「森林総合研究所研究報告」(Bulletin of FFPRI) Vol.11 No.2 (No.423) 51 - 55 June 2012

論　文（Original article）

Differential responses to α-pinene of two horntail wasps, *Urocerus antennatus* and *Xeris spectrum* (Hymenoptera: Siricidae)

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Abstract

Horntail wasps carrying the symbiotic fungus *Amylostereum laevigatum* discolor the wood of conifers by infesting it with the fungus at the time of oviposition. Since wood discoloration cannot be distinguished before the wood has been cut, it is a serious economic problem for foresters. It is important to understand the mechanisms involved in oviposition preference by investigating volatile constituents from host plants and behavioral characteristics of the insects. Therefore, we conducted an olfactometer-based bioassay using two species of horntail wasps (*Urocerus antennatus* and *Xeris spectrum*) to examine their response to α-pinene, which is a major component widely distributed in conifers and which attract for female *U. japonicus*. In the present study, α-pinene attracted female *X. spectrum*, but not *U. antennatus*. These results suggest that *U. antennatus* was attracted by other conditions of the host trees that were utilized by *U. japonicus* and *X. spectrum*.

Key words : α-Pinene, attraction, horntail wasps, host selection, olfactometer, volatiles

Introduction

The Japanese horntail (*Urocerus japonicus* Smith) and the Pine horntail (*U. antennatus* Marlatt) are two species of wasps (Hymenoptera: Siricidae) that cause wood discoloration of Japanese cedar (*Cryptomeria japonica*) and hinoki cypress (*Chamaecyparis obtusa*) through the transmission of the symbiotic fungus *Amylostereum laevigatum* (Tabata and Abe, 1997, 1999), which infects the wood when female horntail wasps oviposit in the trunk (Okuda, 1989; Fukuda, 1997). It was recently elucidated that the discoloration of *Cr. japonica* and *Ch. obtusa* induced by siricid wasps occurs widely throughout Japan (Okuda, 1985; Sano, 1992; Miyata, 1999). The market price of discolored wood is much lower than normal, inflicting considerable economic damage on foresters (Fukuda, 1997; Tabata and Abe, 1997; Fukuda and Maeto, 2001). Therefore, it is necessary to understand the mechanisms involved in the behavior and habits of horntail wasps, and to prevent these insects from causing damage.

On the other hand, *Xeris spectrum* Linnaeus (Hymenoptera: Siricidae) carries neither symbiotic fungi nor mycangia. *X. spectrum* oviposits in wood in which other horntail wasps have oviposited and introduced the symbiotic fungus, so the life cycle of *X. spectrum* is a kind of social parasitism using the symbiotic fungi of other horntail wasps (Fukuda, 1997; Fukuda and Hijii, 1997).

Horntail wasps are classified as secondary wood-feeding insects (Knight and Heikkenen, 1980), which can attack and utilize only weakened, stressed or freshly cut trees as their hosts. In general, secondary wood-feeding insects utilize the characteristics and specific secondary volatile constituents emitted from weakened and/or stressed trees, and when trees are cut down (Haack and Slansky, 1987; Hanks, 1999). To elucidate the mechanisms of damage by the pest insects and prevent such damage, it is important to determine the specific volatile constituents emitted from the hosts and investigate the functions of the volatiles. Alpha-Pinene in terpenoid compounds is a major characteristic component widely distributed in conifers, and is used as a kairomone by horntail wasps that utilize conifers as their host plants. Sato and Maeto (2006), using adhesive traps in field tests, reported that α-pinene attract for female adults of *U. japonicus*.

To investigate the volatile constituents from host plants and the behavior of insects, it is important to develop appropriate methods for bioassay (Kennedy, 1977; Ikekawa et al., 1984), because this can reveal the insects' responses induced by volatile constituents and clarify the relationship between the bioactive substances emitted from the hosts and the habitual behaviors of the insects mediated by the

原稿受付：平成 24 年 1 月 19 日 Received 19 January 2012　原稿受理：平成 24 年 4 月 12 日 Accepted 12 April 2012
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chemical cues. In general, bioassay is conducted using an olfactometer in order to investigate the insects' responses to volatile constituents. This usually involves a dual-choice type olfactometer to measure the insects' sensitivy to volatile constituents (Ikekawa et al., 1984). To estimate the detailed responses to volatile constituents, it is important to set up the equipment according to the behaviors and habits of the insect. In particular, highly accurate equipment is required for the olfactometer bioassay of horntail wasps, because it is difficult to obtain many adult wasps from the host trees.

In our earlier study, we used a dual-choice olfactometer created for horntail wasps and demonstrated that α-pinene attracted *U. japonicus* females (Matsumoto and Sato, 2007). However, it was not known if other horntail wasp species were also attracted to α-pinene in the olfactometer. Thus, in this study, we conducted an olfactometer-based bioassay to investigate the responses of *U. antennatus* and *X. spectrum* to α-pinene, and compared the results with those for *U. japonicus*.

Materials and Methods

Insects. Adult horntail wasps were collected from the logs of trees that were felled in February 2005 in a stand of *Ch. obtusa* in Kami City, Kochi Prefecture. The felled trees were left on the ground of the original stands for about one year to allow horntail wasps to oviposit. The tree logs were then hauled to Shikoku Research Center, Forestry and Forest Products Institute in Kochi City. The logs were cut into lengths of 1 m and placed in outdoor cages. Adult horntail wasps (*U. antennatus* and *X. spectrum*) emerging from the logs were collected daily during the summer of 2006. Unmated females which did not coexist with males in the cages were used for the olfactometer bioassay within one day after they emerged.

Olfactometer attraction method and attractant sample. We used a T-shaped dual-choice olfactometer, which was created for adult *U. japonicus* as shown in Fig. 1 (Matsumoto and Sato, 2007). The olfactometer consists of T-shaped branched tubes (32 mm in diameter), and glass chambers (85 mm in diameter, 130 mm in height) in which to place samples. The T-shaped tubes consisted of a branched part (200 mm in length), a junction part and an introduction part (200 mm in length). The airflow (4 L/min) was regulated by a flow meter with needle valve connected to each branch, was purified by passing through a charcoal tube, and then was humidified by passing through a wash bottle filled with distilled water. The airflow was separated equally at the Y-shaped joint to pass through each chamber in which a sample was placed. During the test, the introduction part was covered with aluminum foil to prevent the entry of light. The tests using the olfactometer were conducted at a temperature of 26 ˚C, under luminance of *ca.* 600 lux and relative humidity of the airflow in the olfactometer of *ca*. 85 %.

A sample of the attractant was placed in the glass chamber and air was flowed for 5 min for conditioning. After the conditioning, an unmated female was gently introduced into the end of the introduction part (Fig. 1). Each test was conducted for 5 min, and finished when the wasp reached the terminal of the branched part (Fig. 1). A wasp that did not make a choice within 5 min was recorded as a non-responder. The procedure was replicated 30 times per female within 150 min by assigning chambers in randomized locations and the number of trials of each female was counted. Both horntail wasps (*U. antennatus* and *X. spectrum*) were assayed using 10 females.

A bottle (40 mm in diameter and 80 mm in height) with six holes (each 1 mm^2 in area) was used as a volatile dispenser. Two grams of cotton and 1 ml of (-)-α-pinene

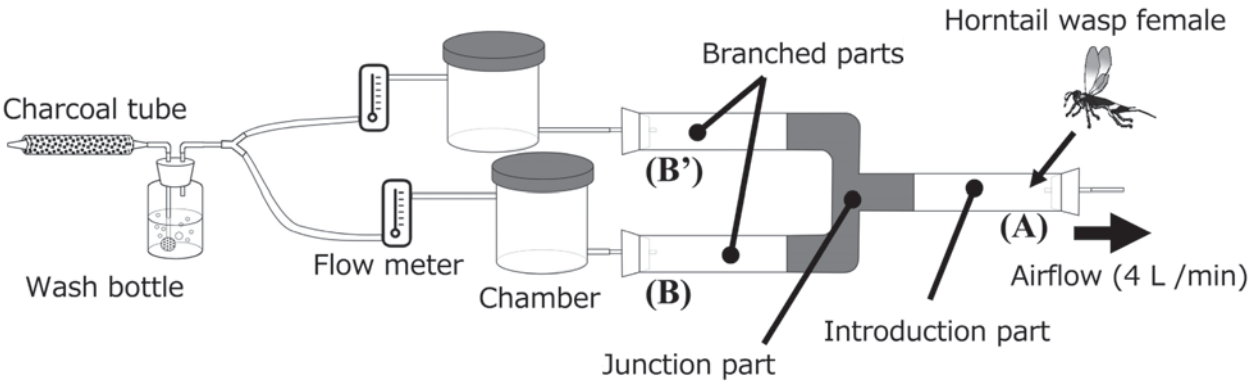

Fig. 1. Schematic diagram of the T-shaped olfactometer used for testing the olfactory responses of horntail wasps. branched parts, junction part and introduction part were all 32 mm in diameter. Horntail wasps could move from the end of the Introduction part (A) to each terminal of the branched part (B) or (B'). This figure was modified from Matsumoto and Sato (2007).

(purity 95 %; Wako Pure Chemical Industries, Ltd.) were inserted in the bottle, which was then placed in one chamber of the olfactometer. As a control, a bottle without α-pinene was placed in the other chamber.

Results and discussion

The attraction responses of the two horntail wasp species are shown in Fig. 2. In this olfactometer-based bioassay for horntail wasps, female *U. antennatus* were not attracted to α-pinene (Fig. 2). The number of trials of female *U. antennatus* to α-pinene was 13.6 ± 3.04 (SD) and that of the control was 16.4 ± 3.0 (Wilcoxon signed-rank test, $df = 9$, $P = 0.10$). This suggests that female *U. antennatus* utilize different volatile components to recognize their host plants. It is also possible that a high concentration of α-pinene in the airflow of the olfactometer prevented attraction to α-pinene, or that female *U. antennatus* prefer different conditions for their host trees. The concentrations of α-pinene in the atmosphere of the plantation of *Cr. japonica* and *Ch. obtusa* were much lower than that in the airflow of the olfactometer (Matsumoto, unpublished data). To estimate the attraction response of *U. antennatus*, it is important to use different concentrations of α-pinene in the olfactometer. In addition, an adhesive trap test should be conducted in the field using α-pinene as an attractant.

Alpha-Pinene significantly attracted female *X. spectrum* (α-pinene, 21.8 ± 2.14; control, 8.0 ± 2.02, Wilcoxon signed-rank test, $df = 9$, $P < 0.001$; Fig.2). Female adult *X. spectrum* have no substantial symbiotic fungi or mycangia in their bodies, and have a characteristic life cycle in which they oviposit on host trees already inoculated with the *Amylostereum* fungi by other horntail wasps (Fukuda, 1997; Fukuda and Hijii, 1997). Thus, the host preference of *X. spectrum* would be strongly dependent on that of other horntail wasps. Female *X. spectrum* search for and choose a host tree already used for oviposition by another species of horntail wasp; therefore, female *X. spectrum* utilize not only α-pinene but also the characteristic specific secondary volatile constituents emitted from the host tree oviposited by other horntail wasps.

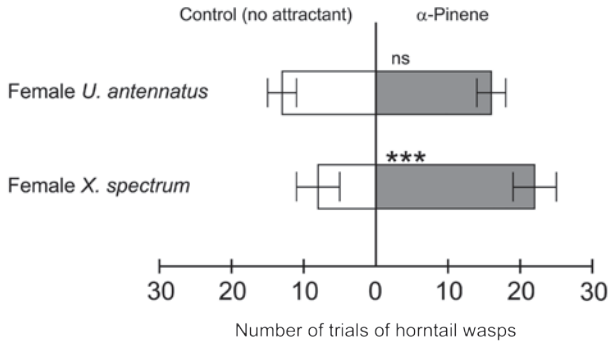

Fig. 2. Olfactory responses of female adults of two horntail wasp species, *U. antennatus* and *X. spectrum*, to α-pinene according to the olfactometer. *** $P < 0.001$; ns, not significant by Wilcoxon signed-rank test, $n = 10$.

We previously reported that α-pinene attracted female *U. japonicus* under the olfactometer (α-pinene, 23.1 ± 1.52; control, 6.9 ± 1.52, Wilcoxon signed-rank test, $df = 9$, $P < 0.001$; Matsumoto and Sato, 2007). Thus, the response to α-pinene as the component widely distributed in conifers was clarified for three major horntail wasps that utilize *Cr. japonica* and *Ch. obtusa* as a host plant. Since horntail wasps are secondary wood-feeding insects (Knight and Heikkenen, 1980), they would utilize not only the structural constituents like α-pinene but also the characteristic secondary volatile constituents emitted from the host to correctly identify their host plants, which mainly consist of weakened and stressed trees (Haack and Slansky, 1987; Hanks, 1999). Sato et al. (2004) reported the oviposition preference of *U. japonicus* was not related to the diameter of the host tree or the moisture content of the wood, but was strongly related to an unknown factor. It is possible that the unknown factor is the volatile constituents emitted from the trees.

The present experiments using the olfactometer revealed that female *X. spectrum* as secondary wood-feeding insects utilize α-pinene as a secondary volatile constituent emitted from the host tree, but female *U. antennatus* do not. Bioassay using the olfactometer for horntail wasps can reveal the responses of the horntail wasps to other volatile constituents.

Acknowledgements

We thank Dr. M. Tabata of the Forestry and Forest Product Research Institute (FFPRI) and Mr. H. Miyata of the Kochi Prefectural Forest Technology Center. We also thank Mr. T. Hirota and Mr. H. Kadota of Shikoku Research Center, Forestry and Forest Product Research Institute for preparing the samples. We are grateful to Mr. and Mrs. Yamasaki for the use of the research forest. This study was carried out with support from a Grant-in-Aid for Young Scientists (B), (19780127) of the Japan Society for the Promotion of Science (JSPS).

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キバチ科 2 種（ヒゲジロキバチ・オナガキバチ）における α ピネンへの誘引反応の違い

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要 旨

キバチ類は産卵時に接種するキバチ共生菌によってスギ・ヒノキといった主要な人工林構成樹種に対して材変色被害をもたらし、材質劣化害虫として林業経営に対して大きな被害を与えている。キバチ類の産卵選好性を解明していく上で、揮発性成分に対するキバチ類の誘引反応を調べていくことは重要なことだと考えられる。そこで，針葉樹材の主要な揮発性成分であり、またニホンキバチに誘引活性を持つ α ピネンに対して、キバチ類 2 種（ヒゲジロキバチおよびオナガキバチ）の誘引反応をオルファクトメーターを用いて調べた。誘引試験の結果、オナガキバチの雌成虫は α ピネンに誘引されたが、ヒゲジロキバチ雌成虫は α ピネンに誘引されなかった。ヒゲジロキバチは他種キバチと異なる状態の寄主木に誘引される可能性が示唆された。

キーワード：α ピネン、誘引、キバチ類、宿主選好性、オルファクトメーター、揮発性物質

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