The sex pheromone components for mating disruption of the rice leaf bug, *Trigonotylus caelestialium* (Heteroptera: Miridae)

Masashi Kakizaki*

Hokkaido Dohnan Agricultural Experiment Station; Ohono, Hokkaido 041–1201, Japan
(Received 20 December 2002; Accepted 5 December 2003)

Abstract

The sex pheromone components for mating disruption of the rice leaf bug *Trigonotylus caelestialium* were examined in both the laboratory and the field. A rearing cage test in the laboratory showed that the copulation rate of *T. caelestialium* adults in cages treated with a dispenser loaded with the synthetic sex pheromone, a mixture of *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate, and *n*-octyl *n*-butyrate (100:40:3), was lower than that of bugs in untreated cages, and that populations of the next generation were also reduced. In small-scale field tests (9 dispensers loaded with 50 mg of the sex pheromone placed at 5 m intervals in a 10 m × 10 m square area), the numbers of males captured by traps baited with a lure containing the three components of the sex pheromone were reduced to some degree by treatment with either *n*-hexyl *n*-hexanoate or (E)-2-hexenyl *n*-hexanoate alone, or a 100:40 mixture of these two components. Furthermore, male capture by traps baited with the lure were mostly reduced by treatment of the 3-component sex pheromone, and also those baited with 3 virgin females. In large-scale field tests (200 dispensers each containing 300 mg of the 3-component sex pheromone in an area of 10,000 m²), the population densities of *T. caelestialium* and the numbers of males captured by traps in the treated fields were lower than those in the untreated fields.

Key words: *Trigonotylus caelestialium*; mating disruption; *n*-hexyl *n*-hexanoate; (E)-2-hexenyl *n*-hexanoate; *n*-octyl *n*-butyrate

INTRODUCTION

In the true bug (Heteroptera) family Miridae, the female sex pheromones of *Campylomma verbasci* (Smith et al., 1991), *Phytiocoris relutivus* (Millar et al., 1997), and *P. californicus* (Millar and Rice, 1998) have been reported. For *C. verbasci*, studies have been reported on the disruption of male trapping (Judd et al., 1995; McBrien et al., 1996) and population suppression in the field by mating disruption using the synthetic sex pheromone (McBrien et al., 1997).

The rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Miridae), is distributed in Japan, China, Europe, Russia, and North America (Kirkaldy, 1902; Wagner, 1956; Carvalho and Wagner, 1957; Korcz, 1979; Mikhailova, 1979; Wheeler and Henry, 1985; Yasunaga et al., 1993). It is one of the major pests causing pecky rice, and also damages wheat, maize, and gramineous forage grasses in northern Japan. Female *T. caelestialium* adults have been reported to attract conspecific males (Kakizaki and Sugie, 1997), and the female sex pheromone has been identified to be a mixture of *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate, and *n*-octyl *n*-butyrate (Kakizaki and Sugie, 2001). Pest control using the sex pheromone would be useful as an IPM program for *T. caelestialium*. This report describes the sex pheromone components and their effects on mating disruption in *T. caelestialium*.

MATERIALS AND METHODS

Pheromone compounds. The synthesis and purification of the sex pheromone compounds, *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate and *n*-octyl *n*-butyrate, were performed as reported previously (Kakizaki and Sugie, 2001). The purity of each compound for a lure was greater than 99.8% and the isomeric purity was greater than 99.9% by GC analyses, and that for a dispenser of mating disruption was 96.3–99.8% and the isomeric purity was 98.8%, respectively.

* E-mail: kakizams@agri.pref.hokkaido.jp
Bait and trap

**Pheromone lure.** The mixture of n-hexyl n-hexanoate (10 μg), (E)-2-hexenyl n-hexanoate (4 μg), and n-octyl n-butrate (0.3 μg) was loaded into a glass capillary tube (Kakizaki and Sugie, 2001), and used as a pheromone lure.

**Virgin females.** Three females, 3–5 days old after emergence, placed in a stainless steel net cage (‘Soup basket’: 6 cm diam., 6.5 cm ht., 1-mm mesh) with wheat leaves, were also used as a bait.

**Traps.** A sticky net cylinder trap (SNC-trap: cylinder of 5-mm-mesh black polyethylene net 1 mm in thickness, 6 cm in diam. and 30 cm long; Kakizaki and Sugie, unpublished) was used. The trap was fixed vertically on the ground with wires at both sides. The height of the bottom end was adjusted to the height of the top of the surface vegetation (5 cm high). It was coated with a sticky material (‘Kinryu’ spray, Maruzen Chem. Indus. Co., Ltd., Tokyo) at intervals of 1 to 2 weeks; then the lure or bait was hung in the cylinder.

**Dispensers for mating disruption.** A dispenser for mating disruption experiments was made with a polyethylene pipette (OD 10 mm, 20 mm long; ‘Transfer pipette’: No. E-241, Iuchi-Seieido Co., Tokyo), in which 300 mg or 50 mg of the synthetic sex pheromone—a mixture of n-hexyl n-hexanoate, (E)-2-hexenyl n-hexanoate, and n-octyl n-butrate in a ratio of 100:40:3—was loaded. The opening of the dispenser was closed by heating with a gas burner.

**Measurement of release rates of sex pheromone from dispensers.** Three dispensers each loaded with 300 mg or 50 mg of synthetic sex pheromone were put in a 100-ml glass beaker, which was placed in a draft chamber with slow wind flow at room temperature (about 22–23°C) in the laboratory. Release rates of the sex pheromone from the dispenser were calculated from the change in weight for 5,955 h after preparation. Also, three dispensers each loaded with 50 mg were put in a vinyl bag (35 cm×45 cm) inflated with air, placed in laboratory incubators set at 10°C, 15°C, 20°C, 25°C, and 30°C, and their changes in weight were measured for 1,487 h after preparation. The release rates indicated a peak at the 331 h measurement point at many temperatures, after which the release rates at 30°C were reduced and became lower than those at other temperatures. The sex pheromone in the dispenser was completely released at 1,078 h measurement. Therefore, the release rates during 92–331 h after preparation were compared.

**Laboratory tests in a rearing cage.** *T. caelestialium* was collected in the field (Sapporo City in Hokkaido) and reared for a year on wheat seedlings (Ito, 2000) at 22°C under a 16L8D photoperiod.

1. To observe effects on the copulation of *T. caelestialium* adults, 50 virgin females and 50 males 3–5 days old were released at the beginning of the scotophase into a rearing cage (H28×W30×D25 cm; Sanshin Indus. Co. Ltd., Tokyo), from the ceiling of which the dispenser loaded with 50 mg of the 3-component sex pheromone was hung. Because the copulation of *T. caelestialium* occurs more during the first 5 h after sunset than at other times (Kakizaki and Sugie, 1997), the numbers of pairs that copulated in the treated and untreated cages were counted for 5 h after release and lights-off, with 3 replicates.

2. To test the effect on reproduction, a dispenser loaded with 50 mg of the 3-component sex pheromone was hung from the ceiling of a rearing cage, in which 29–88 individuals of *T. caelestialium* matured nymphs (which were just before the adult stage; about equal numbers of males and females) were released. The numbers of *T. caelestialium* nymphs of the next generation grown up in each cage were counted, and the increase rate (the number of next-generation nymphs relative to that of the previous-generation matured nymphs released into each cage) was calculated in comparison with that for the untreated cages, with 3 replicates.

**Field experiments**

**Small-scale field tests.** These experiments (tests 1 and 2) were carried out during the period of the third (October) generation of *T. caelestialium* adults in a 7.5-ha field of Italian rye-grass in 2001 (Takikawa City). For testing the disruptive effects on male trapping by treatment with each pheromone component, nine dispensers were set in a 10 m×10 m square area at 5-m intervals 50 cm above ground level on steel wire bars (OD 2 mm, 70 cm long, Takiron Co. Ltd., Tokyo), and three traps baited with the pheromone lure or 3 females were each placed 2 m apart from the central dispenser (Fig. 1).

Four kinds of dispenser, each loaded with 50 mg
Mating Disruption of Trigonotylus caelestialium

n-hexyl n-hexanoate alone, 50 mg (E)-2-hexenyl n-hexanoate alone, a mixture of 50 mg n-hexyl n-hexanoate and 20 mg (E)-2-hexenyl n-hexanoate, or a mixture of 50 mg n-hexyl n-hexanoate, 20 mg (E)-2-hexenyl n-hexanoate, and 0.15 mg n-octyl n-butyrate, were placed in each area, about 70 m distant from each other.

Large-scale field tests. To test the effect of population suppression by mating disruption using the sex pheromone, experiments were carried out during the periods of the first (June) to the third (October) generations of T. caelestialium adults in a 5-ha field of Italian rye-grass in 2001 (test 3; Takikawa City) and in a 3.5-ha field of the same plant in 2002 and 2003 (tests 4 and 5; Ohono Town). Two hundred dispensers, each loaded with 300 mg of the 3-component sex pheromone, were placed in a 10,000 m² area at intervals of 5 m x 10 m at 50 cm above ground level on steel wire bars from 6 June to 31 October in 2001 and 2002, and from 19 June to 23 September in 2003. The dispensers were replaced by new ones on 14 August in 2002 and at 9 September in 2003. Three traps baited with the pheromone lure or females were placed at intervals of 30–40 m near the center of the treatment area.

The numbers of T. caelestialium adults and nymphs captured by net sweeping were counted in each season for the investigations of population density. The net sweeping was done using a 45 cm diam. insect net with 3 m-wide sweeps on the grass surface, 20 times with 3 or 5 replicates in tests 3 and 4, and 10 times with 5 or 6 replicates in test 5. The numbers of males captured by traps were also counted at intervals of 3–5 days, during the season of their occurrence.

Statistics. The numbers (X) of bugs counted in these investigations were transformed to square root (X+0.5) before analysis of variance, and were compared by Tukey’s test in the case of more than three treatments.

RESULTS

Release rate of sex pheromone from dispensers for mating disruption

The release rates of the sex pheromone from the dispensers loaded with 300 mg and 50 mg sex pheromone were smaller during the first few days after preparation, and those from the dispensers loaded with 300 mg and 50 mg were similar until 648 h; the mean release rates from these dispensers were calculated to be 63.2 µg/h and 64.3 µg/h during 48–648 h after preparation, respectively. However, the sex pheromone in the dispenser loaded with 50 mg was completely released at 984–1,152 h
(Fig. 2). While 48.8 mg of the sex pheromone remained in the dispenser loaded with 300 mg at 5,955 h (248 d) after preparation, the release rates from this dispenser were approximated to a regression line, as shown in Fig. 2. The influence of the amount of the sex pheromone loaded in the dispenser on the release was small in comparison with the 50 mg and 300 mg amounts.

The release rates (Y μg/h) from the dispenser loaded with 50 mg sex pheromone at each temperature (T°C; ranging from 10°C to 30°C) during 92–331 h after preparation were approximated to a regression line: Log(Y) = 0.02854 T + 1.337951 (R² = 0.97638, p < 0.01) (Fig. 3).

**Laboratory experiment**

(1) **Effect of the 3-component sex pheromone on copulation.** During the observation period, the mean copulation rate of *T. caelestialium* in cages treated by the dispenser loaded with 50 mg was 0%, whereas in untreated cages was 9.3% (Table 1), indicating that the treatment with the 3-component sex pheromone disrupted the copulation of *T. caelestialium* adults.

(2) **Effect of the 3-component sex pheromone on reproduction.** When a dispenser was set in the cage during the period from the mature nymph to the adult stage, the numbers of next generation nymphs of the next generation were 9–49 individuals in cages treated with the dispenser and 73–144 individuals in the untreated cages (Table 2). The mean rate of increase was 0.37 in treated cages and 3.22 in untreated cages, respectively. This result indicated that treatment with the 3-component pheromone decreased the reproduction of *T. caelestialium*.

### Disruption of male trapping by sex pheromone components in field experiments

In the small-scale field experiment (9 dispensers placed in a 100-m² area), the numbers of males captured by traps were reduced to some degree by treatment with *n*-hexyl *n*-hexanoate alone or (E)-2-hexenyl *n*-hexanoate alone, or a 2-component mixture of *n*-hexyl *n*-hexanoate and (E)-2-hexenyl *n*-hexanoate (Fig. 4), and furthermore, those by treatment with the 3-component mixture of *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate, and *n*-octyl *n*-butyrate were reduced to zero (Fig. 4). The numbers of males captured by traps baited with 3-virgin females were also reduced by treatment with the 3-component mixture (Fig. 5A and 5B), similarly to those baited with the synthetic sex pheromone lure (Fig. 5A). Disruption effects for male attraction to traps baited with the lure or 3 females in the treated field were also not significantly different in the

<table>
<thead>
<tr>
<th>Table 1. Effect of the sex pheromone treatment on the copulation of <em>Trigonotyulus caelestialium</em> adults in rearing cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment¹</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Treated</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
</tbody>
</table>

¹ Treated with the dispenser loaded with 50 mg of the 3-component sex pheromone.
² The numbers of copulation pairs were observed during 5 h after release at scotophase (*, p < 0.05).

<table>
<thead>
<tr>
<th>Table 2. Effect of the sex pheromone treatment on the reproduction of <em>Trigonotyulus caelestialium</em> adults in rearing cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment¹</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Treated</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
</tbody>
</table>

¹ The bugs were reared on wheat seedling (Ito, 2000) during observation; treated with the dispenser loaded with 50 mg of the 3-component sex pheromone.
³ Rate of increased were calculated from the numbers of the next-generation nymphs per numbers of previous-generation matured nymphs released in each cage (*, p < 0.05).
large scale field experiment (test 5 in Fig. 6), however, the traps baited with females tended to attract a few males in the treated field, but those with the lure did not (Figs. 5 and 6).

Population suppression by treatment with the 3-component sex pheromone in field experiments

In the large-scale (10,000 m²) field experiments, the numbers of males captured by traps baited with the sex pheromone lure in the treated fields were lower than those in the untreated fields after the treatment (test 3 in Fig. 7, test 4 in Fig. 8, and test 5 in Fig. 9). The total numbers of males captured in the treated fields were 7.3% (test 3), 15.1% (test 4), and 3.5% (test 5) of those in the untreated fields.

The numbers of *T. caelestialium* adults captured by net sweeping in the treated fields were also lower than those in the untreated fields during the season of occurrence after treatments of dispensers (test 3 in Fig. 10, test 4 in Fig. 11, and test 5 in Fig. 12), and the total numbers of adults captured in the treated fields were 0% (test 3), 45.0% (from June to Oct.; test 4), and 22.3% (from July to Sept.; test 5) of those in the untreated fields. *T. caelestialium* nymphs did not occur on the investigation date in test 3. The total numbers of *T. caelestialium* nymphs captured in the treated fields were 0% (test 4) and 2.2% (test 5) of those in the untreated fields, indicating that reproduction of *T. caelestialium* was suppressed in the treated fields.

**DISCUSSION**

The treatment with the 3-component sex pheromone, *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate, and *n*-octyl *n*-butyrate, disturbed the copulation of *T. caelestialium* adults and consequently reduced their reproduction under laboratory conditions. Furthermore, the field experiments revealed that treatment with the 3-component sex pheromone interfered with male attraction to the lure and females within the entire 100-m² treated area, and suppressed *T. caelestialium* populations within a 1-ha treated field. Also the results for male trapping disruption showed that treatment with the complete 3-component sex pheromone, *n*-hexyl *n*-hexanoate, (E)-2-hexenyl *n*-hexanoate, and *n*-octyl...
Fig. 7. Numbers of *Trigonotylus caelestialium* males captured by traps baited with sex pheromone lure in the field (test 3; 2001). Two hundred dispensers in 1-ha were set from 6 Jun. to 31 Oct. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (*, p<0.05).

Fig. 8. Numbers of *Trigonotylus caelestialium* males captured by traps baited with the sex pheromone lure in the field (test 4; 2002). Two hundred dispensers in 1-ha were set from 6 Jun. to 31 Oct. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (*, p<0.05).

Fig. 9. Numbers of *Trigonotylus caelestialium* males captured by traps baited with the sex pheromone lure in the field (test 5; 2003). Two hundred dispensers in 1-ha were set during 19 Jun. to 23 Sept. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (*, p<0.05).

Fig. 10. Numbers of *Trigonotylus caelestialium* adults captured by net sweeping (20 times) in the field (test 3; 17 Sept. 2001). MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (*, p<0.05).

Fig. 11. Numbers of *Trigonotylus caelestialium* bugs captured by net sweeping (20 times) in the field (test 4; 14 Jun. to 11 Oct. 2002). MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field.

Fig. 12. Numbers of *Trigonotylus caelestialium* bugs captured by net sweeping (10 times) in the field (test 5; 15 Jun. to 23 Sept. 2003). MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field.
population (McBrien et al., 1996, 1997), using the sex pheromone in amounts of 0.94–1.07 g/ha/d (calculated from 78.9 g/ha/84 d–80.6 g/ha/75 d; McBrien et al., 1997). On the other hand, in *T. caelestialium*, 200 dispensers each loaded with 300 mg of the sex pheromone per hectare were placed in the fields at about 0.80 g/ha/d (calculated from 300 mg×200 dispensers×2 times during 5 months in 2002). The treated amounts were similar to those for *C. verbasci*. However, the dose for male attraction by the sex pheromone lure in *T. caelestialium* was smaller than that for other mirids, e.g., about 4.29 µg/2-weeks (0.306 µg/d) for a glass capillary tube formulation in *T. caelestialium* (Kakizaki and Sugie, 2001), 0.8–2.8 mg/d for a gray septum in *C. verbasci* (McBrien et al., 1994), and 33 µg/2-weeks (2.3 µg/d) for *P. relatuvus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998), respectively. Therefore, it may be possible to reduce the amounts of sex pheromone needed for mating disruption in *T. caelestialium* by controlling the release from the dispensers.

The release amounts of the sex pheromone from a dispenser in the laboratory experiments were estimated at 42.2 mg (June), 59.1 mg (July), 60.6 mg (Aug.), 49.7 mg (Sept.), and 36.1 mg (Oct.) in 2002, and 44.3 mg (June), 47.2 mg (July), 59.6 mg (Aug.), and 48.3 mg (Sept.) in 2003, respectively, based on a regression line in Fig. 3 using atmospheric temperature each hour of the AMeDAS data at Ohono town. Then, the amount loaded in a dispenser was estimated to be sufficient to cover the entire occurrence season (May to November) of *T. caelestialium*. However, the dispensers placed in the fields from June began to run out of pheromone in mid August in 2002 and in early September in 2003. The release rates from a dispenser during these periods placed in the fields were calculated about 178.6 µg/h (300 mg/70 d; 2002) and 152.4 µg/h (300 mg/82 d; 2003), indicating more than twice of those for laboratory conditions (e.g., about 63.2 µg/h at 22–23°C in Fig. 2). Generally, the release rate of the sex pheromone from a dispenser is also greatly affected by wind, solar radiation, and rain (Bierl et al., 1976; Bierl-Leonhardt et al., 1979), and that of this dispenser might be also affected by these factors. As the polyethylene pipette used as the dispenser has a large capacity (maximally 1 ml), it would be a long-lived dispenser covering throughout the entire occurrence season of *T. caelestialium*. Therefore, it was concluded that treatment with the 3-component sex pheromone is necessary for the effective disruption of *T. caelestialium* mating. Kakizaki and Sugie (2001) described that *n*-hexyl *n*-hexanoate and (E)-2-hexenyl *n*-hexanoate are essential components for male attraction, whereas *n*-octyl *n*-butyrate enhances the effect of these two components, and its additional effect for male attraction is small. However, from the present results, it is considered that *n*-octyl *n*-butyrate is also an important component for male attraction by lure, and probably for the attraction process prior to the mating of *T. caelestialium*. The traps baited with females tended to be slightly more attractive than those with the lure containing the 3-component pheromone in the treated field. It was considered that the lower attraction activity of the lure might be related with its prescription; further, it may indicate that unknown factors (e.g., other pheromone components, behavioral signals) are also involved with the attraction process of *T. caelestialium* males.

In *C. verbasci*, the disruptive effect on male trapping was high in fields treated with the complete 2-component sex pheromone, but low in fields treated with each individual component (Judd et al., 1995; McBrien et al., 1996). While the condition was slightly different for *T. caelestialium*, there was also a disruptive effect on male trapping to some degree in fields treated with each individual component. In these Miridae species, it is commonly accepted that treatment with the complete sex pheromone components would be necessary for effective mating disruption. Judd et al. (1995) considered that the mechanism of mating disruption in *C. verbasci* is due to ‘camouflage of natural plumes’ and ‘false trail following’, hypotheses which were summarized by Mink and Cardé (1988) and Cardé (1990). Although these observations revealed the phenomenon that *T. caelestialium* males had difficulty detecting females in an area treated with the sex pheromone, further study is necessary to make clear the mechanism of mating disruption in *T. caelestialium*.

One thousand dispensers each loaded with 118 mg of the synthetic sex pheromone per hectare were placed for the suppressions of a *C. verbasci*
caelestialium, either by loading a larger amount of the sex pheromone or addition of materials for release control.

The present results demonstrated the potential for population control by mating disruption using the 3-component sex pheromone of T. caelestialium. Further, it will be necessary to study the other sex pheromone components, the minimum field size for treatment, dispenser density, method of dispenser placement, and the protective effect against pecky rice in paddy fields and also against infestation of other crops.

ACKNOWLEDGEMENTS

I thank Dr. Hajime Sugie of the National Institute for Agro-Environmental Sciences for his critical reading of the manuscript, Dr. Sadahiro Tatsuki of the Laboratory of Applied Entomology and Zoology, Faculty of Agriculture, University of Tokyo, Dr. Hiroshi Noguchi of the National Institute for Agro-Environmental Sciences, Dr. Yoichi Kazino and Dr. Masaharu Ozaki of Hokkaido Central Agricultural Research Station for their advice.

REFERENCES


