Chapter I   GENERAL INTRODUCTION

The rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae), injures wheat, maize and gramineous forage grasses and it is one of the major pests causing pecky rice. This pest is distributed in Japan, China, Russia, Europe and North America (Kirkaldy, 1902; Wagner, 1956; Carvalho and Wagner, 1957; Korcz, 1979; Mikhailova, 1979; Wheeler and Henry, 1985; Yasunaga et al., 1993). In Japan, *T. caelestialium* is generally found throughout the eastern parts (Yasunaga et al., 1993), but its damage to rice occurs mainly in the north (Okuyama and Inoue, 1974). They reproduce on plants of the family Gramineae and invade rice fields after rice plant heading. Damage caused by *T. caelestialium* feeding produces stained grains or kernel spotting, known as pecky rice (Okuyama and Inoue, 1974; Ito, 2004). Pecky rice is a qualitative injury due to the introduction of pathogens into the kernels by feeding leaf bugs, which results in grain discoloration (Giudici and Villa, 2007). As pecky rice is a threat for rice grade quality, the rice leaf bug must be controlled when it occurs.

Prior to its control, monitoring of this bug has been carried out by net sweeping. Sweeping of vegetation with an insect net is one of the conventional methods of surveying insect pests in rice. However, this is a time- and labor-intensive method requiring expert knowledge and experience to determine the types of insects captured. In contrast, pheromone-baited traps are easy to use and can provide similar data on seasonal population dynamics and densities of target species. Species-specific pheromone traps also eliminate the need for specialized training to detect and identify the captured insects. Therefore, identification of the sex pheromone of the bug is urgently needed to establish a new monitoring tool and to develop a new control method in the future.

Males of true bug (Heteroptera) families, e.g. Miridae, Pentatomidae, Alydidae, Rhopalidae, Reduviidae and Coreidae, often secrete aggregation or sex pheromones (Aldrich, 1988). Some of these pheromones have been chemically identified (aggregation pheromone: Aldrich et al., 1991, 1997; Leal et al., 1995, 1996, 1998; Kochansky et al., 1989; Sugie et al., 1996; sex pheromone: Brèzot et al., 1994). Females have been reported to attract conspecific males in several Miridae species, e.g., *Ligocoris communis* (Knight) (Boivin and Stewart, 1982), *Lygus lineolaris* (Palisot de Beauvois) (Scales, 1968), *Lygus hesperus* (Knight) (Strong et al., 1970), *Lygus desertinus* Knight and *Lygus elisus* Van Duzee (Graham, 1987), *Distantiella theobroma* (Distant) (King, 1973), *Helopeltis clavifer* (Walker) (Smith, 1977) and *Campylomma verbasci* (Meyer-Dür) (Thistlewood et al., 1989, Smith and Borden, 1990). Nothing is known of the mating behavior and possible pheromonal attraction of *T. caelestialium*. Therefore, this study was conducted to investigate whether female *T. caelestialium* attract con-specific males, and whether females produce a sex pheromone that attracts males.

Female sex pheromones had been identified in three species of the family Miridae, i.e., *C. verbasci* (Smith et al., 1991), *Phytocoris relativus* Knight (Millar et al., 1997) and *Phytocoris californicus* Knight (Millar and Rice, 1998) at the time when I conducted this study. Since then, female sex pheromones that attract males in Miridae have been reported in more than 10 species (Pherobase, 2003-2017). In the volatile headspace of plant bugs, many kinds of esters, aldehydes, alcohols and hydrocarbons were detected (Aldrich, 1988). Amongst these compounds, sex pheromone action

has been identified for some of the esters of carboxylic acid: butyl butyrate and (*E*)-2-butenyl butyrate in *C. verbasci* (Smith et al., 1991), and hexyl acetate and (*E*)-2-octyl acetate in *P. relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998). In all of these reports, sex pheromones have been identified by chemical analysis of the extracts, plus field trapping tests using synthetic compounds.

In Miridae, the disruption of mating communication has been applied using sex pheromones. Disruption of mating by male trapping using a synthetic sex pheromone was performed in *C. verbasci* (Judd et al., 1995; McBrien et al., 1996) and it was confirmed to lead to population suppression in the field (McBrien et al., 1997).

In the current study, in order to find female sex pheromone candidates efficiently, extracts of *T. caelestialium* females were analyzed by a coupled Gas Chromatography – Electro-Antennographic Detector system (GC-EAD; Struble and Arn, 1984). This system was also used to elucidate the components and their optimum ratio for sex pheromone traps used in pest monitoring.

Using the sex pheromone thus identified in *T. caelestialium*, I investigated whether mating disruption would be a useful tool for the control of the plant bugs, and I assessed the components necessary for mating disruption, as well as the population suppression effect. In this thesis, I describe for *T. caelestialium* (1) the mating behavior and female attractiveness to males, (2) the chemical identification of the female sex pheromone, and (3) the control by mating disruption using synthetic sex pheromone components.

In Chapter 2, I describe the mating behavior and female attractiveness to males in field attraction tests, confirming that females indeed produce a sex pheromone. In Chapter 3, I describe the female sex pheromone candidates based on GC-EAD analysis, the effectiveness of a synthetic sex pheromone in field condition, and the optimum ratio of the sex pheromone compounds. In Chapter 4, I report the control effects by mating disruption using synthetic sex pheromone of *T. caelestialium* in small and large fields. Finally, Chapter 5 resumes the most important findings of this study and discusses future aspects of eco-friendly control of this bug.

Chapter II   Attraction of males to females in the rice leaf bug, *Trigonotylus caelestialium*

2.1   INTRODUCTION

In Pentatomidae, Alydidae, Rhopalidae, Reduviidae and Coreidae, it is known that males secrete an aggregation pheromone or a sex pheromone (Aldrich, 1988). Some of these pheromones have been chemically identified as attractants (Aldrich et al., 1991, Kochansky et al., 1989, Leal et al., 1995, Sugie et al., 1996). In Miridae, it was reported that females attract males of the same species, e.g., *Lygus lineolaris* (Scales, 1968), *L. hesperus* (Strong et al., 1970), *Distantiella theobroma* (King, 1973), *Helopeltis clavifer* (Smith, 1977), *Ligocoris communis* (Boivin and Stewart, 1982), *L. desertinus* and *L. elisus* (Graham, 1987), and *Campylomma verbasci* (Thistlewood et al., 1989). And the female sex pheromone has been identified in three species, *C. verbasci* (Smith et al., 1991), and *Phytocoris relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998) at the time of this study.

However, the mating behavior in *T. caelestialium* has not been reported. At first, in this chapter, it was examined for a mating behavior and female's attractiveness to males in *T. caelestialium*, and suggested whether female has the sex pheromone.

2.2   MATERIALS AND METHODS
2.2.1   Observation of Mating Behavior

Adults of *T. caelestialium* were collected using insect nets in wheat fields (Hokkaido Central Agricultural Research Station, Naganuma) on 1 August 1995. Mating behavior was observed in the laboratory under natural photoperiod and room temperature. About 110 pairs of adults were released in an acryl rearing box (H28 × W30 × D25 cm, SANSHIN Co. Ltd.) with wheat leaves. Mating behavior was observed for 30 h from 18:00 on 1 August. The number of mating pairs was counted at 15-minute intervals. The duration of mating in 14 pairs was also investigated at one-minute intervals from 18:00 to 24:00 on the first day. The sun set at 18:57 and rose at 4:24.

Further, the behavior of adults clustering in an insect collecting tube (3 cm diam. × 8 cm length) was observed. The adult bugs were collected near a light at night on 10 August 1995.

2.2.2   Insect-Baited Traps

Attractiveness of the adults and nymphs of *T. caelestialium* to conspecifics was examined in the field. These observations were carried out in 1996 on the second generation of *T. caelestialium* in a spring wheat field (variety was 'Haruyutaka') where this species occurred. Field size was 7,500 m^2 (75 × 100 m). Traps were set between 13−21 August.

Ten females, ten males or twenty middle stadium nymphs were used as bait for this examination. Nymphs of this species were collected in the spring wheat field using insect nets. Two to 3-d old adults were put into cages with spring wheat leaves and the bugs were not changed during this examination.

The traps used in this examination were water pan type (white, 35 cm diam. and 6 cm in depth) and were placed on the ground. A wire net cage (7 × 15 cm, 1 mm mesh) was used for maintaining the adults, and a spherical tea strainer made of stainless steel net (7 cm diam., 0.5 mm mesh) was used for the nymphs to prevent escape through the mesh. These cages were hung about 5 cm above the water pans. Three traps were used for each bait and these traps were placed linearly at intervals of 7−8 m on the ground along the wheat field. The numbers of this species captured by each trap were counted at intervals of 2−3 d, and the locations of the traps were rotated at that time.

Trap data (X) was transformed to the square root (X + 0.5) before analysis of variance, and was compared by Tukey's test.

2.3   RESULTS AND DISCUSSION

2.3.1   Mating Behavior

Although mating was observed more in the nighttime than in the daytime during this observation (Fig. 2−1), the peak mating time was not clear. The mean duration of mating for 14 pairs was 37.1 ± 20.5 (SD) min with a range from 19 to 93 min. This species mated as follows. A male near a female jumped and mounted the female instantly. Then, the male adjusted his body axis to that of the female, and attempted to copulate with her. Neither mating dance of males nor calling behavior of females was observed.

When adults attracted to a light were collected using an insect collecting tube, it was observed that most of the males responded to some of the females, and several males mounted one female simultaneously (Fig. 2−2). On the other hand, only a few females stimulated the male's behavior in such a manner. These males responded to the female simultaneously at the beginning of mating. Males which failed to mate also remained on the female until the end of mating. However, other males approaching after the start of mating did not respond to this cluster of adults. When the mating was over, the female began to walk actively and the males on the female scattered away parted from her. From these behaviors, it was considered that males responded to some cue emitted from the female. These behaviors could occasionally be observed in a rearing box, although the number of males mounting one female was smaller than that in the collecting tube.
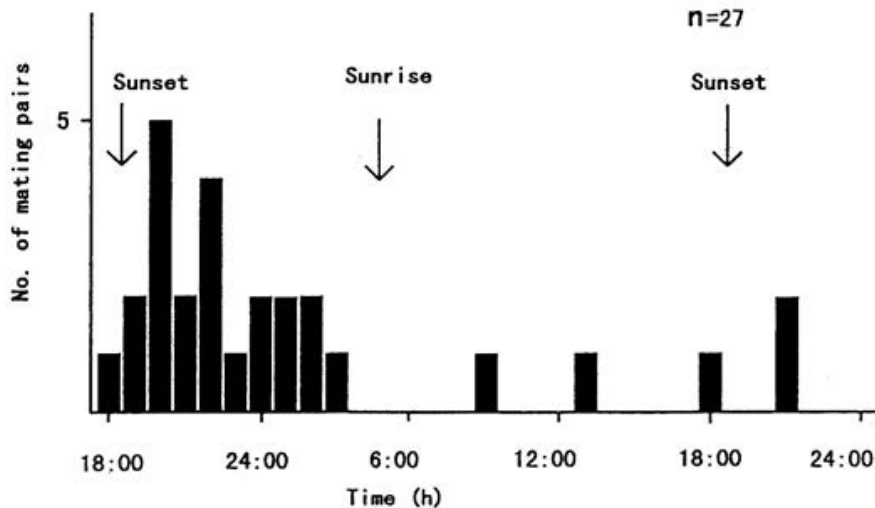

Figure 2−1.   Beginning time of mating of *Trigonotylus caelestialium* observed in a rearing box at the laboratory under natural photoperiod and room temperature (1−2 August 1995).
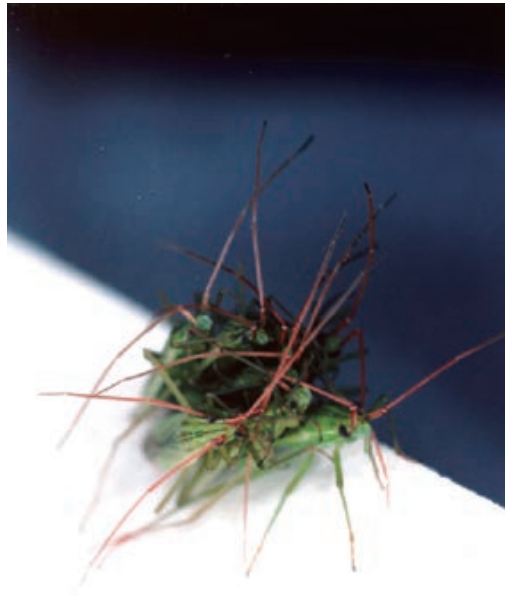
Figure 2−2. Cluster of *T. caelestialium* observed during mating in a glass tube. Eight males are mounting one female. The individual at the bottom of the cluster is the female.

2.3.2 Trapping Tests in the Spring Wheat Field

The female traps using a wire net cage and a tea strainer cage captured significantly more males (57.7 males and 11.3 males/trap during 8 d, respectively) than the other traps (Table 2−1, 2−2). The numbers of males captured by the male traps or the wheat leaf traps were not different from that of the unbaited traps. Also the number of males captured by the nymph traps using the tea strainer cage was not different from that of the unbaited traps (Table 2−2). On the other hand, no traps captured females significantly (Table 2−1, 2−2), and nymphs were not captured at all. These results indicated that the females attract the males selectively for mating.

As the factor of the attractiveness of these female traps, airborne materials, acoustic signals and visual stimuli were expected. However, the body of this species is too small, 5−6 mm length, with protective coloration, to be seen from outside of the cages. And as described above, males were considered to respond to some cue of the female on mating behavior. Thus other stimuli may be more important than visual stimuli in attraction. Although an acoustic signal has not been reported in any species of Miridae, it as an attraction signal cannot be excluded (Gogala, 1985). However, the female sex pheromone as a long-range attractant was identified in *Campylomma verbasci* (Smith et al., 1991). Therefore, it is probable that females of *T. caelestialium* also use a sex pheromone for attracting males.

Table 2–1. Number of adults captured by traps using a wire net cage 1.0 mm mesh) in the second generation of *T. caelestialium* (13 – 21 August 1996)

Bait	No. of adults captured (mean ± SE)[2]	
	Males	Females
10 females	57.7 ± 26.6 a	0.7 ± 0.3 a
10 males	3.7 ± 4.0 b	3.0 ± 1.0 a
Wheat leaves	2.3 ± 0.7 b	0.3 ± 0.3 a
Unbaited	1.7 ± 0.3 b	2.0 ± 1.1 a

1 Bugs used for bait were supplied with wheat leaves.
2 Means followed by the same letter in the same column are not significantly different at the 5% level by Tukey's test.

Table 2–2. Number of adults captured by traps using a tea strainer cage 0.5 mm mesh) in the second generation of *T. caelestialium* (13 – 21 August 1996)

Bait	No. of adults captured (mean ± SE)[2]	
	Males	Females
10 females	11.3 ± 2.5 a	1.0 ± 0.6 a
20 nymphs	0.3 ± 0.3 b	0.0 ± 0.0 a
Unbaited	1.0 ± 1.0 b	0.3 ± 0.3 a

1 Bugs used for bait were supplied with wheat leaves.
2 Means followed by the same letter in the same column are not significantly different at the 5% level by Tukey's test.

Chapter III　Identification of the female sex pheromone of the rice leaf bug, *Trigonotylus caelestialium*

3.1　INTRODUCTION

In twelve species of Miridae, females attract males of the same species (Scales, 1968; Boivin and Stewart, 1982; Thistlewood et al., 1989; McBrien and Millar, 1999). In these, the female sex attractant pheromones were identified for *Campylomma verbasci* (Smith et al., 1991), *Phytocoris relativus* (Millar et al., 1997), and *P. californicus* (Millar and Rice, 1998). The rice leaf bug, *T. caelestialium* males are attracted and sexually stimulated by virgin females, indicating that females possess a sex pheromone (see chapter 2). Therefore, identification of the female sex pheromone of *T. caelestialium* is reported in this chapter.

3.2　METHODS AND MATERIALS

3.2.1　Insects and Pheromone Extraction

Adult bugs of the second generation of *T. caelestialium* were collected in an insect collecting tube (3 cm diam., 8 cm length) near a light from 20:00 to 24:00 at Hokkaido Central Agricultural Experiment Station (HCAES), Naganuma, in mid-August 1992, 1993, and 1994. Females and males were placed in separate tubes. Whole bodies of about 100−200 bugs were dipped into 30 ml of hexane and extracted for 1−2 days at room temperature.

3.2.2　Column Chromatography

The compound groups in the extract were separated by the separation system of lipid classes by column chromatography on the Florisil (Carroll, 1961). The extracts were placed on a glass column tube (2 cm in diam., 45 cm length) packed with 30 g of the Florisil containing 7% H_2O, and successively eluted with 50 ml hexane (elute hydrocarbons), 120 ml 5% ether in hexane (elute esters and aldehydes), 150 ml 15% of that (elute triglycerides), 150 ml 25% of that (elute alcohols),

and 150 ml 50% of that (elute others).

3.2.3 Bioassay by Coupled Gas Chromatography - Electroantennographic Detector (GC-EAD)

The electric responses of antennae of *T. caelestialium* males to each compound peak in fraction of the extract from the Florisil column chromatography were recorded with a GC-EAD system (Struble and Arn, 1984), and the compounds in the extracts were monitored by a gas chromatograph (Hewlett-Packard HP5890) equipped with an HP-INNOWAX capillary column (Hewlett-Packard: 30 m × 0.53 mm ID × 0.25-μm film thickness) and coupled to a thermal conductivity detector (TCD). The carrier gas used Helium. The injector with split less mode was set 250 ºC. The oven temperature was programmed at 5°C/min from 60ºC(1.5 min) to 220ºC (30 min).

3.2.4　Chemical Analysis

Each EAD active compound in the fraction from Florisil column chromatography of female and male body extracts was collected from a vent of the TCD by a glass capillary tube. EAD activities were confirmed by GC-EAD, and the compounds were analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS analysis (EI mode) was performed on a Hewlett-Packard HP5971A with an HP-INNOWAX capillary column (60 m × 0.33 mm ID × 0.25-μm film thickness). The carrier gas used Helium. The injector with split less mode was set 250 ºC. The oven temperature was programmed as above. The interface temperature was 250ºC. Structures of ester compounds were estimated by the characteristic fragment ions, corresponding to the C_m carboxylic moiety (e.g., $C_{m-1}H_{2m-1}COOH_2^+$ and $C_{m-1}H_{2m-1}CO^+$), and the C_n alcohol moiety (e.g., $C_nH_{2n}^+$), and the molecular ion (M^+). Double bond positions in unsaturated compounds were determined by adduction of

dimethyl disulfide (DMDS) (Buser et al., 1983). Geometric isomerism was determined by comparing GC retention times for synthetic compounds with the polar column noted above.

3.2.5 Chemical Synthesis

Esters were synthesized from the same equivalent of the corresponding carboxylic acids and the alcohols by following the method of Hassner and Alexanian (1978). For an example, the ester: *n*-hexyl *n*-hexanoate (H:H) was synthesized as followed as; a solution of *n*-hexanoic acid 1.16 g (0.010 mol: 1 *eq*), as a catalyst: *N,N'*-dicyclo-hexylcarbodiimide (DCC) 2.27 g (1.1 *eq*) and 4-pyrrolidinopyridine 0.15 g (0.1 *eq*) in dichloro-methane (20 ml) had been added slowly with a solution of *n*-hexan-1-ol 1.12 g (0.011 mol: 1.1 *eq*) in dichloromethane (20 ml), and was stirred at room temperature until esterification was complete during about 20 min. The solution was washed with the saturated NaCl water (100 ml) at one time, 3.5% HCl solution (100 ml) at one time, and the saturated $NaHCO_3$ water (100 ml) at three times, and was dried by $MgSO_4$, and the solvent evaporated in vacuuo to give the ester. Other esters were also synthesized in a same procedure. The synthetic compounds were purified by silica gel (Wakogel C-200) column chromatography with a solvent system of hexane–ethyl acetate (10–20 : 1). Geometric isomers were separated by silica gel–silver nitrate (16.7%) column chromatography. The purity of each compound was greater than 99.8%, and the isomeric purity greater than 99.9% by GC. These chemicals using the syntheses were purchased from TCI (Tokyo Chemical Industry Co. Ltd., Japan, Tokyo).

3.2.6 Baits

Solutions containing test samples in 1 μl hexane were loaded in the center of glass capillary tubes (Duran ringcaps 5 μl, 0.021 mm ID, 125 mm long, bent into a 'V' shape at the center by heating with a gas burner; Kozai and Wakamura, 1982). The hexane was evaporated at room temperature overnight, and they were used as lure in all

experiments. Two virgin females in steel net cage ("soup basket"; 6 cm in diameter, 6.5 cm in height and 1.0 mm mesh) also were used in the test for comparison to the synthetic sex pheromone.

3.2.7 Field Experiments

Field experiments were carried out during the second (Aug.–Sep.) and third (Oct.) adult generations of *T. caelestialium* in fields of Italian rye grass at HCAES (22,500 m^2, in 1995–1997), and at Hokkaido Ornamental Plants and Vegetables Research Center (6,000 m^2, Takikawa, in 1998). Water-pan traps (white, 35 cm in diameter and 6 cm deep) were used. The baits were hung at 20 cm above the pans with wire. Blank traps without baits were used as controls. Three to six replications were conducted for each experiment. The traps were placed linearly at intervals of 20–25 m on the ground in fields. The number of bugs captured by each trap was counted at intervals of 1–3 days, and the location of traps was rotated at the same time.

In test I, attraction activity of each of the ten EAD active compounds, a 6-component mixture of *n*-hexyl *n*-hexanoate (5 μg), (*E*)-2-hexenyl *n*-hexanoate (2.5 μg), *n*-hexyl (*E*)-2-hexenoate (5 ng), *n*-octyl *n*-butyrate (150 ng), *n*-octyl *n*-hexanoate (275 ng), (*E*)-2-octenyl *n*-hexanoate (275 ng), and a 10-component mixture of *n*-pentyl *n*-hexanoate (5 ng), *n*-hexyl *n*-butyrate (1.15 μg), (*E*)-2-hexenyl *n*-butyrate (450 ng), and *n*-hexyl (*E*)-2-butenoate (150 ng) mixed with the above mentioned 6-component mixture were tested. In test II, one of the compounds of the 6-component mixture was deleted to evaluate the necessity of the compound. In test III, one of the compounds of a mixture of *n*-hexyl *n*-hexanoate, (*E*)-2-hexenyl *n*-hexanoate, *n*-hexyl (*E*)-2-hexenoate, *n*-octyl *n*-butyrate, and *n*-octyl *n*-hexanoate, which were selected by test II, was deleted and the necessity of the compounds was evaluated. In test IV, attraction activity of a 3-component mixture of *n*-hexyl *n*-hexanoate (3 μg), (*E*)-2-hexenyl *n*-hexanoate (1.5 μg), and *n*-octyl *n*-butyrate (90 ng), which were selected by test III, was evaluated. Effect of the addition of either *n*-pentyl *n*-hexanoate, *n*-hexyl *n*-butyrate, (*E*)-2-

hexenyl *n*-butyrate, *n*-hexyl (*E*)-2-butenoate, or all of these compounds to the 3-component mixture was evaluated in tests V and VI. In test VII, effect of the addition of *n*-octyl *n*-butyrate in variable doses of 0.015–1.5 μg on a mixture of *n*-hexyl *n*-hexanoate (5 μg) and (*E*)-2-hexenyl *n*-hexanoate (2.5 μg) was evaluated. Effect of the addition (*E*)-2-hexenyl *n*-hexanoate in variable doses of 0.5–5 μg on a mixture of *n*-hexyl *n*-hexanoate (5 μg) and *n*-octyl　*n*-butyrate (0.15 μg) were evaluated in test VIII. In test IX, optimum doses of the 3-component mixture in a ratio of 1000 : 400 : 30 ranging from 1.43 μg to 143 μg were evaluated with glass capillary tubes. In test X, attraction activity of the sex pheromone, *n*-hexyl *n*-hexanoate (5 μg), (*E*)-2-hexenyl *n*-hexanoate (2.5 μg), and *n*-octyl *n*-hexanoate (150 ng) loaded into the glass capillary tube was compared with that of traps baited with two virgin females.

Trap data (*X*) were transformed to square root (*X* + 0.5) before analysis of variance, and were compared by Tukey's test.

3.3　RESULTS

3.3.1　EAD Active Peaks in the Extracts

All the EAD active peaks by male antennae were contained in the 5% ether in hexane fraction from the Florisil column chromatography (Table 3–1). In the extracts of one female equivalent, there were two stronger EAD peaks (about 1.05–0.95 mV), three intermediate EAD peaks (about 0.3–0.15 mV), and five weaker EAD peaks (about 0.05–0.025 mV). These EAD peaks with identical retention times to those of above peaks were also detected in male body extracts.

3.3.2　Identification of the EAD Active Compounds

These EAD active compounds were identified as in Table 3–1: Two stronger EAD active compounds are *n*-hexyl　*n*-hexanoate [H:H, Abbreviation in all Tables; the retention time (Rt) = 30.67 min; *m/z* 117 (Relative intensity: 100% (base peak)), 99 (52%), 84 (40%), 200 (M$^+$, 0.3%)], and

(*E*)-2-hexenyl *n*-hexanoate [*E*2H:H; Rt = 32.38 min; *m/z* 117 (3%), 99 (100%), 82 (20%), 198 (M$^+$, 2.5%); DMDS adduct: *m/z* 292 (M$^+$), 103 ($C_3H_7CH=S^+CH_3$), 189 ($CH_3S^+=CHCH_2OCO$-C_5H_{11}); synthetic *E*-isomer, Rt = 32.38 min, *Z*-isomer, Rt = 32.17 min]. Three intermediate EAD active compounds are *n*-hexyl (*E*)-2-hexenoate [H:*E*2H; Rt = 34.46 min; *m/z* 115 (100%), 97 (36%), 84 (5%), 198 (M$^+$, 0.1%); DMDS adduct: *m/z* 292 (M$^+$), 189 ($C_6H_{13}OCOCH=S^+CH_3$), 103 ($CH_3S^+=$CHC_3H_7$); synthetic *E*-isomer, Rt = 34.46 min], *n*-octyl *n*-hexanoate [O:H; Rt = 36.45min; *m/z* 117 (100%), 99 (40%), 112 (38%), 228 (M$^+$, 0.2%)], and (*E*)-2-octenyl *n*-hexanoate [*E*2O:H; Rt = 32.38 min; *m/z* 117 (3%), 99 (100%), 110 (10%), 226 (M$^+$, 2.3%); DMDS adduct: *m/z* 320 (M$^+$), 131 ($C_5H_{11}CH=S^+CH_3$), 189 ($CH_3S^+=CHCH_2OCO$-C_5H_{11}); synthetic *E*-isomer, Rt = 38.10 min, *Z*-isomer, Rt = 37.11 min]. Five weaker EAD active compounds are *n*-octyl *n*-butyrate [O:B; Rt = 30.82 min; *m/z* 89 (100%), 71 (98%), 112 (24%), 201 (M^{+1}, 0.5%)], *n*-pentyl *n*-hexanoate [P:H; Rt = 27.65 min; *m/z* 117 (100%), 99 (62%), 70 (59%), 186 (M$^+$, 0.3%)], *n*-hexyl *n*-butyrate [H:B; Rt = 24.91 min; *m/z* 89 (100%), 71 (85%), 84 (45%), 172 (M$^+$, 0.1%)], (*E*)-2-hexenyl *n*-butyrate [*E*2H:B; Rt = 26.70 min; *m/z* 89 (4%), 71 (100%), 82 (15%), 170 (M$^+$, 1.0%); DMDS adduct: *m/z* 264 (M$^+$), 103 ($C_3H_7CH=S^+CH_3$), 161 ($CH_3S^+=CHCH_2OCOC_3H_7$); synthetic *E*-isomer, Rt = 26.70 min, *Z*-isomer, Rt = 26.50 min], and *n*-hexyl (*E*)-2-butenoate [H:*E*2B; Rt = 28.98 min; *m/z* 87 (83%), 69 (100%), 84 (4%), 171 (M^{+1}, 1.0%); DMDS adduct: *m/z* 264 (M$^+$), 189 ($C_6H_{13}OCOCH=S^+CH_3$), 75 ($CH_3S^+=CHCH_3$); synthetic *E*-isomer, Rt = 28.98 min].

These EAD active compounds were identified from whole body extracts of both sexes, and female-specific compounds showing EAD activity could not be detected.

3.3.3　Ratio of The Compounds in Extracts

The ratios of the ten compounds in female and male body extracts were quantified as Table 3–1. The relative ratios of (*E*)-2-hexenyl *n*-hexanoate, (*E*)-2-octenyl *n*-hexanoate, and *n*-octyl *n*-butyrate

to *n*-hexyl *n*-hexanoate tended to be high in female extracts, and that of *n*-hexyl (*E*)-2-hexenoate to *n*-hexyl *n*-hexanoate in male extracts.

Mean amounts of *n*-hexyl *n*-hexanoate in whole-body extracts were 127 ng per female ($N = 10$) and 27 ng per male ($N = 10$) at 7-days after emergence.

Table 3–1   EAD Active Compounds in 5% Ether in Hexane Fraction from the Florisil Column Chromatography to Male Antennae in *Trigonotylus caelestialium*

Compound[a]	Retention time (min)[b]	Relative ratio in extracts[c]		EAD activity in one female extract (mV)
		Female	Male	
H:H	30.67	1000	1000	1.05
E2H:H	32.38	414–491	271–342	0.95
H:E2H	34.46	tr.–5	10–43	0.15
O:B	30.82	5–11	1–3	0.05
O:H	36.45	55–71	58–78	0.2
E2O:H	38.10	50–63	14–19	0.3
P:H	27.65	tr.–3	tr.	0.05
H:B	24.91	225	178	0.05
E2H:B	26.70	90	36	0.05
H:E2B	28.98	32	26	0.025

[a] Abbreviations: H:H, *n*-hexyl *n*-hexanoate; E2H:H, (*E*)-2-hexenyl *n*-hexanoate; H:E2H, *n*-hexyl (*E*)-2-hexenoate; O:B, *n*-octyl *n*-butyrate; O:H, *n*-octyl *n*-hexanoate; E2O:H, (*E*)-2-octenyl *n*-hexanoate; P:H, *n*-pentyl *n*-hexanoate; H:B, *n*-hexyl *n*-butyrate; E2H:B, (*E*)-2-hexenyl *n*-butyrate; H:E2B, *n*-hexyl (*E*)-2-butenoate.

[b] GC: one female equivalent of 5% ether in hexane fraction from the Florisil column chromatography was injected and separated on an HP-INNOWAX column, programmed at 5°C/min from 60°C (1.5 min) to 220°C (30 min).

[c] Investigations were performed for first six compounds in 1992 and 1993, and for all ten compounds in 1994.

3.3.4   Attraction Activity of EAD Active Compounds in the Field

None of the ten EAD active compounds alone attracted males significantly, but the 6- and 10-component mixture were equally attractive to males (test I in Table 3−2). When *n*-hexyl *n*-hexanoate or (*E*)-2-hexenyl *n*-hexanoate were deleted from the 6- or 5-component mixture (tests II and III in Table 3−3), the numbers of males captured were as low as those in blank traps. By adding *n*-octyl *n*-butyrate to the mixture of *n*-hexyl *n*-hexanoate and (*E*)-2-hexenyl *n*-hexanoate (test IV), the number of captured males increased.

However, the deletion of (*E*)-2-octenyl *n*-hexanoate, *n*-octyl *n*-hexanoate, or *n*-hexyl (*E*)-2-hexenoate from the 6- and 5-component mixture (tests II and III), and the addition of each compound of *n*-pentyl *n*-hexanoate, *n*-hexyl *n*-butyrate, (*E*)-2-hexenyl *n*-butyrate, and *n*-hexyl (*E*)-2-butenoate (test V in Table 3−4), and of a mixture of these 4 compounds (test VI) to the 3-component mixture, did not affect male attraction. No females or nymphs of *T. caelestialium* were attracted to these mixtures in any of the experiments.

Table 3−2.   Attractiveness of the EAD Active Compounds and Their Mixtures to *T. caelestialium* in the Field (Test I, 11−25 Aug. 1995)

Baits[a]	Amount (μg)	Bugs caught/trap[b]		
		Males	Females	Nymphs
H:H	5	2.7 b	0.7 a	0.3 a
H:H	1	2.7 b	0.3 a	0.3 a
*E*2H:H	5	4.3 b	0.3 a	0 a
*E*2H:H	1	6.3 b	0.7 a	0.7 a
H:*E*2H	1	1.7 b	1.3 a	0.7 a
O:B	1	3.0 b	1.0 a	0.7 a
O:H	1	3.0 b	0.3 a	0.3 a
*E*2O:H	1	5.3 b	0.7 a	0.3 a
P:H	1	2.3 b	0.7 a	1.0 a
H:B	1	4.7 b	1.0 a	0.3 a
*E*2H:B	1	2.7 b	0.7 a	0.7 a
H:*E*2B	1	3.3 b	0.7 a	0.7 a
6-component[c]	8.205	13.3 a	0.3 a	0.7 a
10-component[d]	9.960	12.7 a	0.7 a	1.7 a
Blank		4.0 b	0.7 a	0.3 a

[a] These compounds and mixtures were loaded in glass capillary tubes. Abbreviations are same as in Table 1.
[b] Values are means from three traps during the period of the investigation. The means followed by the same letter in the same column are not different at the 5% level by Tukey's test.
[c] Mixture of 5 μg H:H, 2.5 μg *E*2H:H, 5 ng H:*E*2H, 150 ng O:B, 275 ng O:H and 275 ng *E*2O:H.
[d] Mixture adding of 5 ng P:H, 1.15 μg H:B, 450 ng *E*2H:B and 150 ng H:*E*2B on the above six-component mixture.

Table 3–3.   Attractiveness of   6-, 5-, 4-, 3-, and 2-Component Mixtures of the EAD Active Compounds to *T. caelestialium* in the Field

H:H (μg)	E2H:H (μg)	O:B (ng)	H:E2H (ng)	O:H (ng)	E2O:H (ng)	Males	Females	Nymphs
Test II, August 8–15, 1995								
5	2.5	150	5	275	275	15.3 ab	2.0 a	0.3 a
5	2.5	150	5	275		17.0 a	2.2 a	0.2 a
5	2.5	150	5		275	12.7 abc	1.7 a	0 a
5	2.5	150		275	275	11.8 abc	1.7 a	0.3 a
5	2.5		5	275	275	13.0 ab	1.8 a	0.3 a
5		150	5	275	275	7.8 cd	2.5 a	0.7 a
	2.5	150	5	275	275	7.8 cd	2.5 a	0.5 a
	Blank					5.5 d	1.7 a	0.5 a
Test III, August 13–27, 1996								
5	2.5	150	5	275		16.5 ab	1.2 a	1.3 a
5	2.5	150	5			22.7 a	1.2 a	2.0 a
5	2.5	150		275		23.2 a	1.7 a	2.3 a
5	2.5		5	275		16.5 ab	1.8 a	1.7 a
5		150	5	275		7.2 c	2.3 a	1.0 a
	2.5	150	5	275		9.8 bc	2.3 a	0.8 a
	Blank					3.7 c	1.0 a	0.8 a
Test IV, August 9–23, 1996								
3	1.5	90				32.3 a	0 a	0 a
3	1.5					20.7 b	0.3 a	0 a
	Blank					0.7 c	0 a	0 a

The header spans: Baits[a] (H:H, E2H:H, O:B, H:E2H, O:H, E2O:H) and Bugs caught/trap[b] (Males, Females, Nymphs).

[a] Mixtures were loaded in glass capillary tubes. Abbreviations are same as in Table 1.
[b] Values are means from six traps (test II and III) or three traps (test IV) during the period of the investigation. The means followed by the same letter in the same column in each test are not different at the 5% level by Tukey's test.

Table 3–4.   Effect on Attractiveness to *T. caelestialium* by Adding Four EAD Active Compounds to the 3-Component Mixtures in the Field

H:H (μg)	E2H:H (μg)	O:B (ng)	P:H (ng)	H:B (μg)	E2H:B (ng)	H:E2B (ng)	Males	Females	Nymphs
Test V, August 15–28, 1997									
5	2.5	150	5				12.6 a	1.8 a	0 a
5	2.5	150		1.15			10.4 a	0.6 a	0.2 a
5	2.5	150			450		11.0 a	0.2 a	0.6 a
5	2.5	150				150	12.4 a	0.8 a	0.6 a
5	2.5	150					11.2 a	0.6 a	0.4 a
	Blank						1.2 b	0.8 a	0.2 a
Test VI, August 15–25, 1997									
5	2.5	150	5	1.15	450	150	15.3 a	0 a	0.3 a
5	2.5	150					16.0 a	0.7 a	0.3 a
	Blank						0 b	0.3 a	0 a

The header spans: Baits[a] (H:H, E2H:H, O:B, P:H, H:B, E2H:B, H:E2B) and Bugs caught/trap[b] (Males, Females, Nymphs).

[a] Mixtures were loaded in glass capillary tubes. Abbreviations are same as in Table 1.
[b] Values are means from five traps (test V) and three traps (test VI) during the period of the investigation. The means followed by the same letter in the same column in each test are not different at the 5% level by Tukey's test.

3.3.5 Optimum Ratio and Doses of the Three Compounds for Male Attraction

The addition of 0.05–0.5 μg *n*-octyl *n*-butyrate in a 2-component mixture of 5 μg *n*-hexyl *n*-hexanoate and 2.5 μg (*E*)-2-hexenyl *n*-hexanoate (in ratios of 10–100 : 1000 : 500) (test VII in Table 3–5), and the addition of 2–2.5 μg (*E*)-2-hexenyl *n*-hexanoate in a 2-component mixture of 5 μg *n*-hexyl *n*-hexanoate and 0.15 μg *n*-octyl *n*-butyrate (in ratios of 400–500 : 1000 : 30) (test VIII), led to more males being captured than in other ratios.

Doses 42.9 μg or more of the 3-component mixture captured smaller numbers of males than doses within the range 1.43–14.3 μg (test IX in Table 3–6), indicating that attraction was inhibited by excessive amounts. At all doses used, glass capillary tubes containing doses of 4.29 μg and 14.3 μg caught the largest numbers of males.

3.3.6 Comparison of Numbers of Bugs Captured by the Synthetic Sex Pheromone and Two Virgin Females

The numbers of male bugs captured by traps baited with 7.65 μg of the 3-component mixture were greater than those by traps baited with two virgin females (test X in Table 3–7).

Table 3–5. Attractiveness of the 3-Component Mixture with Variable Ratios to *T. caelestialium* in the Field

Baits[a]			
H:H (μg)	*E*2H:H (μg)	O:B (μg)	Male bugs caught/trap[b]
Test VII, October 2–16, 1997			
5	2.5	0	5.3 b
5	2.5	0.015	5.7 b
5	2.5	0.05	10.0 a
5	2.5	0.15	6.7 ab
5	2.5	0.5	6.0 ab
5	2.5	1.5	4.7 b
	Blank		0 c
Test VIII, October 1–12, 1997			
5	0.5	0.15	8.0 d
5	1	0.15	8.7 cd
5	1.5	0.15	8.7 cd
5	2	0.15	16.0 a
5	2.5	0.15	12.7 abc
5	3	0.15	8.7 cd
5	3.5	0.15	7.3 d
5	4	0.15	8.0 d
5	4.5	0.15	10.7 bcd
5	5	0.15	14.7 ab
	Blank		0.7 e

[a] Mixtures were loaded in glass capillary tubes. Abbreviations are same as in Table 1.
[b] Values are means from three traps during the period of the investigation. The means followed by the same letter in the same column in each test are not different at the 5% level by Tukey's test.

Table 3−6.   Attractiveness of the 3-Component Mixture with Variable Amounts to *T. caelestialium* in the Field (Test IX, 9−23 Oct. 1998)

Baits[a]				
H:H (μg)	*E*2H:H (μg)	O:B (μg)	Total amounts (μg)	Male bugs caught/trap[b]
100	40	3	143	10.7 b
30	12	0.9	42.9	12.3 b
10	4	0.3	14.3	23.7 a
3	1.2	0.09	4.29	32.3 a
1	0.4	0.03	1.43	15.0 b
	Blank			0.7 c

[a]Mixtures were loaded in glass capillary tubes. Abbreviations are same as in Table 1.
[b]Values are means from three traps during the period of the investigation. The means followed by the same letter are not different at the 5% level by Tukey's test.

Table 3−7. Attractiveness of the Synthetic Sex Pheromone and Two Virgin Females in *T. caelestialium* in the Field (Test X, 12−16 Oct. 1998)

Baits	Bugs caught/trap[a]		
	Males	Females	Nymphs
Sex pheromone[b]	15.7 a	0 a	0 a
2 females[c]	8.0 b	0 a	0 a
Blank	0 c	0 a	0 a

[a]Values are means from three traps during the period of the investigation. The means followed by the same letter in the same column are not different at the 5% level by Tukey's test.
[b]Mixture of 5 μg H:H, 2.5 μg *E*2H:H and 150 ng O:B was loaded in glass capillary tube. Abbreviations are same as in Table 1.
[c]Two virgin females were kept in a steel net cage with the germinal forage grasses.

3.4 DISCUSSION

The ten compounds identified stimulated a response from male antennae. However, *n*-hexyl (*E*)-2-hexenoate, *n*-octyl *n*-hexanoate, (*E*)-2-octenyl *n*-hexanoate, *n*-pentyl *n*-hexanoate, *n*-hexyl *n*-butyrate, (*E*)-2-hexenyl *n*-butyrate, and *n*-hexyl (*E*)-2-butenoate did not affect the attraction activity and these compounds were shown not to be necessary for attraction. In conclusion, only three compounds are responsible for male attraction. *n*-Hexyl *n*-hexanoate and (*E*)-2-hexenyl *n*-hexanoate were the essential components, and *n*-octyl *n*-butyrate enhanced the male attraction in combination with the two essential components. Neither females nor nymphs were attracted to the 3-component mixture. Therefore, the 3-component mixture appears to be the sex pheromone of female *T. caelestialium*. Although clear optimum ratio of *n*-hexyl *n*-hexanoate and (*E*)-2-hexenyl *n*-hexanoate was not shown, the mixtures of *n*-hexyl *n*-hexanoate: (*E*)-2-hexenyl *n*-hexanoate: *n*-octyl *n*-butyrate in ratios of 1000: 400–500 : 10–100 were attractive to males.

In Miridae, the mixture of *n*-butyl *n*-butyrate and (*E*)-2-butenyl *n*-butyrate in *Campylomma verbasci* (Smith et al., 1991), the mixture of *n*-hexyl acetate and (*E*)-2-octenyl *n*-butyrate in *Phytocoris relativus* (Millar et al., 1997), and the mixture of *n*-hexyl acetate and (*E*)-2-octenyl acetate in *P. californicus* (Millar and Rice, 1998), were reported as attractant pheromones. The pheromones in these species contain at least one female-specific compound. On the other hand, in the case of *T. caelestialium*, the components of the attractant pheromone were common in body extracts of both sexes. However, traps baited with *T. caelestialium* males do not attract conspecific males (Kakizaki and Sugie, 1997), and there may be other factors disturbing the attraction by the males.

In a number of true bugs, many esters have been detected from extracts or secretions (Aldrich, 1988). *n*-Hexyl *n*-hexanoate and/or (*E*)-2-hexenyl *n*-hexanoate were reported in secretions of *Blepharidopterus angulatus* (Miridae) (Knight et al., 1984) and *Homoeocerus unipunctatus* (Coreidae) (Kitamura et al., 1984). (*E*)-2-Hexenyl *n*-hexanoate was reported to be an alarm pheromone in *Riptortus clavatus* (Alydidae) (Leal and Kadosawa, 1992). However, *n*-octyl *n*-butyrate has not been reported from other true bugs. The existence of *n*-hexyl *n*-butyrate or (*E*)-2-hexenyl *n*-butyrate was reported in other mirids (Smith et al., 1991; Millar et al., 1997; Gueldner and Parrott, 1978; Knight et al., 1984).

This pheromone would be useful for monitoring of *T. caelestialium* adult populations. The attraction period of the lures with the glass capillary tubes was less than two weeks, and a more long-lived pheromone dispenser should be developed for practical monitoring.

Chapter IV   Mating disruption using the sex pheromone of the rice leaf bug, *Trigonotylus caelestialium*

4.1   INTRODUCTION

In species of Miridae, the female sex pheromones of *Campylomma verbasci* (Smith et al., 1991), *Phytocoris relativus* (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).

Female *T. caelestialium* adults have been reported to attract conspecific males (see chapter 2), 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 (see chapter 3). Pest control using the sex pheromone would be useful as an IPM program for *T. caelestialium*. Therefore, in this chapter, it was examined for the sex pheromone components and their effects on mating disruption in *T. caelestialium*.

4.2   MATERIALS AND METHODS

4.2.1   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 [chapter 3]). 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.

4.2.2   Bait and trap
4.2.2.1   Pheromone lure

The mixture of *n*-hexyl *n*-hexanoate (10 μg), (*E*)-2-hexenyl *n*-hexanoate (4 μg), and *n*-octyl *n*-butyrate (0.3 μg) was loaded into a glass capillary tube (Kakizaki and Sugie, 2001 [chapter 3]), and was used as a pheromone lure.

4.2.2.2   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.

4.2.2.3   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 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.

4.2.3   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*-butyrate in a ratio of 100 : 40 : 3 − was loaded. The opening of the dispenser was closed by heating with a gas burner.

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 of each dispenser 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.

4.2.4　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 of 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 [chapter 2]), 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.

4.2.5　Field experiments
4.2.5.1　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 (Ohono town). 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. 4−1).
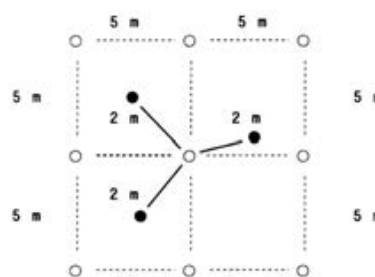

Figure 4−1.　Schema of a small-scale (100 ㎡) field test set in a 5-ha field of Italian rye-grass. A white circle (○) indicates the position placed by the dispenser, and a black circle (●) indicates the position placed by the trap.

　　Four kinds of dispenser, each loaded with 50 mg *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.

4.2.5.2　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) and in a 3.5-ha field of the same plant in 2002 and 2003 (tests 4 and 5). Two hundred dispensers, each loaded with 300 mg of the 3-component sex pheromone, were placed in a 10,000 m^2 area at intervals of 5 m × 10 m at 50 cm above ground level on steel wire bars from 5 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 on 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.

4.2.6   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.

4.3   RESULTS

4.3.1   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. 4–2). 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. 4–2. 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 (*r* = 0.9881, *p* < 0.01) (Fig. 4–3).
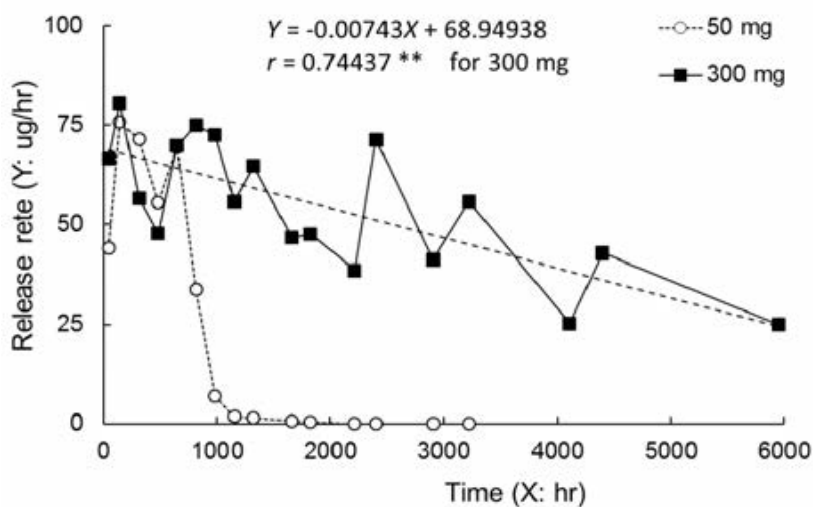
Figure 4–2. Release rate of the sex pheromone from a dispenser loaded with 300 mg and 50 mg of the 3-component sex pheromone at room temperature (** $p < 0.01$).
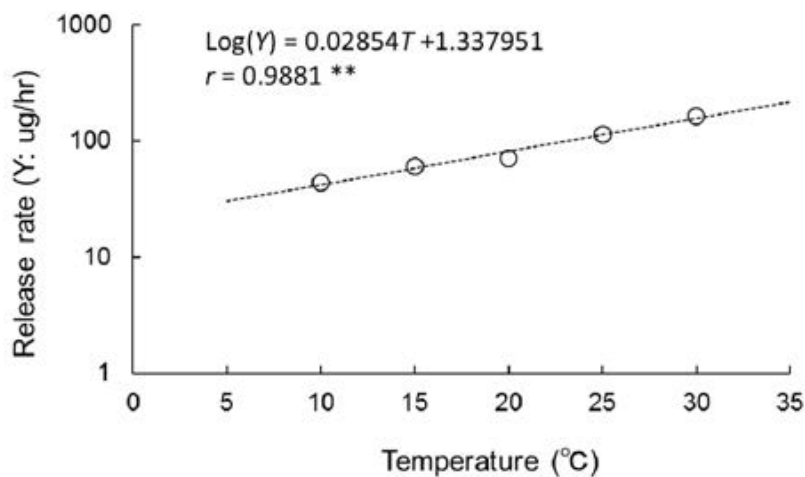

Figure 4–3. Release rate of the sex pheromone from a dispenser loaded with 50 mg of the 3-component sex pheromone at each temperature during 92–331 hr after preparation. (** $p < 0.01$).

4.3.2 Laboratory experiment

4.3.2.1 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 that in untreated cages was 9.3% (Table 4−1), indicating that the treatment with the 3-component sex pheromone disrupted the copulation of *T. caelestialium* adults.

4.3.2.2 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 *T. caelestialium* nymphs of the next generation were 9−49 individuals in cages treated with the dispenser and 73−144 individuals in the untreated cages (Table 4−2). The mean rates of increase were 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*.

Table 4−1. Effect of the sex pheromone treatment on the copulation of *Trigonotylus caelestialium* adults in rearing cages

Treatment[a]	Replicates	No. of females and males released	No. of copulation pairs[b] (mean±S.E.)	% of copulation (mean±S.E.)
Treated	3	50 and 50	0±0	0±0] *
Untreated	3	50 and 50	4.7±1.2	9.3±2.4

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

Table 4−2. Effect of the sex pheromone treatment on the reproduction of *Trigonotylus caelestialium* adults in rearing cages

Treatment[a]	Replicates	No. of bugs released (range)	No. of bugs grown up (range)	Rate of increase[b] (mean±S.E.)
Treated	3	79.7 (67−88)	26.3 (9−49)	0.37±0.19] *
Untreated	3	32.7 (29−51)	105.3 (73−144)	3.22±0.90

[a] 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.
[b] Rate of increased were calculated from the numbers of the next-generation nymphs per numbers of previous-generation matured nymphs released in each cage (*, $p < 0.05$).

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

In the small-scale field experiment (9 dispensers placed in a 100-m^2 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. 4–4), 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 further reduced to zero (Fig. 4–4). The

numbers of males captured by traps baited with 3-virgin females were also reduced by treatment with the 3-component mixture (Fig. 4–5A, B), similarly to those baited with the synthetic sex pheromone lure (Fig. 4–5A). 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 large scale field experiment (test 3 in Fig. 4–6), however, the traps baited with females tended to attract a few males in the treated field, but those with the lure did not (Figs. 4–5 and 4–6).
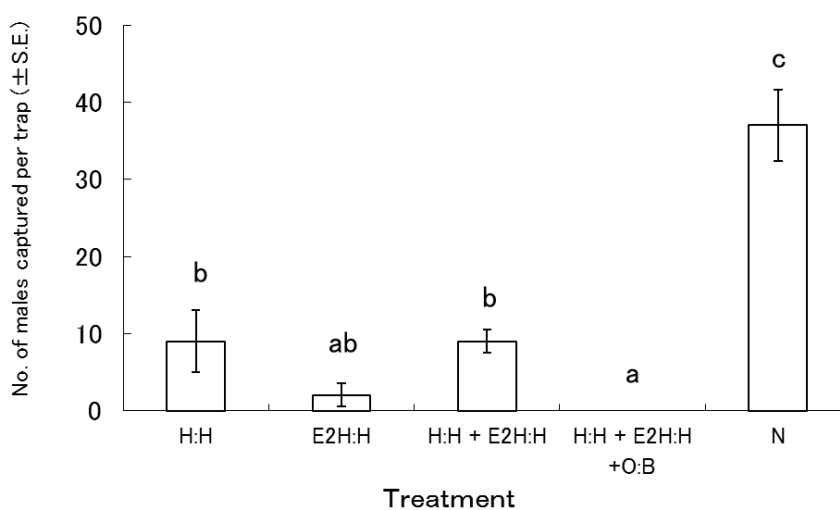
Figure 4–4.　Effect of trapping disruption of *Trigonotylus caelestialium* males attracting to the 3-component pheromone trap by high concentration treatments of the sex pheromone components in small-scale field experiment (12 Aug. to 22 Sep. 2001). Abbreviations: H:H, *n*-hexyl *n*-hexanoate; *E*2H:H, (*E*)-2-hexenyl *n*-hexanoate; O:B, *n*-octyl *n*-butyrate; N, untreated. The means followed by the same letter are not different at the 5 % level by Tukey's test.
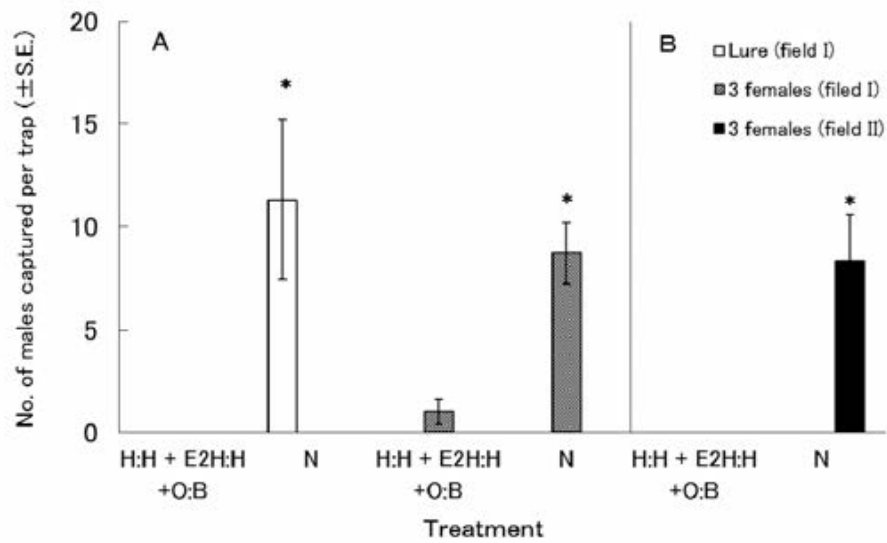
Figures 4–5 A and B.   Effect of trapping disruption of *Trigonotylus caelestialium* males attracting to traps baited with the lure and females by high concentration treatments of the 3-component sex pheromone in small-scale field experiment (A: field I, 21 Sep. to 20 Oct., 2001; B: field II, 30 Sep. to 20 Oct., 2001) (* $p < 0.05$).
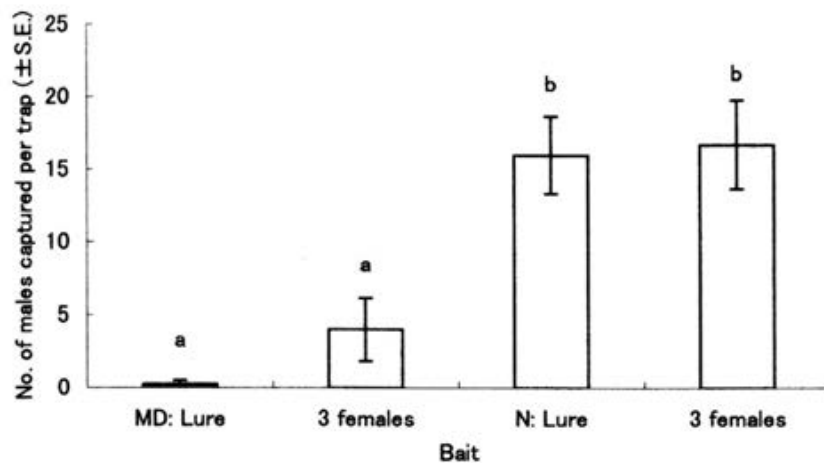

Figure 4–6.   Effect of trapping disruption of *Trigonotylus caelestialium* males attracting to the 3-component pheromone trap by high concentration treatments of the sex pheromone components in small-scale field experiment (test 5: 15–29 Aug. 2003). MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N, untreated field. The means followed by the same letter are not different at the 5 % level by Tukey's test.

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

In the large-scale (10,000 m^2) 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. 4–7, test 4 in Fig. 4–8, and test 5 in Fig. 4–9). 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. 4–10, test 4 in Fig. 4–11, and test 5 in Fig. 4–12), 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.
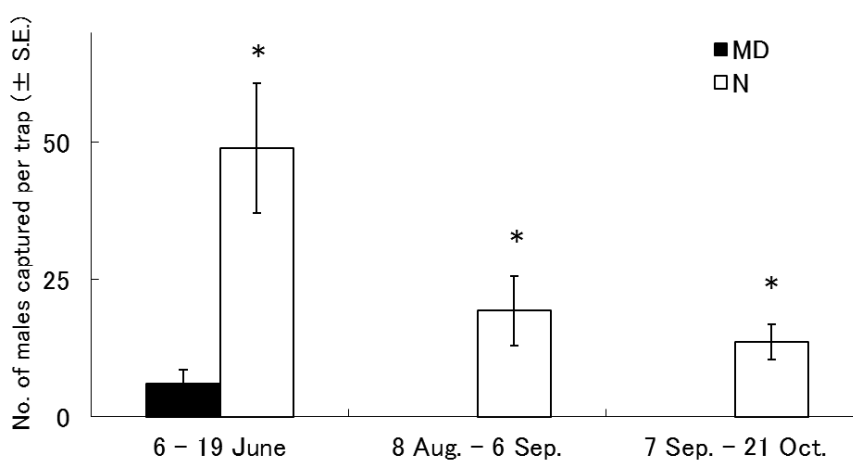
Figure 4–7. Numbers of *Trigonotylus caelestialium* males captured by traps baited with the sex pheromone lure in the field (test 3; 2001). Two hundred dispensers in 1-ha were set during 6 June to 31 Oct. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (** *p* < 0.05).
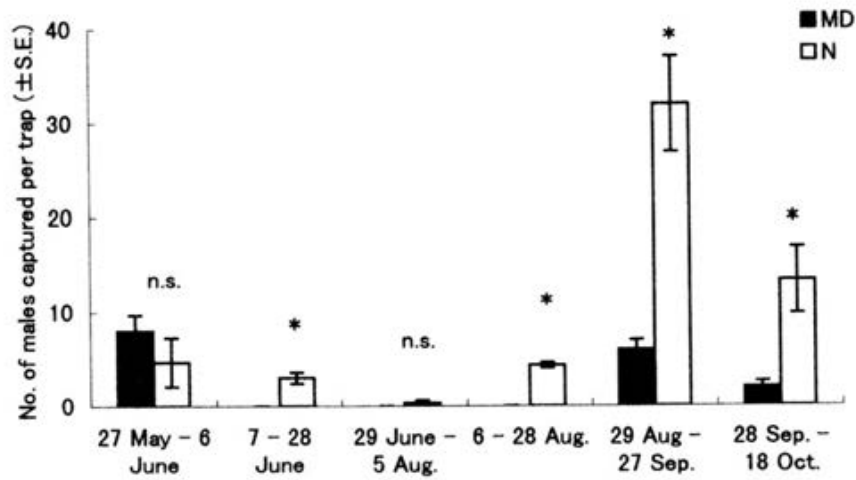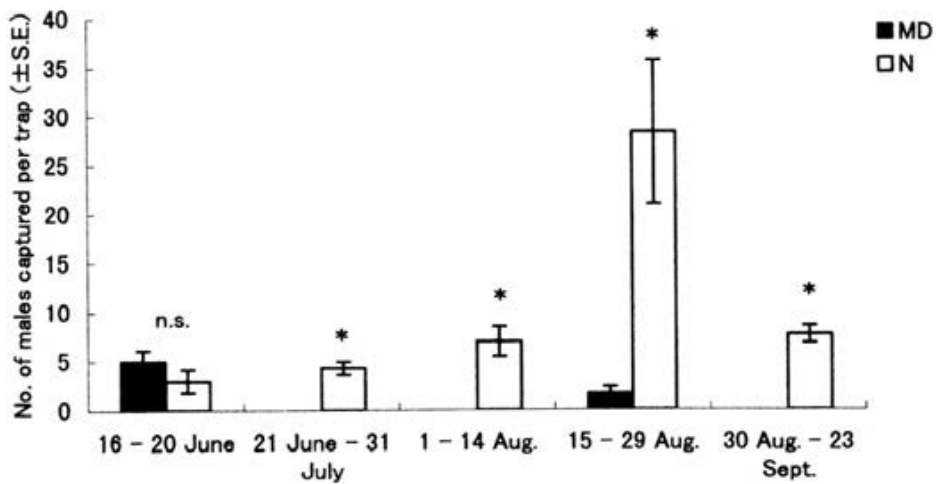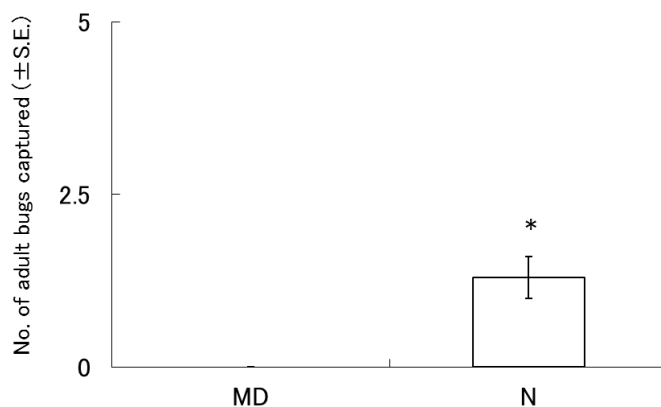
Figure 4–8.　Numbers of *Trigonotylus caelestialium* males captured by traps baited with the sex pheromone lure in the field (test 4; 2002). Two hundred dispensers in 1-ha were set during 6 June to 31 Oct. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (* $p < 0.05$).

Figure 4–9.　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 June to 23 Sept. MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field (* $p < 0.05$).

Figure 4–10. 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 (* $p < 0.05$).
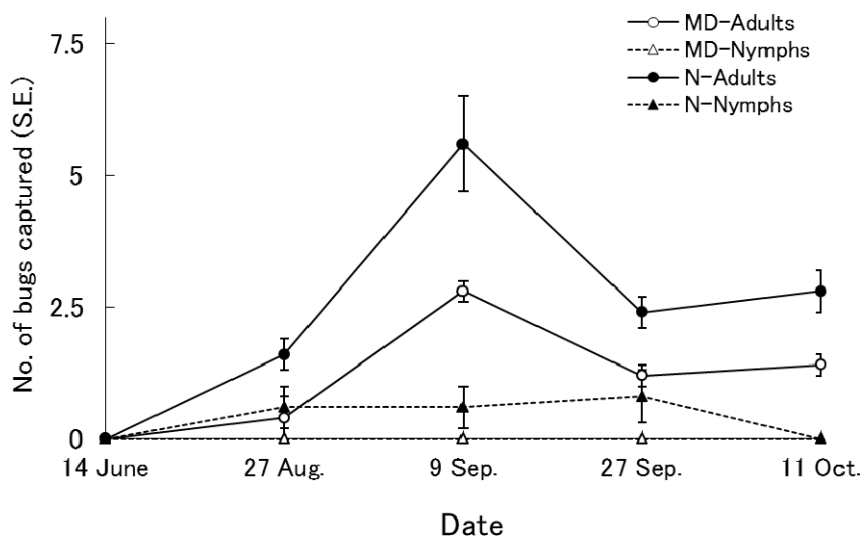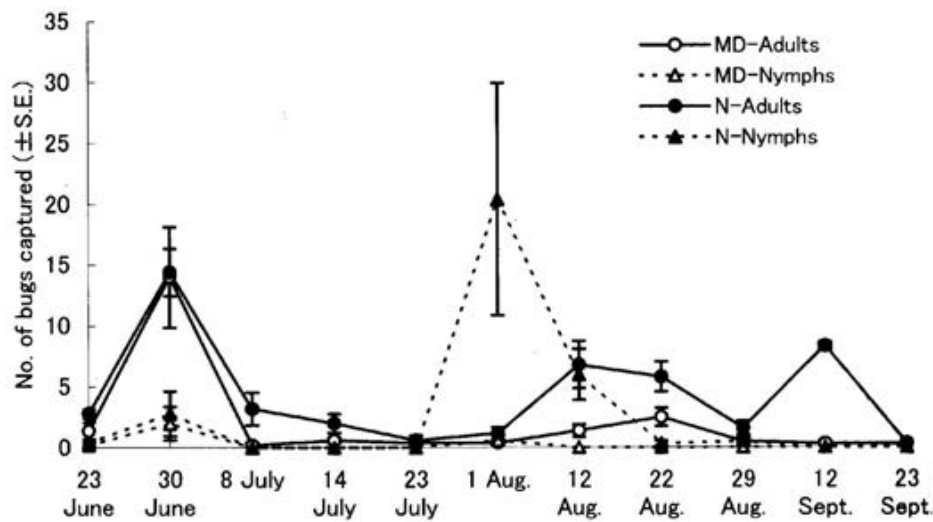

Figure 4–11.   Numbers of *Trigonotylus caelestialium* bugs captured by net sweeping (20 times) in the field (test 4; 14 Jun. to 11 Oct. 2002). MD: field treated with the dispenser loaded with 300 mg of the 3-component sex pheromone; N: untreated field.

Figure 4–12.   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.

4.4   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-m^2 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 *n*-butyrate, was more effective than treatments with *n*-hexyl *n*-hexanoate alone, (*E*)-2-hexenyl *n*-hexanoate alone, or a mixture of these two components. 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 Minks 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* 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/84d – 80.6 g/ha/75d; 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 [chapter 3]), 0.8–2.8 mg/d for a gray septum in *C. verbasci* (McBrien et al., 1994), and 33 mg/2-weeks (2.3 mg/d) for *P. relativus* (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. 3−3 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/70d; 2002) and 152.4 µg/h (300 mg/82d; 2003), indicating more than twice of those for laboratory conditions (e.g., about 63.2 µg/h at 22−23°C in Fig. 3−2). 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*, 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 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.

Chapter V   GENERAL DISCUSSION

5.1 The female sex pheromone

Generally, sex pheromones are highly species specific and low toxic compounds that have great potential in the control of bugs. They may be used in detection, monitoring, mass trapping and mating disruption of economically important species, and in attract-and-kill control strategies. In this study, it was confirmed that male adults were attracted to a trap baited with female adults of *T. caelestialium* (Kakizaki and Sugie, 1997 [chapter 2]), as reported in other species of Miridae (Boivin and Stewart, 1982; Scales, 1968; Strong et al., 1970; Thistlewood et al., 1989). This demonstrated the existence of a female sex pheromone in *T. caelestialium*.

Sex pheromones of Miridae species have been identified by analyzing compound extracts and sex pheromone components have been clarified in various species, e.g., *C. verbasci* (Smith et al., 1991), *P. relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998). In this study (Kakizaki and Sugie, 2001 [chapter 3]), 10 sex pheromone candidates induced an EAG response on the male antennae: *n*-hexyl *n*-hexanoate (H:H); (*E*)-2-hexenyl *n*-hexanoate (*E*2H:H); *n*-hexyl (*E*)-2-hexenoate (H:*E*2H); *n*-octyl *n*-butyrate (O:B); *n*-octyl *n*-hexanoate (O:H); (*E*)-2-octenyl *n*-hexanoate (*E*2O:H); *n*-pentyl *n*-hexanoate (P:H); *n*-hexyl *n*-butyrate (H:B); (*E*)-2-hexenyl *n*-butyrate (*E*2H:B); and *n*-hexyl (*E*)-2-butenoate (H:*E*2B), in the ratios of 1000 : 414–491 : trace–5 : 5–11 : 55–71 : 50–63 : trace–3 : 225 : 90 : 32 in female body extracts. In field attraction tests using synthetic compounds of these sex pheromone candidates, a mixture of *n*-hexyl *n*-hexanoate (H:H) and (*E*)-2-hexenyl *n*-hexanoate (*E*2H:H) – the main components of the sex pheromone – proved a potent male attractant, and adding *n*-octyl *n*-butyrate (O:B) enhanced the attraction activity. A mixture of H:H, *E*2H:H and O:B in the ratio of 1000 : 400–500 : 10–100 displayed the highest attraction activity. It was concluded that these three

components compose the female sex pheromone, because only male adults were attracted to the mixture. Also, a synthetic sex pheromone trap containing a mixture of these three components in a ratio of 100 : 40 : 3 displayed attractiveness equal to or higher than a trap with two *T. caelestialium* females.

Many esters have been detected from extracts or secretions in true bugs (Aldrich, 1988). The esters H:H and/or *E*2H:H were reported in secretions of *Blepharidopterus angulatus* (Fallén) (Miridae) (Knight et al., 1984) and *Homoeocerus unipunctatus* (Thunberg) (Coreidae) (Kitamura et al., 1984), but it is unknown whether these compounds are the sex pheromone for these two species. *E*2H:H was also reported to be an alarm pheromone in *Riptortus clavatus* (Thunberg) (Alydidae) (Leal and Kadosawa, 1992). In subsequent studies on Miridae, H:H and *E*2H:H were reported as the components of the sex pheromone of *Creontiades dilutus* (Stål) (Lowor et al., 2009), and *E*2H:H was one of the components of the sex pheromone of *Adelphocoris suturalis* (Jakovlev) (Zhang et al., 2016). The ester *n*-octyl *n*-butyrate (O:B), which was identified as an enhance substance of the two main sex pheromone compounds in *T. caelestialium*, is not known from other true bugs; that is, O:B appears a specific substance for *T. caelestialium*.

In species of the family Miridae, sex pheromone compounds with reported attraction activity are mixtures of *n*-butyl *n*-butyrate (B:B) and (*E*)-2-butenyl *n*-butyrate (*E*2B:B) for *C. verbasci* (Smith et al., 1991) and mixtures of *n*-hexyl acetate (H:Ac) and (*E*)-2-octenyl *n*-butyrate (*E*2O:Ac) for *P. relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998). These compounds are different from those of *T. caelestialium*.

n-Hexyl *n*-butyrate (H:B) and (*E*)-2-hexenyl *n*-butyrate (*E*2H:B) were detected from extracts or secretions in many Miridae species (Smith et al.,

1991; Millar et al., 1997; Gueldner and Parrott, 1978; Knight et al., 1984). Thereafter, H:B and/or *E*2H:B are detected as common major compounds in 16 species of mirid sex pheromones: *Adelphocoris fascilaticollis* Reuter, *Adelphocoris lineolatus* (Goeze), *Apolygus lucorum* (Meyer-Dür) (Zhang, 2011), *A. suturalis* (Zhang et al., 2016), *Apolygus spinolae* (Meyer-Dür) (Yang et al., 2014), *Halticus minutus* Reuter (Zhang et al., 2017), *Liocoris tripustulatus* (Fabricius), *Lygocoris pabulinus* (L.) (Fountain et al., 2014), *L. hesperus*, *L. desertinus*, *L. elisus* (Byers et al., 2013), *Lygus pratensis* L. (Innocenzi et al., 2005), *Lygus rugulipennis* Poppius (Innocenzi et al., 2004), *Stenotus rubrovittatus* (Matsumura) (Yasuda et al., 2008), *Orthops campestris* (L.) and *Taylorilygus apicalis* (Fieber) (Yang et al., 2015).

Although sex pheromones serve a purpose for intra-specific attraction, inter-specific attraction has occasionally been observed among *L. hesperus*, *L. desertinus* and *L. elisus* (Graham, 1987). This may be explained by the sex pheromone components that they have in common: H:B and *E*2H:B. The ratio and amount of H:B and *E*2H:B differ among the three species, therefore inter-specific attraction does not occur frequently (Byers et al., 2013). *T. caelestialium* males had slight EAG responses to H:B and *E*2H:B from female extracts, but these compounds did not elicit any attraction in males, and they were not part of the sex pheromone of this species. Until now, no inter-specific attraction activity is known for the sex phenomenon of *T. caelestialium*. Therefore, it seems unlikely that *T. caelestialium* females attract heterospecific males, and it seems unlikely that *T. caelestialium* males are attracted to heterospecific females that produce H:B and *E*2H:B.

Before this study, the ester compounds of the sex pheromone had been identified in three species, i.e., *C. verbasci* (Smith et al., 1991), *P. relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998). Although the esters, e.g., H:B or *E*2H:B, had been found in extracts in several species, at first there was no report showing attractiveness of these compounds. Prior to this study, a variety of chemical compounds – such as aldehydes, hydrocarbons, terpenes, other esters, etc. – were also reported from the extracts of various Miridae species, and all of these compound groups had been considered as sex pheromone candidates, without confirmation of their attractiveness. Studies included in this thesis have clarified that the esters of carboxylic acid are the main compound group of the sex pheromone in Miridae species. Their publication has led to many subsequent investigations, identifying sex pheromones in other Miridae species.

5.2　Monitoring and mating disruption using the sex pheromone

As expected, we found that the sex pheromone of *T. caelestialium* attracts conspecific males, not conspecific females, nor non-target bugs. Therefore, for the monitoring of *T. caelestialium* lures containing this species-specific sex pheromone may be used. Based on the present study, a new sex pheromone trap has been developed, consisting of a sticky net cylinder (SNC) and a long-lived lure, effective for 1.5 months (Kakizaki, 2013). This SNC trap can capture more than 3 times the number of bugs in comparison with a vertical sticky plate. Furthermore, in the monitoring system using the SNC trap, 3 to 5 times the number of bugs are captured, compared with the sweep-net sampling method (Kakizaki, 2006, 2013). As a possible alternative to sweep-net sampling of vegetation in and around paddy fields, we investigated the potential of synthetic sex pheromone traps for monitoring *T. caelestialium*. It turned out that the sex pheromone trap effectively assesses the occurrence of the rice leaf bug and of pecky rice in the paddy fields with more stable survey accuracy than the net sweeping method (Kakizaki, 2006, 2013). Furthermore, we set the control threshold for the occurrence of pecky rice in the paddy fields based on monitoring of the sex pheromone trap catches, for a decision of spaying insecticide (Kakizaki et al., 2008). In addition, the sex pheromone compounds identified in this study are being used for the development of another type of

sex pheromone trap, which uses a vertical sticky plate (Higuchi et al., 2004).

On this study for mating disruption (Kakizaki, 2004 [chapter 4]), treatment with the 3-component sex pheromone – containing *n*-hexyl *n*-hexanoate (H:H), (*E*)-2-hexenyl *n*-hexanoate (*E*2H:H) and *n*-octyl *n*-butyrate (O:B) – disturbed the copulation of *T. caelestialium* adults and consequently reduced their reproduction under laboratory conditions. Field experiments demonstrated that the 3-component sex pheromone interfered with male attraction to the traps baited with either the lure or with two adult females in the 100-m^2 field. Application of the 3-component sex pheromone in a 1-ha (10,000-m^2) field indicated a continuous effect on the inhibition of males being attracted towards females, resulting in decreased *T. caelestialium* population densities to 22–45% for adults and 0–2% for nymphs, in comparison with untreated fields, based on sweep-net samplings. The results of the mating disruption field trials also showed that treatment with the 3-component sex pheromone (H:H, *E*2H:H and O:B) was more effective than treatments with H:H alone, *E*2H:H alone, or a mixture of these two components. Therefore, it was concluded that treatment with the 3-component sex pheromone is necessary for effective disruption of *T. caelestialium* mating. Mating disruption by the 3-component lure also inhibits the attraction of males to females. However, the disruption effect of the trap containing females was slightly lower than that of the sex pheromone trap. Hence, the option of control by communication disruption using the sex pheromone appears viable.

Disturbing male attraction to traps has been reported in *C. verbasci* (Judd et al., 1995; McBrien et al., 1996) and population suppression by mating disruption using synthetic sex pheromone in the field was shown (McBrien et al., 1997). This is similar to the potential for population control by mating disruption using the 3-component sex pheromone of *T. caelestialium*. Many studies have reported the control by communication disturbance using the sex pheromone of lepidopteran pests; however, few studies have been done in heteropterans. This study demonstrating population density control based on sex pheromone is the first report after that of McBrien et al. (1997).

In Miridae species, it is commonly observed that treatment with the complete sex pheromone blend is necessary for effective mating disruption. Judd et al. (1995) considered that the mating disruption in *C. verbasci* is due to 'camouflage of natural plumes' and 'false trail following', two mechanisms that were hypothesized by Minks and Cardé (1988) and Cardé (1990). Although our observations revealed that *T. caelestialium* males had difficulty in detecting females in an area treated with sex pheromone, further study is necessary to make clear the mechanism of mating disruption. For this, it will be necessary to study 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.

Finally, elucidating the sex pheromone of *T. caelestialium* has enhanced the possibility of developing new monitoring tools, bringing closer its control in an environmentally safe manner, without the use of pesticides.

Summary

The rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae), is one of the major pests causing pecky rice, and its control is necessary. A new monitoring system needs to replace the laborious net sweeping. In this study, it was confirmed that male adults were attracted to a trap with adult females of *T. caelestialium*, indicating that a female sex pheromone is produced, as reported in other mirid species. The female sex pheromone was analyzed by Coupled Gas Chromatography–Electroantennographic Detection (GC-EAD) and its effectiveness was confirmed by field attraction tests using synthetic sex pheromone candidates. The main sex pheromone components were identified as *n*-hexyl *n*-hexanoate (H:H) and (*E*)-2-hexenyl *n*-hexanoate (*E*2H:H). The addition of *n*-octyl *n*-butyrate (O:B) to these compounds was shown to enhance the attraction of males. A mixture of H:H, *E*2H:H and O:B in the ratio of 1000: 400–500: 10–100 demonstrated high attraction activity. Only male adults were attracted to the mixture of these three components, just as females attracted only conspecific males, and not females. Traps containing a mixture of these three synthetic sex pheromone components in a ratio of 100: 40: 3 were shown to attract males in numbers equal to or higher than traps with two females of the bug.

Trials on mating disruption demonstrated that treatment with the 3-component sex pheromone (H:H, *E*2H:H and O:B) disturbed the copulation of *T. caelestialium* adults and consequently reduced their reproduction under laboratory conditions. Experiments in a 100-m^2 field revealed that the 3-component sex pheromone interfered with male attraction to traps baited with either the lure or with three adult females. Application of the 3-component sex pheromone in a 1-ha (10,000-m^2) field indicated a continuous effect on the inhibition of attraction towards the trap, resulting in decreased *T. caelestialium* population densities to 22–45% for adults and 0–2% for nymphs, in comparison with untreated fields, based on sweep-net samplings. Hence, the option of control by communication disruption using the sex pheromone appears viable.

As a possible alternative to sweep-net sampling of vegetation in and around paddy fields, the potential for using pheromone-baited traps for monitoring of *T. caelestialium* was effective. To elucidate the mechanism of mating disruption, it will be necessary to study 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. Finally, the elucidation of the sex pheromone of *T. caelestialium* has enhanced the possibility of developing new monitoring tools, bringing closer the bug's control in an environmentally safe manner, without the use of pesticides.

和 文 要 旨

アカヒゲホソミドリカスミカメ *Trigonotylus caelestialium* (Kirkaldy)は，北日本における水稲の斑点米を発生させる重要害虫である．従来，水田ほ場では捕虫網によるすくい取り調査により発生のモニタリングを行う方法が行われているが，降雨や風などの気象条件やほ場での調査位置，調査員による捕獲数のふれがあるなど安定した調査精度を得るには問題があった．しかし，本種の性フェロモンが解明され、合成性フェロモンを利用できるようになれば，安定した精度で発生のモニタリングが可能になり，さらに農薬によらない防除法の開発にもつなげることができる．そこで，本研究では，アカヒゲホソミドリカスミカメの性フェロモンの構造解析を行い，さらに合成性フェロモンを用いた交信攪乱による防除の可能性を検討した．

アカヒゲホソミドリカスミカメが性フェロモンを放出しているのかどうかを明らかにするために，野外のイネ科牧草ほ場において，雌成虫，雄成虫をそれぞれ金網ケージに入れて誘引源とした生体トラップへの捕獲調査を行った．雌成虫トラップに雄成虫が特異的に誘引される現象を観察できたことから，本種の雌が誘引性の性フェロモンを放出している可能性が確認できた．

次に，性フェロモンの構造解析のため，雌成虫をヘキサンに虫体浸漬し，その抽出物の各成分に対する雄成虫触角の触角電位(Electro Antenno Gram: EAG)応答をガスクロマトグラフ触角電位測定法（coupled gas chromatography–electroantennographic detection ：GC-EAD）によって調査した．このうち，抽出物中の主成分 2 物質を含む 10 物質のピークに EAG 応答がみられ， GC-MS 分析の結果，これらの応答物質は炭素数が C_5，C_6，C_8 のアルコールと，炭素数が C_4，C_6，C_8 のカルボン酸とのエステルであることがわかり，これらの物質は，*n*-hexyl *n*-hexanoate; (*E*)-2- hexenyl *n*-hexanoate; *n*-hexyl (*E*)-2-hexenoate; *n*-octyl *n*-butyrate; *n*-octyl *n*-hexanoate; (*E*)-2-octenyl *n*-hexanoate; *n*-pentyl *n*-hexanoate; *n*-hexyl *n*-butyrate; (*E*)-2-hexenyl *n*-butyrate; *n*-hexyl (*E*)-2-butenoate と同定され，雌抽出物中の成分比は 1000 ： 414–491 ： trace–5 ： 5–11 ： 55–71 ： 50–63 ： trace–3 ： 225 ： 90 ： 32 であった．

これらの物質の化学合成品を抽出物中の成分比に準じてガラス細管に担持した誘引製剤を用いて，野外誘引試験によって雄成虫に対する誘引活性を調査した．その結果，抽出物中の主要な 2 成分である *n*-hexyl *n*-hexanoate と(*E*)-2-hexenyl *n*-hexanoate の混合物に基本的な誘引活性があり，副成分の *n*-octyl *n*-butyrate はこれら主要 2 成分の混合物の誘引性を高める効果があることを突き止めた．それらの成分比が，1000 ： 400–500 ： 10–100 の時に誘引性が最も高くなった．これら 3 成分の混合成分には，雄成虫のみが誘引され，雌成虫トラップに雄成虫が誘引される現象と一致したことから，これらの成分が本種の雌性性フェロモンであると同定した．また，これら 3 成分の 100:40:3 の混合物を担持した合成性フェロモントラップは，雌成虫 2 頭のトラップと同等以上の誘引性を示した．

次に，防除への応用を目的として，この 3 成分による交信攪乱法の検討を行った．室内の飼育ケージ内に 3 成分の化学合成品を処理すると，飼育ケージ内の本カメムシの交尾率の低下や増殖が抑えられる傾向が観察された．野外牧草地における小規模試験（100 ㎡に 5m 間隔に 9 個の交信攪乱用ディスペンサーを処理）において，その試験区の中央に設置した 3 成分を担持した誘引製剤の合成性フェロモントラップへの雄成虫の捕獲は，交信攪乱剤の成分として主要 1 成分 *n*-hexyl *n*-hexanoate または(*E*)-2-hexenyl *n*-hexanoate を処理した場合，あるいは，主要 2 成分 *n*-hexyl *n*-hexanoate と(*E*)-2-hexenyl *n*-hexanoate の混合成分を交信攪乱剤として周りに処理した場合のいずれでも，試験区内に設置した合成性フェロモントラップへの捕獲数を減少させる効果がみられたが，3 成分の交信攪乱剤を用いた場合には，合成性フェロモントラップの捕獲数を減少させる効果が最も高かった．また，雌成虫トラップでの捕獲数を減少させる効果がみられ，交信攪乱処理区内での雌成虫への誘引を阻害する効果が確認された．しかし，合成性フェロモントラップにおける捕獲数に比較して雌成虫トラップでの捕獲数はやや多く，交信攪乱処理の雌成虫に対する誘引阻害効果は，合成性フェロモントラップに対する誘引阻害効果ほど完全ではなかった．

次に，3 成分の交信攪乱剤での大規模処理試験（10,000 ㎡に 5 m × 10 m 間隔に 200 個の交信攪乱用

ディスペンサーを処理）を行った．3 成分の交信攪
乱処理は処理区内での合成性フェロモントラップで
の捕獲数を持続的に抑制し，処理区内での捕虫網に
よるすくい取り成虫数は無処理区の 22.3–45.0%，幼
虫数は 0–2.2％となり低密度に抑えられていたこと
から，これら 3 成分の交信攪乱剤処理には密度抑制

効果があることを確認できた．

　本研究においてアカヒゲホソミドリカスミカメの
性フェロモンが同定されたことにより，これを利用
したモニタリングや減農薬につながる新たな防除法
の開発等，現場における総合防除の構築が可能にな
ったと考えられる．

Acknowledgements

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