratios of *cis*-3-hexenyl acetate between the volatiles of *L. chinense* and host plants, or the presence of compounds masking the attractancy of *cis*-3-hexenyl acetate. The main volatile component of *R. sativus* was *cis*-3-hexenyl acetate; same as that of host plants. However, 4-(methylthio)-3-butenyl isothiocyanate, which was one of the characteristic components of brassicaceous plants, isothiocyanates, was contained in the headspace. This compound may mask the attractancy of *cis*-3-hexenyl acetate.
G. vittaticollis may be attracted to plant volatiles characterized by cis-3-hexenyl acetate and containing no inhibitors to attractancy of cis-3-hexenyl acetate, and reach the prospective plants as their host.

cis-3-Hexenyl acetate may be a characteristic component of not only their host plants but also of some non-host plants because it is a general green leaf volatile. Larvae of Bombyx mori are attracted to mulberry leaf odor, and its components, cis-3-hexen-1-ol, trans-2-hexenal (Watanabe, 1958), citral, linalyl acetate, linalool and terpinyl acetate (Hamamura and Naito, 1961). These components are distributed in many non-host plant volatiles, and the larvae are attracted to some non-host plant odors (Hamamura, 1963; Hirao and Ishikawa, 1964). Similarly, the beetles may sometimes be attracted to non-host plants whose volatiles are characteristic of cis-3-hexenyl acetate. However, the larvae of B. mori attracted to non-host plant leaves do not actually bite the leaves. A biting factor and a swallowing factor are necessary to induce the feeding behavior (Hamamura, 1963). G. vittaticollis are stimulated to feed by quercetin glycosides contained in the polygonaceous plants and F. ananassa (Matsuda, 1976, 1982; Ohta et al., 1998), indican present in P. tinctorium (Matsuda, 1983) and some leaf surface wax components of F. ananassa (Adati and Matsuda, 1993). It is assumed that G. vittaticollis are stimulated to feed by these components, and that they feed on host plant leaves after reaching the host by using the olfactory cue, volatile characterized by cis-3-hexenyl acetate.

cis-3-Hexenyl acetate significantly attracted the beetles at concentrations of 0.01, 0.03 and 0.05%, but did not attract them at concentrations of 0.005 and 0.1%. The concentration of cis-3-hexenyl acetate is important for attractancy to G. vittaticollis. The amounts of this compound volatilized from the host plants may be important for the finding behavior of the beetles.

The compounds that play important roles in the sequence of host selection behavior of G. vittaticollis, from finding to feeding on the host plants, were clarified in previous studies (Matsuda, 1976, 1982, 1983; Adati and Matsuda, 1993; Ohta et al., 1998) and this study. G. vittaticollis orientates to F. ananassa and polygonaceous plants by the attractancy of cis-3-hexenyl acetate, which is a common main volatile to these plants. After reaching the plants, the beetle feeds on them by feeding stimuli of quercetin glycosides contained in both F. ananassa and polygonaceous plants. From these results, G. vittaticollis interestingly selects these plants belonging to different families as the host plants.

ACKNOWLEDGEMENTS

We wish to thank Dr. Toshio Masuda, of the Miyagi Prefectural Agriculture and Horticulture Research Center, for kindly supplying the strawberry seedlings.

REFERENCES


Matsuda, K. and Y. Matsumoto (1975) Feeding stimulation


