Roles of α-Farnesene in the Behaviors of Codling Moth Females

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Reproduction and olfactory behavioral responses of codling moth, *Cydia pomonella* (L.), females to synthetic α-farnesene were observed in the laboratory as well as their reproduction behaviors in an apple orchard. Calling levels were lifted and ovipositional peaks were advanced in codling moth females at presence of 1 μg and 0.1 μg of α-farnesene, respectively. Mated females of codling moth more actively responded to 0.01 μg α-farnesene with walking and wing-fanning while walking than to other doses (0.001, 0.1, 1, 10 μg) and control. The results show that α-farnesene plays important roles in the behaviors of codling moth females. However, the differences between responses to α-farnesene and those to apple volatiles by codling moth females indicate that components other than α-farnesene in apple volatiles also have biological activities.

Key words: Reproduction, Olfactory Responses, *Cydia pomonella*

Introduction

Applications of insecticides to prevent crops from phytophagous insect pests have created lots of disastrous problems. Attempts in searching for environmentally friendly pest control strategies have long been made mainly from naturally occurring products, including insect pheromones and plant allelochemicals. Pheromone applications are already well documented, but females are basically not affected in the disruption or mass-trapping techniques with synthetic pheromone blends (e. g., Witzgall and Arn, 1997). Volatiles from host plants are crucial for host searching and the reproduction of phytophagous insects; and for the gravid females, host plants are extremely important for their progeny (Hendrikse and Vos-Bunnemyer, 1987; Renwick, 1989; McNeil and Delisle, 1989; Bernays and Chapman, 1994; Hartlieb and Rembold, 1996; Landolt and Philips, 1997). Therefore, there are great potentials in developing supplementary control strategies of insect pests with plant allelochemicals (e. g., Yokoyama and Miller, 1991).

Codling moth, *Cydia pomonella* (L.), is the most serious pest of pome fruit orchards worldwide (Chapman, 1973). Only in the presence of host plants can females of codling moth call, mate and oviposit in the field (Wildbolz, 1958; Gehring and Madsen, 1963; Geier, 1963; Jackson, 1979). Laboratory experiments have shown that volatiles from mature apple fruits stimulate oviposition of codling moth females and attract newly hatched codling moth larvae (Wearing *et al.*, 1973; Wearing and Hutchins, 1973). Yan *et al.* (1999) demonstrated that green apple volatiles could advance periodicity of reproduction behaviors and stimulate olfactory responses of virgin and mated codling moth females. α-Farnesene, among many components in apple volatiles, has been proven to play major roles in host plant searching and reproduction of codling moth.

α-Farnesene was first reported as a bioactive volatile released from the wax of apple skin in 1960s (Heulin and Murray, 1964; Murray and Heulin, 1964). This sesquiterpene component was later isolated by Murray (1969) from natural coating of apples. Anet (1970) synthesized 3 of 6 farnesene stereoisomers and confirmed that (*Z,E*)-α-farnesene was predominant and (*E,E*)-α-farnesene was minor in apples, and only (*E,E*)- and (*Z,E*)-α-farnesene were subsequently found to be attractive to newly hatched larvae and to be able to stimulate oviposition of gravid females (Sutherland, 1972; Sutherland and Hutchins, 1972; Sutherland and Hutchins, 1973; Sutherland *et al.*, 1974; Suski and Sokolowski, 1985; Bradley and Suckling, 1995). Recently, Landolt (2000) demonstrated that codling moth injury could increase concentration of (*E,E*)-α-farnesene in the apple fruits. However, the role of α-farnesene in the olfactory behaviors and the periodicity of reproduction of codling moth females has not yet been reported. Our experiments were undertaken in attempt to understand action mode of α-farnesene on the pheromone releasing, attraction and oviposition stimulation of codling moth females. In order to compare behavioral responses to α-farnesene with those to natural apple volatiles, some observations were also conducted in an orchard.

Material and Methods

Insects

Codling moths were reared on a semiartificial diet (Mani *et al.*, 1978) at 25 °C, 65–75% RH and 18:6 (L:D) photoperiod. In the climatic room, the light went off at 12:00 and on at 18:00. Several hundred wild insects were introduced into and interbred with laboratory population each year.

Field observations

The field assays were conducted in May and June 1998 in an apple orchard at Alnarp in southern Sweden. Insects in pupal stage were kept in a net house where photo rhythm, temperature and humidity were as same as in the orchard. Newly emerged adults were sexed everyday and supplied with sucrose solution (ca. 10%, w/v). Insects were introduced into a 4 × 30-cm glass tube stopped with nets at two ends. The tubes were hung horizontally on the apple trees where green apples were growing. Insects in tubes hung outside of the orchard were used as control. For calling assays, 10 2-d old virgin females were used in each tube. Number of calling individuals was counted at 30 min intervals from 17:00 to 22:00 for two days. For the oviposition assays, 2-d old females were mated during sunset, and 5 mated females were introduced into each glass tube. Newly laid eggs were counted and marked at 2 h intervals from 21:00 on the first day upon mating to 23:00 on the third day. Three and 5 replicates were used in calling and oviposition assays, respectively. The time from 20:30 to 21:00 was sunset.

Laboratory assays

Test arena. All laboratory assays were conducted in an olfactometer room with 25 °C, 60% RH and 2 lux for scotophase and 400 lux for photophase. Glass tubes (4 × 60 cm) were placed parallel on a table. Charcoal-filtered air was blown into the tubes through an air distributor. Wind speed inside the tubes was 20 cm/s. Air from the down wind end of the tubes was blown out from the room with a fan. Metal nets were used for stopping insects at two ends of each tube. The glass tubes and metal nets were washed with detergent, and burnt at 400 °C for 8 h before use.

Chemicals. Synthetic α-farnesene (85% in purity, *EE* 73%, *ZE* 27%) was kindly provided by Prof. Heinrich Arn. The purity was determined with capillary gas chromatography; GC-FID was performed on a HP 5890 GC equipped with a 30 m × 0.25 mm id DB-Wax column (J & W Scientific, Folsom, CA 96830, USA), programmed from 50 °C (hold 5 min) at 10 °C/min to 230 °C. α-Farnesene was dissolved in and diluted with hexane (HPLC grade). The solution was stored at −20 °C until used. Ten μl of solution was applied onto one piece of filter paper (1.5 × 1.5 cm) or a rubber septum. The filter paper or rubber septum with hexane alone was used as control. The solvent (hexane) was allowed to vaporize completely at room temperature for 3–5 min. The filter paper or rubber septa were prepared 1–3 h before tests and kept in vials or aluminum paper.

Calling assays. Observation was conducted for 10 h each day, 4 h photophase (from 8:00 to 12:00) plus 6 h scotophase (from 12:00 to 18:00). Ten 2-d old virgin females were introduced into each glass tube. Rubber septa with 1 μg of α-farnesene and control septa were placed at upwind ends of glass tubes, respectively. Number of calling individuals was counted at 30 min intervals. The female was considered to be calling when she lifted the abdomen and protruded the ovipositor (Fluri *et al.*, 1974; Niemczyk *et al.*, 1977; Castrovillo and Cardé, 1979). Forty virgin females were used for treatment and control, respectively.

Oviposition assay. A rubber septum with 0.1 μg of α-farnesene and a control septum were placed at upwind ends of control and treatment glass tubes, respectively. Five 2-d old females, mated at beginning of scotophase, were introduced into each glass tube and stopped at both ends of the tube with metal nets. Egg numbers and their locations were recorded at 2 h intervals within 50 h. The experiment was repeated 5 times.

Olfactory assays. One piece of filter paper with one of the doses (0.001, 0.01, 0.1, 1.0 or 10.0 μg) of α-farnesene was placed at upwind end of the glass tubes. Two-day old females were mated, and tested on the following day. One insect was released into downwind end of control and treatment tubes, respectively. Activities of insects in control and treatment tubes were observed simultaneously at 10 sec intervals during 5 min. The behavioral responses of females were assessed with wing-fanning, walking, wing-fanning while walking. For the scoring of upwind orientation or displacement, the olfactometer tube was divided into 6 sections of 10 cm. The insects were released at section 1, and section 6 was at the upwind end. Twenty-one, 30, 45, 27, 33 and 20 mated females were used for observation at doses of 0 (control), 0.001, 0.01, 0.1, 1, 10 μg α-farnesene, respectively.

Statistics. All data were analyzed by the STATGRAPHICS 6.0 with the level of differences between treatments set at $P = 0.05$ under Mann-Whitney U test.

Results

Calling. The calling peak of virgin codling moth females in orchard was at 19:00–20:00, 1 or 2 h earlier than that of control (Fig. 1, A). The calling patterns of 2- and 3-d old females in both treatment and control group were similar, but more calling individuals were found in 3-d old females. Compared to the natural apple odors, α-farnesene apparently had less effects on calling behaviors of codling moth females (Fig. 1, B). When 1 μg of α-farnesene was applied in rubber septa in the laboratory, more calling females were found at some time point in treatment than in control, but for rest of the time, there were almost no differences in calling levels between treatment and control groups.

Oviposition. In the field, the insects laid eggs in the orchard earlier than outside during 3 day observations. Oviposition periodicity and location of eggs were significantly influenced at presence of α-farnesene (Fig. 2). Peak of oviposition at presence of α-farnesene was shifted 2 or 3 h earlier

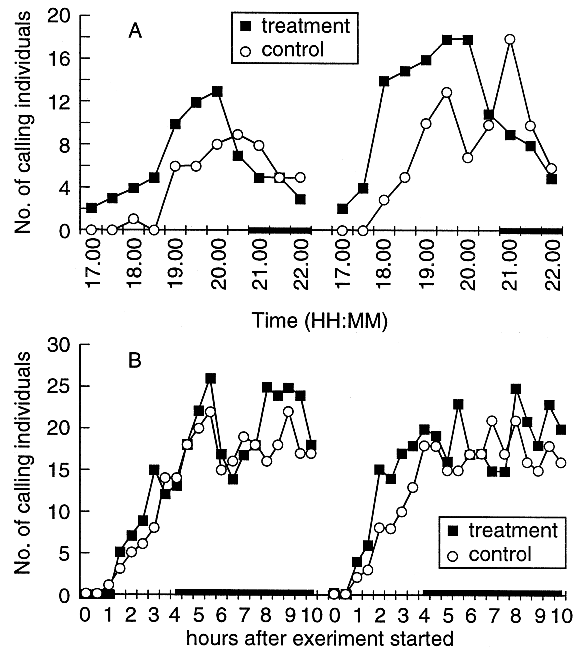

Fig. 1. Comparison of effects of apple volatiles and α-farnesene on the calling of 2- and 3-d old codling moth female adults (black bars indicate scotophase). A. calling patterns in an orchard; B. effects of 1 μg α-farnesene.

prior to onset of scotophase, while in clean air most eggs were laid during first 2 h of scotophase (Fig. 2, A). There was no significant difference in total egg numbers laid per insect between treatment (52.80 ± 6.67) and control (64.88 ± 2.56). As to the egg location at laboratory observations, eggs were distributed almost evenly at each of 6 sections in control tubes (14.79 ± 2.93, 17.33 ± 2.83, 16.59 ± 3.47, 11.52 ± 2.12, 16.82 ± 3.07, 22.95 ± 2.11 eggs in section I to section VI, respectively), while in treatment tubes, the closer to the odor source (Section VI is closest to odor source), the more eggs were found (7.51 ± 0.60, 12.30 ± 0.49, 17.20 ± 2.61, 15.83 ± 1.90, 23.45 ± 1.68 and 23.66 ± 2.84) (Fig. 2, B).

Behavioral response of mated females to doses of α-farnesene. Series of doses (0, 0.001, 0.01, 0.1, 1 and 10 μg) of α-farnesene were applied on filter paper to evaluate olfactory responses of mated codling moth females in an olfactory room. The times for walking and wing-fanning while walking during 5 min were significantly different for 0.01 μg from those for rest of the doses; and 1 μg α-farnesene also showed some activity in these

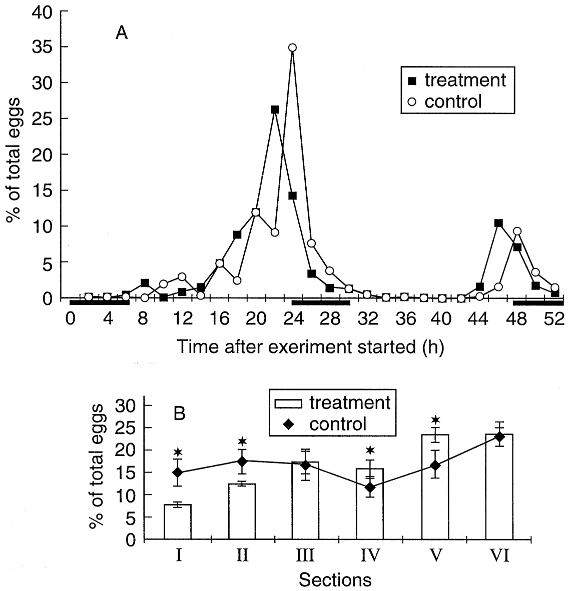

Fig. 2. Ovipositional patterns of codling moth females in the presence of 0.1 μg α-farnesene. The chemical was applied in a rubber septum placed at upwind end of a glass tube in an olfactometer room. A. Temporal pattern of oviposition (black bars indicate scotophase). B. Location of eggs in glass tubes (The tube was divided into 6 sections of 10 cm. Section VI was at upwind end of the tube). Asterisks indicate significant differences between treatment and control at same section at $P = 0.05$ level under Mann-Whitney U test).

two parameters. The dose 0.01 μg of α-farnesene showed some activity on displacement, but the differences from rest of the doses were not significant. There were no differences in activities among the doses for wing-fanning (Fig. 3).

Discussion

Ovipositional stimulation of codling moth by apple volatiles and α-farnesene has been demonstrated in many experiments for decades. Wearing *et al.* (1973) counted the activity number with electrical recording device, but failed to demonstrate the upwind orientation of codling moth adults to apple odors. Whether α-farnesene is an attractant needs to be determined (Wearing and Hutchnis, 1973). Periodicity of reproductive behaviors and olfactory response (including orientation) of codling moth females in the presence of apple volatiles and α-farnesene have been demonstrated in our previous results (Yan *et al.*, 1999) and in the present paper. The presence of apple volatiles or their components indicate the food sources, and therefore without any host plant stimulants, the delay of reproductive behaviors is the adaptive strategy of insects. However, temperature, among other factors, can change such responses of insects to host plants (Batiste *et al.*,

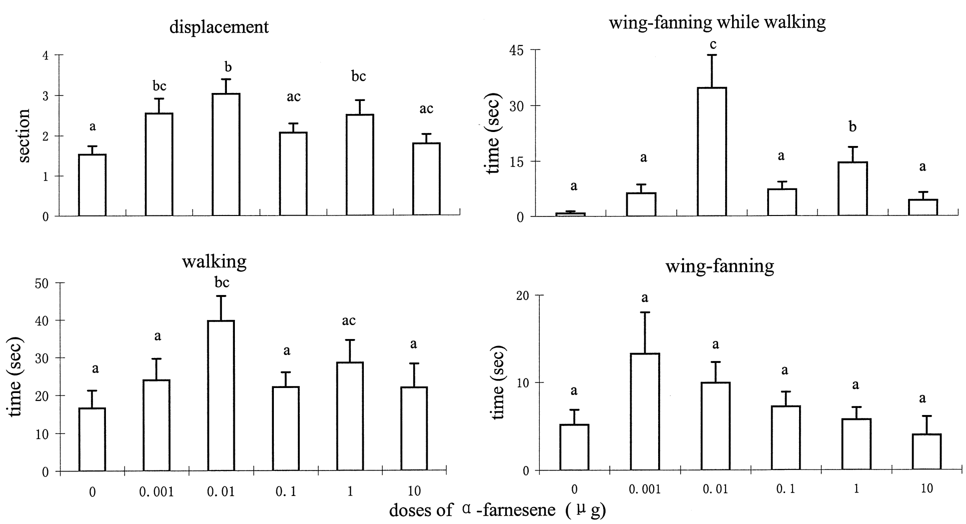

Fig. 3. Olfactory responses of mated codling moth females to different doses of α-farnesene (μg) applied on filter paper at upwind end of a glass tube in an olfactometer room in scotophase. Significant differences between treatment and control are indicated by different letters (N = 21, 30, 45, 27, 33 and 20 for 0 (control), 0.001, 0.01, 0.1, 1, 10 μg α-farnesene, respectively. Mann-Whitney U test, $P = 0.05$).

1973; Castrovillo and Cardé, 1979). In the field, ovipositional peaks normally occur on the 2nd day after mating (Gehring and Madsen, 1963; Weissling and Knight, 1996; Yan *et al.*, 1999), but could be postponed if the temperature was below 15 °C. Even in such a case, the differences in oviposition peaks between insects in orchard and insects outside were still distinct.

Apple volatiles consist of complex blend of compounds (e.g., Flath *et al.*, 1969; Yahia *et al.*, 1990; Mattheis *et al.*, 1991; Boeve *et al.*, 1996). Sutherland *et al.* (1974) noticed that attraction of larvae and gravid female oviposition responses to pure α-farnesene were less intense than they were to natural apple odor or extracts. The authors pointed out that components other than α-farnesene could also play roles in attraction and stimulation. Our data confirmed such an assumption. In laboratory assays, lift and time advancement of calling were not so clear as in field observations. In olfactory assays, we also noticed that females responded less intensely to pure α-farnesene than they did to natural apple odor. More work should be done on other components of apple odors, alone, or in combination with α-farnesene, to further understand the role of apple volatiles in the behaviors of codling moths females. A novel control strategy is expected upon fully understanding the action mechanisms of apple volatiles on codling moths.

In laboratory assays, chemicals were applied onto filter paper or rubber septa; filter paper was used in olfactory assays (5 min), while the rubber septa were used in longer time assays (from several hours to several days). Because the releasing rate of chemicals in these two types of material are different, the doses used in these assays are not comparable. However, release rate from filter paper is 100 times higher than from rubber septa. Codling moth females responded best to 0.01 μg of α-farnesene in filter paper in our preliminary olfactory tests, so we used 1 μg in rubber septa in calling assays. But 0.1 μg α-farnesene on rubber septa was enough to stimulate oviposition (Fig. 2).

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