Bradysia Odoriphaga Copulatory Behavior and Evidence of a Female Sex Pheromone

Hong-Jun Li, Xiong-Kui He, Ai-Jun Zeng, Ya-Jia Liu, and Shu-Ren Jiang

ABSTRACT  Bradysia odoriphaga Yang and Zhang is the most serious pest of Chinese chive, Allium tuberosum Rottle ex Spreng. (Liliaceae) in North China. Pesticide residues in this vegetable are very high following excessive use of organophosphorate insecticides. Because there have been no reports on the sex pheromones of B. odoriphaga, sex pheromone and mating behavior of B. odoriphaga were investigated as a possibility of developing semiochemical-based monitoring and control of this pest. In laboratory bioassays, live B. odoriphaga virgin females stimulated 78% of males to vibrate their wings and 67% of males to attempt to mate. Methylene dichloride washes of female whole bodies and excised ovipositors also attracted males. In field test, many B. odoriphaga males were attracted to the traps containing live B. odoriphaga virgin females or methylene dichloride washes of female whole bodies. Most flies mated only once, while a few mated as many as six times. After mating, females were still attractive to males. Flies’ sexual behavior showed a daily rhythm. The higher mating activity was from 2200 to 0600 h and the lower from 1200 to 1800 h. These results indicate the presence of a female sex pheromone in B. odoriphaga with the ovipostor as the most likely source of pheromone production. It is possible to collect the maximum amount of sex pheromone between 2200 and 0600 h. There is the possibility that this sex pheromone may be used to monitor and control of B. odoriphaga in the future.

KEY WORDS  Diptera, Sciaridae, Bradysia, odoriphaga, sex pheromone, mating behavior

Introduction

Many sex pheromones have been identified within the order Diptera, especially in the families Cecidomyiidae (Hillbur et al. 1999, McKay & Hatchett 1984), Drosophilidae (Nemoto et al. 1994), Glossinidae (McDowell et al. 1981), and Muscidae (Uebel et al. 1975). However, only three species of Sciaridae have been reported to have sex pheromones. Casartelli et al. (1971) reported a sex pheromone in Bradysia tritici (Coquillett). Heptadecane was identified as the major component of a sex pheromone of Lycoriella mali (Fitch) (Kostelc et al. 1980). Gotoh et al. (1999) reported that heptadecane did not attract unmated adult males L. mali in Japan. Bradysia impatiens (Johannsen) was also shown to have a sex pheromone by Alberts et al. (1981) and Liu et al. (2002), but the

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chemical structure has not been identified. Overall, little is known about the sex pheromones of sciarids, probably because of their short lifespan and tiny bodies makes them difficult to work with.

The sciarid fly, *B. odoriphaga*, is a serious pest of Chinese chive, *Allium tuberosum* Rottle ex Spreng. The larvae live in the roots and stems of Chinese chive, making it difficult to control with common strategies. It is common practice for farmers to add organophosphate insecticides into the soil to kill larvae. Thus, pesticide residues are very high, and there have been many reports of pesticide poisoning from consuming the Chinese chive in recent years (Wang et al. 2006a, Wang et al. 2006b). Therefore, new and less poisonous alternative control methods have been developed, including an insecticidal-engineered bacterium (Wu et al. 2003), botanical secondary metabolites (Yu et al. 2003), a pesticide-degrading bacterium (Jiang et al. 2004), and an entomopathogenic nematode (Sun et al. 2004).

However, these new substances do not provide adequate control, so better methods to prevent this fly are required. This paper describes the research on whether or not there is a sex pheromone in the female *B. odoriphaga*, and some factors that affect sexual behavior of the adults.

**Materials and methods**

**Test insects and rearing methods.** Sciarid flies, *B. odoriphaga*, were collected from a glasshouse in a Beijing suburb (40°13'N, 116°12'E) in China and were reared in an incubator. Rearing methods were similar to those described by Mu et al. (2003). The culture was maintained at 20±1°C, 50±5% RH, with a 12-h photoperiod from 0600 to 1800 h CST.

**Effect of time-of-day on copulation and other mating behavior.** From 0000 to 0500h, newly emerged flies in plastic pot were placed in plastic tubes (4 cm long and 0.5 cm diam, one fly per tube). The tubes were labeled with date and stored in the incubator. One virgin male and one virgin female were placed together in a clean plastic tube before 0600 h on 0 day (day of emergence), 1 day and 2 day, and then the tubes were placed in the incubator. Mating activities of all flies were observed for 20 min every 2 h from 0600 h of 0 day to 1200 h of 2 day. If flies died during an experiment, they were replaced by flies of the same age. There were 4 replications and 40 couples per replication for each day. A dim red light was used in night during observation.

**Bioassay procedure.** Sexual attraction of sciarid flies was studied using a glass Y-tube olfactometer similar to the one described by Chaudhury et al. (1972). The two arms were 10 cm long and the base was 20 cm long, both had a diameter of 1.8 cm (Fig. 1). The two arms and the base of one Y-tube were closed by a glass stopper (3 cm long) with a cone hole (the diameters of two bottom of the hole were 1 cm and 0.4 cm, respectively). All holes were blocked with a thin layer of glass wool to prevent the flies from escaping. Two arms of this Y-tube were connected to a bottle filled with distilled water through another Y-tube, such that humidified air could pass through the arms. A charcoal filter was put before the bottle to purify the air. The velocity of air in the arms was about 22 cm sec⁻¹ and the test temperature was 20 ± 2°C.

**Attractiveness of live flies.** The attractiveness of virgin females, mated females, mated males, and virgin males to virgin males and the attractiveness of
virgin and mated males to virgin females were tested separately. Virgin flies were collected every 2 h following removal of all the flies to ensure that all flies were unmated. One fly was held in a plastic tube (4 cm long and 0.5 cm diam) with two flies of the opposite sex for 2 h and was observed the mating activities at intervals. The flies would not be further tested if no copula was observed. Groups of five attracting flies or other designed attracting materials as mentioned in Table 1 were placed in the hole of the stopper of two arms. Four responding flies were introduced to the Y-tube from the base following 2 min of air flow. Each group of responding flies was monitored for 8 min, and three activities, orientation, wing vibration and attempted copulation, were recorded (Landolt et al. 1985). If one fly entered half length of one arm, this would be recorded as orientation; hastily fly and frequently wing-flap of a fly would be considered as the activity of wing vibration; when a fly curved its abdomen and stuck its clasper to the glass wool, this indicated that the responding fly attempted to copulate. If one fly responded to both arms, each arm was recorded as one response. The live male fly’s respond to both arms without any flies in them were also observed to provide information about bias in the setup. Each attraction test was replicated 12 to 26 times using new flies. For a new replication, the Y-tube was reversed 180° to eliminate the unclear asymmetry, and the stoppers were rinsed with acetone to remove possible trace chemicals of flies. All observations were made between 0600 and 1000 h. The results were analyzed using chi-square tests (version 12.0, SPSS Inc) to compare the difference of two numbers.

**Attractiveness of methylene dichloride washes of female whole bodies and excised ovipositors.** Methylene dichloride washes of whole bodies, excised ovipositors, and whole bodies without ovipositors were bioassayed to compare their attractiveness to that of the virgin females. The preparation of methylene dichloride washes was similar to McKay & Hatchett (1984). One-hundred virgin females were collected and cooled at 0°C for 5 min. Females with extended ovipositors were placed on clean glass slides. Ovipositors were fully extended by gently pressing the abdomens, excised with a sharp scalpel, and pulled away from the bodies. The ovipositors and bodies of 100 females were soaked separately in 1 ml methylene dichloride for 30 min at 0°C. A 50-μl (5 FE) wash was pipetted onto filter paper (1 cm long and 0.5 cm wide) and the solvent was evaporated for about 20 s in air. The filter paper was then placed in the hole of stopper of one arm. Three groups of material, methylene dichloride solvent versus air, whole body wash versus methylene dichloride solvent, and ovipositor wash versus whole body without ovipositor wash were selected in this experiment. Observations and data recorded were the same as the previous test.
The test was replicated 14 to 16 times, using a new filter paper and flies for each replication. The results were analyzed using chi-square tests.

**Field test.** Self-made \( \triangle \)-shaped sticky traps (10 cm length of triangle side and 30 cm length of trap) were used in greenhouse in a Beijing suburb in China. Ten virgin females, a filter paper with 10FE methylene dichloride wash, or a filter paper with the same volume methylene dichloride solvent was placed in a cage of 1 mm mesh, and the cage was put in center of the \( \triangle \)-shaped trap. An empty cage was used as control test. Traps were set 30 cm above the ground at ca. 5 m intervals. Twenty-four hours later, numbers of trapped males were accounted. Each treatment was replicated five times. The results were analyzed using Duncan’s tests (version 12.0, SPSS Inc) to compare the difference of four treatments.

**Results**

**Effect of time-of-day on copulation and other mating behavior.** *B. odoriphaga* mating activity had a W-shape fluctuation (Fig. 2). The most active period was in the late at night, and the peak copulation was at 0200 h of 1 day (28.8%). In contrast, the lower period of copulation was between noon and evening. For example, the lowest copulation time of 0 day and 1 day were at 1200 h (0.0%) and 1800 h (1.3%), respectively.

In the 0-day mating test with 160 pairs of *B. odoriphaga*, 104 couples mated. Among the mated flies, 52 pairs copulated once, 25 pairs mated twice, and one pair mated up to 6 times.

**Attractiveness of live flies.** In most cases, responding flies walked in both arms, while they vibrated their wings and attempted to copulate to a certain arm. Thus, numbers of orienting and wing-vibrating flies were combined as one data, and numbers of attempted copulation flies were as another data (Table 1). The

**Table 1. Response of virgin *Bradysia odoriphaga* males to virgin females, mated females, mated males, and virgin males.**

<table>
<thead>
<tr>
<th>Attraction test</th>
<th>Total number of ( \sigma )</th>
<th>Orienting and vibrating wings</th>
<th>Attempted copulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percentage of ( \sigma ) to</td>
<td>Percentage of ( \sigma ) to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A vs. B</td>
<td>A vs. B</td>
</tr>
<tr>
<td>control ( (A)^3 ) vs. control ( (B)^3 )</td>
<td>56</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Virgin ( \varphi ) (A) vs. control ( (B)^3 )</td>
<td>104</td>
<td>78</td>
<td>27</td>
</tr>
<tr>
<td>Mated ( \varphi ) (A) vs. control ( (B)^3 )</td>
<td>48</td>
<td>54</td>
<td>8</td>
</tr>
<tr>
<td>Virgin ( \varphi ) (A) vs. Mated ( \varphi ) (B)</td>
<td>56</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>Mated ( \sigma ) (A) vs. control ( (B)^3 )</td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Virgin ( \sigma ) (A) vs. control ( (B)^3 )</td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\)There were 12 to 26 replications for each test, with four males for each replication.

\(^2\)Means for each two-choice situation were compared using \( \chi^2 \)-test, except when means were not available (0%).

\(^3\)Control was air without flies.
sum of A and B sometimes was larger than 100%, for one fly was recorded twice responses if it responded to both arms.

In blank control vs. blank control test, virgin *B. odoriphaga* males did not respond to either arm. Virgin *B. odoriphaga* males responded to virgin females, with 78% of males orienting and vibrating wings in response to virgin females, whereas, only 27% of males responded to the control (Table 1). Attempted copulation by virgin males was 67% in the presence of virgin females, but only 2% in the presence of the control. Virgin males also vibrated their wings to 77% of both unmated and mated females, but attempted copulation to mated females was less than that to virgin females (54% vs. 71%). Neither virgin males nor mated males attracted virgin males and females.

The mean time for males to orient, vibrate their wings, and to mate with females in the Y-tube test was 1.5 min, 2.0 min, and 2.8 min, respectively. The shortest time for males from being released to copulation was only 10 s.

**Attractiveness of methylene dichloride washes of female whole bodies and excised ovipositors.** Methylene dichloride alone was not any more attractive to virgin *B. odoriphaga* males than the control (Table 2). The methylene dichloride wash of female whole bodies was highly attractive to virgin males with 64% of males orienting and vibrating their wings and 36% of males attempting copulation. In comparison, the methylene chloride solvent alone only caused 13% of males to vibrate their wings and only 4% of males attempted mating. Also, virgin males showed a much greater response to the ovipositor wash than the wash of the whole body without ovipositor, with 58% of males exposed to the ovipositor wash vibrating their wings and 33% attempting copulation while the whole-body-without-ovipositor wash only stimulated 13% male wing vibrations and 5% attempted copulation.

**Field test.** In 24 h period in field test, 10 virgin females or 10FE CH$_2$Cl$_2$ wash attracted about 74 or 57 *B. odoriphaga* males, respectively (Table 3). While only 11 or 8 males were attracted by an empty cage or a paper with CH$_2$Cl$_2$ alone.
Table 2. Response of virgin *Bradysia odoriphaga* males to methylene dichloride (CH$_2$Cl$_2$) washes of whole bodies and excised ovipositors of virgin females.$^1$

<table>
<thead>
<tr>
<th>Attraction test</th>
<th>Total number of $\sigma$</th>
<th>Orienting and vibrating wings</th>
<th>Attempted copulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_2$Cl$_2$ solvent (A) vs. control (B)</td>
<td>56</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Whole body wash (A) vs. CH$_2$Cl$_2$ solvent (B)</td>
<td>56</td>
<td>64</td>
<td>36</td>
</tr>
<tr>
<td>Ovipositor wash (A) vs. whole body w/o ovipositor wash (B)</td>
<td>64</td>
<td>58</td>
<td>33</td>
</tr>
</tbody>
</table>

$^1$There were 14 to 16 replications for each test. Other parameters and statistical analyses were as described in Table 1.

The differences among the numbers of caught males by live females, female wash, and CH$_2$Cl$_2$ are significant ($P<0.01$).

**Discussion**

Test of effect of time-of-day on copulation of *B. odoriphaga* showed that during the imago period of 3 days, most flies mated in night from midnight to dawn, and in daytime copulation frequency reduced significantly. Compared with *B. odoriphaga*, the highest sexual activity time of *B. impatiens* was from 4 h before the scotophase to 1 h after the scotophase (Alberts et al. 1981). The lowest sexual activity time was at noon, which was about 12 hours after the highest activity time. And in the next 12 hours, the mating frequency increased stably. Therefore the mating behavior of *B. odoriphaga* is greatly affected by the time-of-day, and the copulation cycle is about 24 hours.

The non-significance between male's responses to blank control vs. blank control indicated the glass Y-tube olfactometer had little bias in this test. Heterogeneity between males to virgin females and the control suggested that virgin females had attracted the males. In contrast, virgin males did not respond

Table 3. Catches *Bradysia odoriphaga* males in traps containing virgin females, CH$_2$Cl$_2$ solvent, or female body wash of CH$_2$Cl$_2$.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Control$^1$</th>
<th>10 virgin $\varphi$</th>
<th>CH$_2$Cl$_2$ solvent</th>
<th>10 FE CH$_2$Cl$_2$ wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catches $\sigma$(mean±SE)</td>
<td>10.8±1.6 c$^2$</td>
<td>74.4±2.7 a$^2$</td>
<td>8.0±1.3 c$^2$</td>
<td>57.4±2.6 b$^2$</td>
</tr>
</tbody>
</table>

$^1$Control was empty cage without flies or filter paper.

$^2$Means followed by different letter are significantly different at $P < 0.01$. 


to virgin males. Furthermore, the whole female body wash had much more attraction to virgin males than that of the solvent alone. Also, males ran to females and mated with females only in 10 s after being released. Thus, it was virgin females, not virgin males that attracted males. These results strongly indicate that *B. odoriphaga* females produce a sex pheromone that attracts males.

Orienting and wing-vibrating by virgin *B. odoriphaga* males to mated females were more than that to the control, which indicated that mated females also attracted virgin males. In the comparison of the attraction of unmated and mated females, males were strongly attracted to both arms. Mating times showed females had attraction to males; even the females were mated for several times. The multi-time copulation of the same fly suggested that the attraction of females to males was not affected too much by copulation behavior. Therefore, there was no difference in the attraction of virgin males to mated and unmated females. This response differed from Hessian fly, *Mayetiola destructor* (Say), which belongs to the same suborder of Nematocera, because its female sex pheromone was transferred to males during mating (McKay & Hatchett 1984). Gotoh et al. (1999) also reported that copulatory behavior of unmated sciarid males of *L. mali* decreased quickly after the female had mated once. It is presumed that the sex pheromone in female *B. odoriphaga* is not lost or transferred to the male during mating.

More males responded to ovipositor wash than that of whole female body without ovipositor wash. The whole female body wash also had attraction for males. Thus, the inference from these results is that the ovipositor is the site of pheromone release and possibly the source of pheromone production. By contrast, it was presumed that the female sex pheromone of *L. mali* was presented on all parts of female flies (Gotoh et al. 1999). The male sciarid fly, *B. paupera* also responded to all parts of female flies (Liu et al. 2002).

In field test, 10 virgin females had more attractiveness to males than 10FE CH₂Cl₂ wash, which was similar to the test in laboratory. It could be explained that the live females released sex pheromone all through 24 h period, while the sex pheromone in CH₂Cl₂ faded away in the air as time passed.

Overall, the results clearly demonstrated that in laboratory and in field female sciarid fly, *B. odoriphaga* produced a sex pheromone and that the ovipositor was the probable site of pheromone production and release. Mating behavior was found to follow a daily rhythm, with male flies most responsive between 2200 to 0600 h. Both males and females could mate more than one time.

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