INTRODUCTION

Fruit-piercing moths are distributed worldwide (Muniappan et al., 1995). In Japan, Oraesia excavata (Butler), O. emarginata (Fabricius) and Adris tyrannus amurensis (Staudinger) are the main species that attack ripe fruits, including peach, orange, grape, and pear, among others (Nomura, 1962; Omori and Mori, 1962; Uchida et al., 1978). These moths live in the hills around orchards in the daytime and fly into orchards to suck ripe fruit juice for egg maturation at night (Nakajima and Shimizu, 1956; Ohmasa et al., 1991), suggesting that the moths utilize the volatile compounds emitted from ripe fruits to search for food. It is well known that fruit-piercing moths are attracted to various fruit odors (Kohno, 1962; Saito and Munakata, 1970; Miyazaki et al., 1972; Uchida et al., 1978); in particular, among the various kinds of fruits, the moths are strongly attracted to peach fruit (Uchida et al., 1978) and are mostly captured in peach orchards (Park et al., 1988). In a previous experiment, fruit-piercing moths were captured by a trap with ripe peach fruit, showing that olfactory stimuli in ripe peach fruits may play an important role in the attraction of the moths (Tian et al., 2007); however, no moths were captured by traps with unripe peach or were observed to suck unripe peach fruits in an orchard (Tian et al., unpublished observation).

Several lactones, such as \(\gamma\)-hexalactone, \(\gamma\)-octalactone, \(\delta\)-octalactone, \(\gamma\)-decalactone, \(\delta\)-decalactone and \(\gamma\)-dodecalactone, constituting the fruity aromas increase rapidly in peach fruits with ripening (Horvat et al., 1990; Zhang and Jia, 2005); however, it is still unclear how the fruit-piercing moth reacts to these fruit odors. In the present study, therefore, we examined electroantennogram (EAG) responses of

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Electroantennographic responses and field attraction to peach fruit odors in the fruit-piercing moth, Oraesia excavata (Butler) (Lepidoptera: Noctuidae)

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Abstract

The olfactory responses of adult males and females of the fruit-piercing moth, Oraesia excavata, to lactones as specific components among ripe peach fruit odors were recorded by electroantennogram (EAG) techniques and trap captures in the field. Six lactones (\(\gamma\)-hexalactone, \(\gamma\)-octalactone, \(\delta\)-octalactone, \(\gamma\)-decalactone, \(\delta\)-decalactone and \(\gamma\)-dodecalactone) and a green leaf volatile compound (cis-3-hexen-1-ol) as the reference compound for normalization were used to measure EAG responses. The EAG response to \(\gamma\)-hexalactone, shown to be the highest among the six lactones tested, did not reach as high as that to a mixture of five lactones when 10% concentrations (v/v) of the lactones were used. There was no significant difference between males and females in EAG responses to those compounds. In the field experiment, the number of moths captured by traps baited with a mixture of the five lactones (\(\gamma\)-hexalactone, \(\gamma\)-octalactone, \(\gamma\)-decalactone, \(\delta\)-decalactone and \(\gamma\)-dodecalactone = 142 : 7 : 145 : 70 : 28, v/v) was about half that captured with ripe peach fruit; however, the moths were not captured by traps with individual lactones. These results show that O. excavata is attracted by a mixture of lactones, but not by individual lactones, although individual lactones are recognized by antennal receptors.

Key words: Attractant; electroantennogram; lactone; Oraesia excavata; peach fruit odor
adult *O. excavata* moths to lactones contained in ripe peach fruit, and compared the number of moths captured by traps using lactones and ripe peach fruit in the field. Because we detected δ-octalactone in ripe and overripe peach fruit in our preliminary experiment, we used the compound as well as five individual lactones to elicit the EAG response.

**MATERIALS AND METHODS**

**Insects.** *O. excavata* larvae were reared on an artificial diet composed mainly of dried leaf powder of the host plant (*Cocculus trilobus* DC.), bean powder, wheat germ and dried brewer’s yeast at 25°C under a photoperiod 16L : 8D (Ohmasa et al., 1991). Furthermore, the larvae were also reared on the leaves of the host plant under the same conditions. Pupae were sexed and placed in screen cages (30×30×30 cm) until adult emergence. Adult moths were kept in the same screen cages and provided with a whole apple fruit as food until use. The antennae of adult females and males 2 to 4 days after eclosion were used for EAG experiments.

**Chemicals.** A green leaf volatile compound and six lactones were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). The chemical purities were as follows: cis-3-hexen-1-ol, 98%; trans-2-hexenal, 98%; γ-hexalactone, 98%; γ-octalactone, 96%; δ-octalactone, 97%; γ-decalactone, 96%; δ-decalactone, 97% and γ-dodecalactone, 95%. Paraffin oil was obtained from Nacalai Tesque, Inc. (Japan).

**EAG recordings.** An antenna was excised at its base and mounted between two metal electrodes using electrically conductive gel (Spectra®, 360, Parker Lab., Inc., Fairfield, New Jersey 07004, USA). EAG signals through a preamplifier were fed into a 16-bit analog to digital converter (IDAC-2, Syntech®, Germany) and transferred to a PC (IBM Aptiva® 2255) for further amplification and analysis with EAG software version 2.7 (Syntech®). A green leaf volatile compound, cis-3-hexen-1-ol (1% v/v) was used as the reference compound for normalization of all responses relative to the responses to the reference compound (100%). Stimulation with the reference compound was given at the beginning and end of each individual series of odors. Responses to individual compounds were normalized from the decline curves of the responses to the reference at the beginning and end of each series. For all experimental procedures, an interstimulus interval of 60–120 seconds was maintained between successive stimulations. Each antenna was only used once for each compound.

Green leaf volatile compounds were prepared by dilution with paraffin oil at 1% concentration (v/v) for normalization. Lactones were dissolved in paraffin oil at concentrations of 10% and 1% (v/v). A mixture of 10% lactones was prepared by adding one portion of the five individual lactones to five portions of paraffin oil. Furthermore, the resultant mixture was diluted with paraffin oil for 1% concentration. One microliter of the solution was applied to a piece of filter paper (0.6 cm×1.2 cm) (Toyo Roshi Kaisha, Ltd., Japan) inserted into a glass Pasteur pipette. The same amount of paraffin oil was used as a blank control. The tip of the pipette was inserted into the side hole of the mixing tube where a continuous charcoal-filtered air flow (1.2 l/min) was blown through onto the antenna. Using a stimulus controller (Type CS-55, Syntech®), a 0.5-second puff of air flow (0.6 l/min) was injected through the pipette to simulate the antenna positioned 20 mm from the outlet of the tube.

**Field trap test.** The field trap test was conducted in the surroundings of peach orchards on a hill located in Tamashima in Kurashiki city, Japan (34.3°N; 133.4°E). The plastic funnel trap (Uni-trap, Sankei Chemical Co. Ltd., Japan) consisted of a bucket (semiclear, 16 cm dia.×12.5 cm ht.) attached to a funnel (yellow, top; 12 cm and bottom; 16 cm dia.×7 cm ht.) with a round lid (green, 16 cm dia.). The eleven traps, baited with ripe peach fruit (ripeness was determined from sugar content, although this quantitative standard changed in different years; ripe peach fruits harvested in 2006 contained more than 13% sugars) and individual lactones (0.5 ml) or a mixture of five lactones (0.5 ml) with or without the two green leaf volatile compounds contained in glass vials (0.8 mm dia.×40 mm ht.), were hung on branches 1–1.5 m above the ground and at a distance of about 15 m (Tian et al., 2007). Two sets of these funnel traps were prepared in this experiment. The glass vial was placed in the bottom of the funnel trap. The relative proportions of the five lactones (γ-hexalactone : γ-octalactone : γ-decalactone : δ-decalactone : γ-dodecalactone = 142 : 7 : 145 : 70 :
and two green leaf volatile compounds (cis-3-hexen-1-ol : trans-2-hexenal = 14 : 29, v/v) were prepared according to the report by Jia et al. (1999). The ripe peach fruit was renewed at intervals of two or three days and all chemical compounds were changed weekly. The hanging locations of the traps were randomly changed at intervals of 6 days. Traps were checked every two or three days. After the number of captured moths was recorded, the moths were removed from the traps. The field test was conducted from 1 to 22 August 2005 and 5 August to 5 September 2006.

Data analysis. Normalized EAG responses (%) were analyzed using ANOVA after square root transformation of the data. The transformed values were compared by Tukey’s methods when ANOVA was significant at the 5% level. The numbers of moths captured by the traps on each collecting day were analyzed using ANOVA with factors of a day (22 times/trap) and test samples (peach fruit, a mixture of five lactones, six individual lactones, two individual green leaf volatiles and paraffin oil as a control) followed by the Bonferroni test.

RESULTS

EAG responses

Electroantennogram responses elicited by paraffin oil were 0.88 ± 0.07 mV in females and 0.71 ± 0.06 mV in males (n = 10). EAG amplitudes in response to (the standard stimulus) 1 μl of 1% cis-3-hexen-1-ol were 2.96 ± 0.21 mV (mean ± SE, n = 10) and 2.70 ± 0.15 mV (mean ± SE, n = 10) on female and male antennae, respectively. Both sexes showed dose-dependent EAG responses to cis-3-hexen-1-ol and trans-2-hexenal, and the response profiles were similar for alcohol and aldehyde (data not shown).

The mixture of five lactones elicited stronger response than all individual lactones tested (Fig. 1). EAG responses to individual lactones decreased with increasing carbon chain length among the six lactones. No significant differences in EAG responses were observed between lactone concentrations of 1% and 10%, except γ-octalactone, in both sexes (Tukey test, p > 0.05). Furthermore, no significant differences in EAG responses were observed between γ-octalactone and δ-octalactone, and between γ-decalactone and δ-decalactone.

Field trap test

In the experiment conducted from 1 to 22 Au-

![Fig. 1. EAG responses (mean ± SD) on O. excavata (n = 10) to 1 μl of lactones at concentrations of 10% and 1% (v/v). Mixture, mixture of five lactones (see Materials and Methods); γ-Hex., γ-hexalactone; γ-Oct., γ-octalactone; δ-Oct., δ-octalactone; γ-Dec., γ-decalactone; δ-Dec., δ-decalactone; γ-Dodec., γ-dodecalactone. EAG responses were normalized to 1 μl of 1% (v/v) cis-3-hexen-1-ol. Different letters are significantly different at the 5% level by the Tukey test.](image)
August 2005, although two green leaf volatile compounds (cis-3-hexen-1-ol and trans-2-hexenal) were added to a mixture of five lactones, there was no significant difference in the numbers of *O. excavata* moths captured by traps baited with the lactone mixture with and without two green leaf volatiles and paraffin oil as a control were analyzed by the Bonferroni test and different letters show significant difference at the 5% level. Lactone mixture; mixture of five lactones (see Materials and Methods). Glv, cis-3-hexen-1-ol and trans-2-hexenal.

**DISCUSSION**

In the present study, no significant difference in EAG responses to the six lactones was observed between females and males. Furthermore, EAG responses to these lactones showed almost the same dose-dependent patterns. These results suggest that both females and males of *O. excavata* can recognize these compounds emitted from ripe peach fruits. In several lepidopteran species, female and male antennae responded to plant volatile compounds with similar patterns (Van Der Pers, 1981; Hansson et al., 1989; Topazzini et al., 1990; Elke and Heinz, 1996).

Among the individual lactones that were specific components produced in ripe peach fruit (Jia et al., 1999), EAG responses decreased with increasing carbon chain length. However, in the same carbon chain lactones, there was no significant difference in EAG responses between $\gamma$ and $\delta$ isomers, suggesting that both sexes do not discriminate the ring structures of these lactones on the antenna.

Plant odors can be classified into general and specific volatile compounds (Visser, 1986). Although insects respond to a wide variety of plant-derived volatile compounds, they often use specific compounds as cues to find or avoid certain plants. In the present experiment, *O. excavata* moths were captured by a trap baited with a mixture of five lactones; however, the green leaf volatile compounds did not affect the number of moths captured. These results show that the moth may use lactones as specific cues to find ripe peach fruit as food. However, the number of moths captured by a trap baited with a mixture of five lactones was almost half of that
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captured with ripe peach fruit, showing that other volatile compounds which attract moths are present in the fruit. In fact, the moths attack various fruits with or without trace lactones (Nomura, 1962; Omori and Mori, 1962; Uchida et al., 1978). Furthermore, the EAG response to \( \gamma \)-hexalactone, which is one of the major lactones in ripe peach fruit (Jia et al., 1999), was nearly as high as that to a mixture of five lactones, but no moths were captured by the compound trap. These results also indicate that the moths may require multiple components to find ripe peach fruits. We are currently planning to analyze the volatile compounds released from the surface of peach fruit during ripening and monitor these compounds using traps.

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REFERENCES


