博士学位論文

Studies on development of the new method of control using behavior regulators of *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae)

山下賢一

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（クモヘリカメムシの行動制御物質を使った新たな防除法の研究）

平成29年5月17日

山下賢一

Contents

Introduction

While chemical pesticide use has increased agricultural production and productivity, its use, overuse and misuse have caused negative externalities on human health and the environment, as well as food safety (Kiritani 2000; Oida 2016). In particular, the overuse of chemical pesticides has led to pest resistance, resurgence and secondary outbreaks, which push farmers to use more new pesticides. To ease the negative issues associated with pesticide use, integrated pest management (IPM) technology, which aims to maximize farms' economic profits, is introduced and implemented in agricultural production worldwide. IPM refers to an ecologically-based approach that makes the best use of all available technologies to sustainably manage pest problems. The primary objective of IPM technology is to minimize chemical pesticide use in relation to pest management, while maintaining or enhancing farms' net returns with minimal environmental degradation. Previous studies have shown that IPM adoption significantly lowers pesticide use, saves production costs and maintains farm productivity for adopters (Oida 2016). Thus, steps must be taken to further progress the IPM program.

Peck (Fig. 1), primarily caused by stink bugs, is one of the key quality factors for rice grading and marketability (Yao 2002; Ito 2004). Pecky grains are discolored and shriveled, and have poor milling quality. As the amount of pecky rice increases, the quality and value of the crop are reduced. The adequate control of rice stink bugs during heading can improve rice grade, quality and selling price, as kernels become discolored when fed on by rice stink bugs in the milk or soft dough stages.

Fig. 1. Damage by stink bugs

The rice stink bug, *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae) (Fig. 2), is a major cosmetic pest. *Leptocorisa chinensis* is now recognized as one of the main pests to cause pecky rice (Suzuki 2001). In Japan, this species occurs in mainland areas (Takeuchi 2007), and is bivoltine throughout most of its distribution. During spring, wild Gramineae species host *L. chinensis*, and the first generation of offspring predominantly uses these plants for reproduction. The second generation of adults invades paddy fields and feeds on grains in the milk or soft dough stages, causing pecky rice (Takeuchi et al. 2004b; Takeuchi 2007). While chemical insecticides are available, the effective and environmentally benign control of this species in paddy fields is also sought.

Fig. 2. *Leptocorisa chinensis* (Dallas) in life, on the top of Italian ryegrass, *Lolium multiflorum* Lam.

Pheromones (with Greek roots meaning ‘carrier of excitation’) are the chemicals that an animal secretes or excretes that release a specific reaction, for example, a definite behavior or developmental process’ in a member of the same species. Wilson and Bossert (1963) divided pheromones into two groups: releasers, which induce an immediate behavioral change including alarm, and primers, which initiate changes in development, such as sexual maturation. The former does not result in immediate behavioral changes, but means that the animal is predisposed to such changes (note here that a releaser pheromone is not necessarily the same as a ‘releaser’ in the sense used by ethologists such as Lorenz and Tinbergen to describe a trigger of instinctive behavior). Knowledge that non-toxic and species-specific pheromones do not harm beneficial species can be used to establish efficient and sustainable insect management strategies.

Disturbed stink bugs emit pungent volatile compounds that could have several ecological functions including defense against predators or behavior regulation (Blum 1985; Aldrich 1995). A behavior regulator function might be used for the behavioral control of stink bugs in agroecosystems. For example, in Sri Lanka, farmers collect and squash stink bugs, placing them in bags around the field to reduce damage (Yamashita personal communication). The volatiles from smashed bugs probably repel stink bugs in the area. I hypothesized that the volatiles emitted from disturbed *L. chinensis* could be used to repel conspecifics in rice fields and reduce the number of pecky rice grains. Leal et al. (1996) identified the volatiles emitted from *L. chinensis* that were anaesthetized with CO_2 to minimize the release of defensive secretion, and found that a 5:1 mixture of (*E*)-2-octenyl acetate and octanol, major components of the emissions of undisturbed *L. chinensis*, was an attractant pheromone. However, the chemistry and ecological functions of the volatiles from disturbed *L. chinensis* individuals have not yet been studied.

The aim of the present study was to develop a new method of control using the behavior regulator in *L. chinensis*. First, I examined the life history of *L. chinensis* to predict and control *L. chinensis* invasion during the rice heading stage (Chapters 1 and 2). Second, I examined whether *L. chinensis* escaped from disturbed conspecifics in an observation arena under laboratory conditions. Then, I analyzed the volatiles emitted by both disturbed and undisturbed *L. chinensis* and observed the responses of undisturbed *L. chinensis* to the components in the volatiles from disturbed conspecifics (Chapter 3). Finally, I tried this control method using the behavior regulator of *L. chinensis* in paddy fields and argued the significance of a new control method using the behavior regulator in *L. chinensis* (Chapter 4).

Chapter 1

Estimation of the number of annual generations using effective heat unit of development for the rice bug, *Leptocorisa chinensis* (Dallas)

Introduction

Understanding the life history of *L. chinensis* is an important prerequisite to understanding the population dynamics of the pest in the field when using the behavior regulator. With a detailed knowledge of the development of *L. chinensis*, it is possible to predict the population fluctuations of this pest and then construct an effective control program (Yao 2002). *Leptocorisa chinensis* invades paddy fields during heading, and the end result is pecky rice is caused by *L. chinensis* (Takeuchi et al. 2004a). Thus, it is important to predict and control *L. chinensis* invasions during the rice heading stage. In particular, the thermal requirements (day-degrees) for development are often used to estimate developmental periods because temperature has a major influence on the rate at which insects develop (Howe 1967; Zaslavski 1988; Gordon 1998). However, little is known about *L. chinensis* development.

The objectives of this study are to estimate the developmental threshold temperatures and heat unit requirements (degrees) for the development of the life history stages of *L. chinensis*. Such information could provide a means to construct a practical model of the development of *L. chinensis* to establish integrated management.

Materials and Methods

Insects

Adults of *L. chinensis* were collected from a paddy field in Hyogo, in September 2002. The insects were kept at 25°C under a 16L–8D photoperiod in a plastic cage (9 cm dia.×5 cm height) with defrosted rice and distilled water (Yamashita 2010).

Effect of temperature on development of eggs, nymphs and pre-oviposition

Developmental periods of *L. chinensis* eggs, nymphs and pre-oviposition were studied at four constant temperatures (±0.5°C): 18, 22, 25 and 30°C (16L–8D). Newly laid eggs (<24 h old) were collected from the adults of the stock culture mentioned above. Newly hatched nymphs (<24 h old) and newly emerged and mated female adults (<24 h old) were placed individually in the plastic cage as mentioned above. The bugs were provided with unhulled rice frozen at the milky stages every 2 d. All eggs and nymphs were checked at 24 h intervals for survival and the presence of exuviae, which was used to determine time of molting. A female and a male that emerged on the same day were confined in the plastic cage and maintained under the same conditions until the female laid the first egg.

Estimation of pre-oviposition period after overwintering

I attempted to estimate the preoviposition period after overwintering under two sets of conditions for adults of *L. chinensis*. Thirty-six adult males and 32 adult females were collected from fields just before they were placed in a cage (9 cm dia.×5 cm height) at a site in a paddy field in Kasai, Hyogo Prefecture from 5 November 2003 to 12 February 2004. The 19 pairs of adults that survived in the field were moved to cages (6 cm dia.×4 cm height) with one pair in the incubator at 25°C (16L–8D) in the laboratory, and whether females laid eggs was recorded every day.

On 9 November 2001, 27 adults that survived in the field were placed in cages (6 cm dia.×4 cm height), with one pair of adults in the incubator at 25°C (16L–8D) in the laboratory. Whether *L. chinensis* females laid eggs was also recorded every day.

Results

Table 1-1 shows the developmental period for eggs, nymphs and the pre-oviposition period of *L. chinensis*. The period for each stage decreased as the temperature increased from 18 to 30°C.

Table 1-1. Effects of temperature on developmental periods of eggs, nymphs and pre-oviposition of adults in *Leptocorisa chinensis*

Temp.	Eggs		Nymphs		Pre-ovispoition	
(℃)	d (mean±SD)	n[a]	d (mean±SD)	n[a]	d (mean±SD)	n[a]
18	14.9 ± 2.6	89	47.1 ± 1.9	81	29.5 ± 3.6	14
22	10.4 ± 1.2	34	31.5 ± 1.7	29	21.1 ± 2.3	11
25	8.7 ± 0.7	250	25.2 ± 2.5	142	17.3 ± 5.3	14
30	6.7 ± 0.7	197	18.7 ± 2.1	172	12.4 ± 3.2	20

[a] Number of individuals tested.

Table 1-2 shows the relationship between rearing temperature (*T*) and the rate of development of *L. chinensis*. Developmental rates for eggs, nymphs and pre-oviposition period increased linearly as the rearing temperature rose from 18 to 30°C. The rate of development of the different life history stages in relation to temperature is expressed by the linear regression equation ($Y=a\times bT$), where Y is the reciprocal of the number of days (= development rate) and T is temperature (°C) (Patel and Schuster, 1983). There was a significant linear relationship between temperature and development rates of eggs, nymphs and pre-oviposition. The developmental zeros in eggs and nymphs were at 8.1 and 10.1°C, respectively, and the effective heat units were 147 day-degrees and 370 day-degrees, respectively. The developmental zero and effective heat unit for the pre-oviposition period of nondiapause females were 9.6°C and 256 day-degrees, respectively.

Table 1-2. Regression equations of developmental rate (Y) to rearing temperature (T) in *Leptocorisa chinensis*, developmental zero and total effective heat units calculated from the regression equation

Stage	Regression equation	r^2	Developmental zero (°C)	Total effective heat units (day-degrees)
Egg	$Y=0.0068T-0.0552$	0.99^{**}	8.1	147
Nymph	$Y=0.0027T-0.0274$	0.99^{**}	10.1	370
Pre-oviposition period	$Y=0.0039T-0.0374$	0.99^{**}	9.6	256

**p<0.01

The cumulative percentage of ovipositing females collected on 9 November 2001 and 12 February 2004 gradually increased (Fig.1-1). All females had laid their eggs by 41 d after incubation. There was no significant difference in the days when egg laying started after incubation between the populations collected on 9 November 2001 (28.6 d±5.7 SD) and 12 February 2004 (33.3 d ±4.9 SD) (p>0.05, t-test). From these results, an effective heat unit of 469.7 day-degrees above 9.6°C, which is the threshold for females of non-reproductive diapause, was assumed to be equal to that of reproductive diapause, and was estimated to be required for the pre-oviposition period of overwintering females.

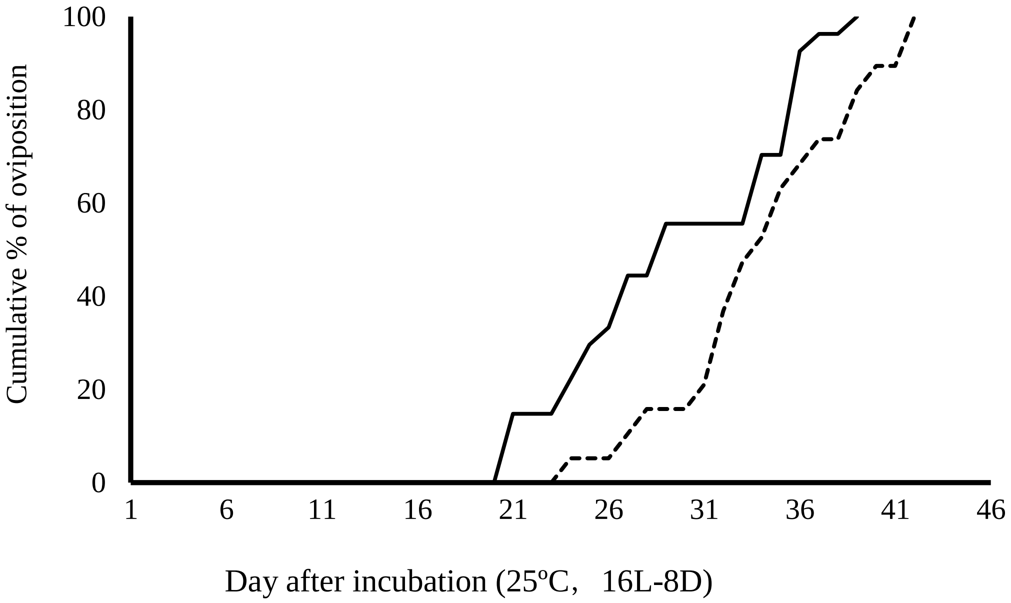

Fig. 1-1. Pre-oviposition period of female, *Leptocorisa chinensis* transferred from the field to laboratory conditions. Solid and dashed lines indicate females collected on 9 November 2001 and 12 February 2004, respectively.

Discussion

In *L. chinensis*, Hasegawa et al. (1976) reported that this bug overwinters as adults. In the present study, we recognized that all the adult females collected in winter gradually began to lay eggs after incubation (Fig. 1-1). An effective heat unit of 469.7 d-degrees was required for the pre-oviposition period of overwintering females, although the effective heat unit for the pre-oviposition period of undiapaused females was 256 day-degrees. We have never observed the level of egg maturation in overwintering females, but Hasegawa et al. (1976) observed that the females have no mature eggs in the winter. The same genus, *Leptocorisa oratorius* Fabricius is known to have reproductive diapause (Ito et al. 1993). It is considered that adult *L. chinensis* would undergo reproductive diapause in the winter. There was no significant difference in the timing of egg laying after incubation between the populations collected on 9 November 2001 and 12 February 2004. It is thought that the level of ovary maturity between both of the populations is the same. Since there was not much difference, the effective heat unit for the pre-oviposition period of *L. chinensis* females in the meteorological data for November to February from 2001 to 2004 was recorded with the AMeDAS system in Kasai City, Hyogo Prefecture. A more intensive experiment should be conducted for assessing the termination of reproductive diapause.

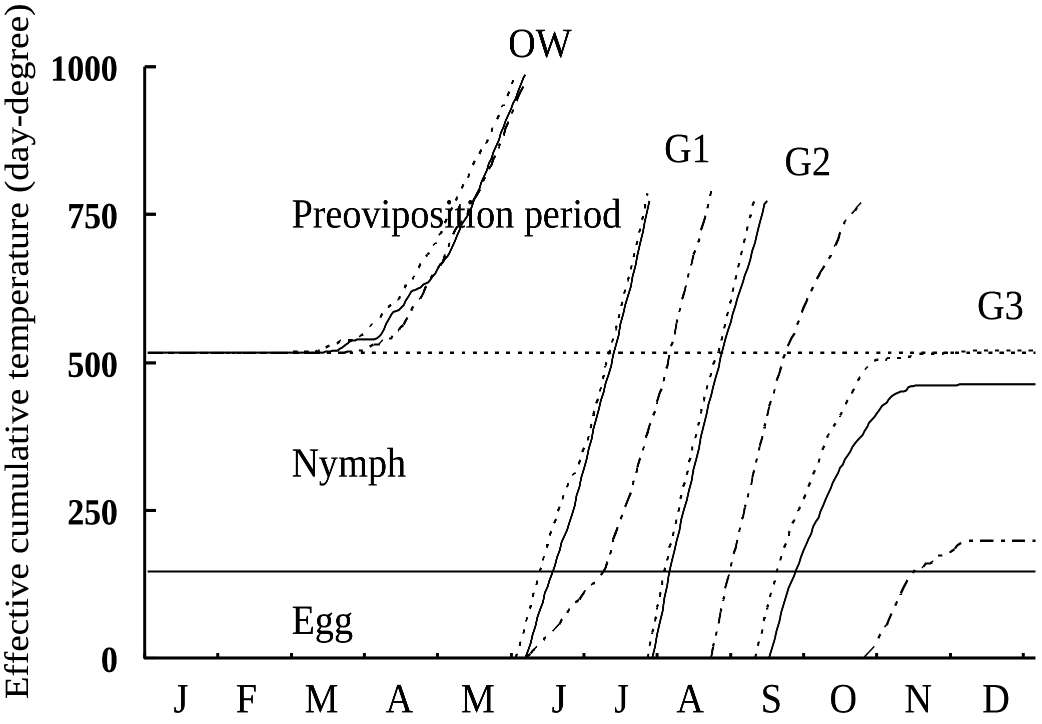

Fig. 1-2. Schematic seasonal advancement of *Leptocorisa chinensis* generations from 2001 to 2003 in Hyogo Prefecture, estimated from the developmental zero and effective heat units of eggs, nymphs, and the pre-oviposition period. OW: Overwintering generation, G1: First generation, G2: Second generation, G3: Third generation. 2001: solid lines, 2002: broken lines, 2003: single dotted lines.

Here, the number of annual generations of *L. chinensis* was estimated based on these data and the meteorological data from 2001 to 2003 recorded by the AMeDAS system in Kasai City, Hyogo Prefecture. The starting point for the calculation was 1 January. The results indicated that adults of the first generation and second generation emerged in mid-July and late August, respectively (Fig. 1-2). The possibility also showed that adults of the third generation would appear depending on temperature. In the earlier study, two peaks were observed in July and September in Shiga Prefecture (Hasegawa et al., 1976), and also in Hyogo Prefecture (Yamashita, unpublished) in the Kinki region. The present results are consistent with these reports, in that the number of annual generations of *L. chinensis* was two.

Earlier studies investigated the effects of constant temperature on the rate of development of *L. chinensis* reared on unhulled rice in milky stages every 7 d (Ishizaki et al., 2002). Developmental thresholds of 13.5, 12.0 and 18.0°C, and thermal constants of 92.4, 316.2 and 247.7 d-degrees were estimated for the eggs, nymphs and pre-oviposition period, respectively. Developmental rates obtained from those results did not provide a good estimate of the developmental times of populations in the field. A direct extrapolation of the results to the field did not fit the field fluctuation of *L. chinensis*. The development of *L. chinensis* reported by Ishizaki et al. (2002) was longer than that in the present results. The estimated lower developmental threshold temperatures recorded in the present study differed slightly from those estimated using the results of Ishizaki et al. (2002). These differences may be due to differences in the quality and quantity of food materials the tested insects consumed or/and differences in developmental thresholds between the geographic populations of *L. chinensis* from Hyogo and Ibaraki prefectures.

These results would be useful to estimate the occurrence of each stage of *L. chinensis*. However, to apply the developmental simulation to *L. chinensis*, further studies are needed for the measurement of another developmental parameter as well as different geographical populations in various climatic regions.

Chapter 2

Temperature and photoperiodic effects on induction and termination of diapause in female *Leptocorisa chinensis*

Introduction

In Chapter 1, the developmental threshold temperature (9.6°C) and the heat unit requirement (469.7 day-degrees) during the preoviposition period of female *L. chinensis* were estimated. It was also estimated that there are two or three annual generations. However, the field population phenology of *L. chinensis* was predicted from these results without considering the consequences of diapause. Both the induction and the termination of diapause in insects can influence the seasonal timing of growth and reproduction in the generation immediately after diapause and in subsequent generations (Tauber et al. 1986). Therefore, it is important to understand diapause induction and termination in *L. chinensis* to determine the field phenology. In a recent study, Tachibana and Watanabe (2007) reported that adult *L. chinensis* collected from Tsukuba (36.02°N, 140.10°E), Ibaragi Prefecture, Japan, undergo a winter reproductive diapause, and the critical day length for diapause induction was 13.75 h/d. However, they did not test the nymphs of the *L. chinensis* for photoperiodic conditions. The nymphal stage is one of the sensitive stages of insects that undergo reproductive diapause (de Wilde 1954, Tauber and Tauber 1976, Numata 2004, Danks 2007). In Japan, nymphal and adult *L. chinensis* occur in the fields in autumn when the day length is shorter than 13.75 h (Takeuchi et al. 2005, Yamashita et al. 2005, Tachibana and Watanabe 2007).

This chapter investigated the influence of photoperiod and temperature on the induction and termination of reproductive diapause in female nymphal and adult *L. chinensis.* These results contribute to understanding the life cycle of *L. chinensis* in the field and to the establishment of integrated management strategies to counteract this major pest to rice.

Materials and Methods

Temperature and photoperiodic effects during nymphal and adult stages on female *L. chinensis* survival and oviposition

A culture of *L. chinensis* was established from adults collected in a paddy field and neighborhood sites in Kasai (34.55°N, 134.59°E), Hyogo Prefecture, Japan, during October 2005. Before experimentation, the insect culture was maintained for two generations at 25 ± 0.5°C under a photoperiod of 16:8 (L:D) h in a plastic cage (9 cm dia.×5 cm height), with defrosted frozen rice panicles in the milk-ripe stage as food and distilled water for drinking (Yamashita, 2010). Randomly selected, newly emerged nymphs (<1 d old) from the third filial generation were maintained under either a constant long-day (16:8 [L:D] h) or short-day (12:12 [L:D] h) photoperiod at 25±0.5°C. Adults that emerged from each photoperiod group (<1 d old) were then randomly allocated to one of the two photoperiods at 25±0.5°C, 20±0.5°C, or 15±0.50.5°C, for a total of 12 experimental adult groups. The initial number of females per group was 11－24. All adults were reared as male-female pairs in plastic cups (6 cm dia.×4 cm height) sustained on frozen, then defrosted, milk-ripe stage rice, along with distilled water. Mortality and the number of females that oviposited were recorded daily for 100 d. Fisher exact probability test was used to determine the significance of the differences in the survival rate among the different conditions.

Photoperiodic effects on survival and postdiapause reproductive recovery of female *L. chinensis*

L. chinensis- cultures were established from adults collected from a wild grass habitat near a paddy field in Kasai, Hyogo Prefecture, Japan, on 8 December 2005. Until experiments began, they were maintained in an outdoor cage (9 cm dia.×5 cm height) on defrosted frozen rice in the milk-ripe stage and distilled water. These overwintering adults were transferred to the laboratory for experiments on 1 February and 29 March 2006, where they were randomly allocated into male-female pairs and maintained under either a constant long-day (16:8 [L:D] h) or short-day (12:12 [L:D] h) photoperiod at 25±0.5°C and sustained on defrosted frozen rice in the milk-ripe stage and distilled water for the duration of the experiment. The initial number of females was 10-12 per cohort. For comparison, field-collected adults from Kasai were transferred to the laboratory on 10 June 2005 and maintained under either a constant long-day (16:8 [L:D] h) or short-day (12:12 [L:D] h) photoperiod at 25±0.5°C. Mortality and the number of females that oviposited were recorded daily for the duration of the experiment (150 d).

Results

Temperature and photoperiodic effects during nymphal and adult stages on female *L. chinensis* survival and oviposition

The effects of temperature and photoperiod on survival and oviposition of adults reared as nymphs under a long-day photoperiod (16:8[L:D] h) are shown in Fig. 2-1. *L. chinensis* females started ovipositing on day 11 after adult emergence at 25°C under a long-day photoperiod, and >60% of the females oviposited during the experimental period (Fig. 2-1a). At 20°C under a long-day photoperiod, adults started ovipositing on day 21 after adult emergence, and the number of ovipositing females increased gradually (Fig. 2-1c). Females under a long-day photoperiod at 15°C laid no eggs, although they survived for the duration the experimental period (100 d; Fig. 2-1e). Females under a short-day (12:12 [L:D] h) photoperiod at 25°C started ovipositing on day 10 after adult emergence. They ceased ovipositing within a few days but survived for the duration of the experimental period (Fig. 2-1b). Females under a short-day photoperiod at 20 or 15°C laid no eggs (Fig. 2-1d and f).

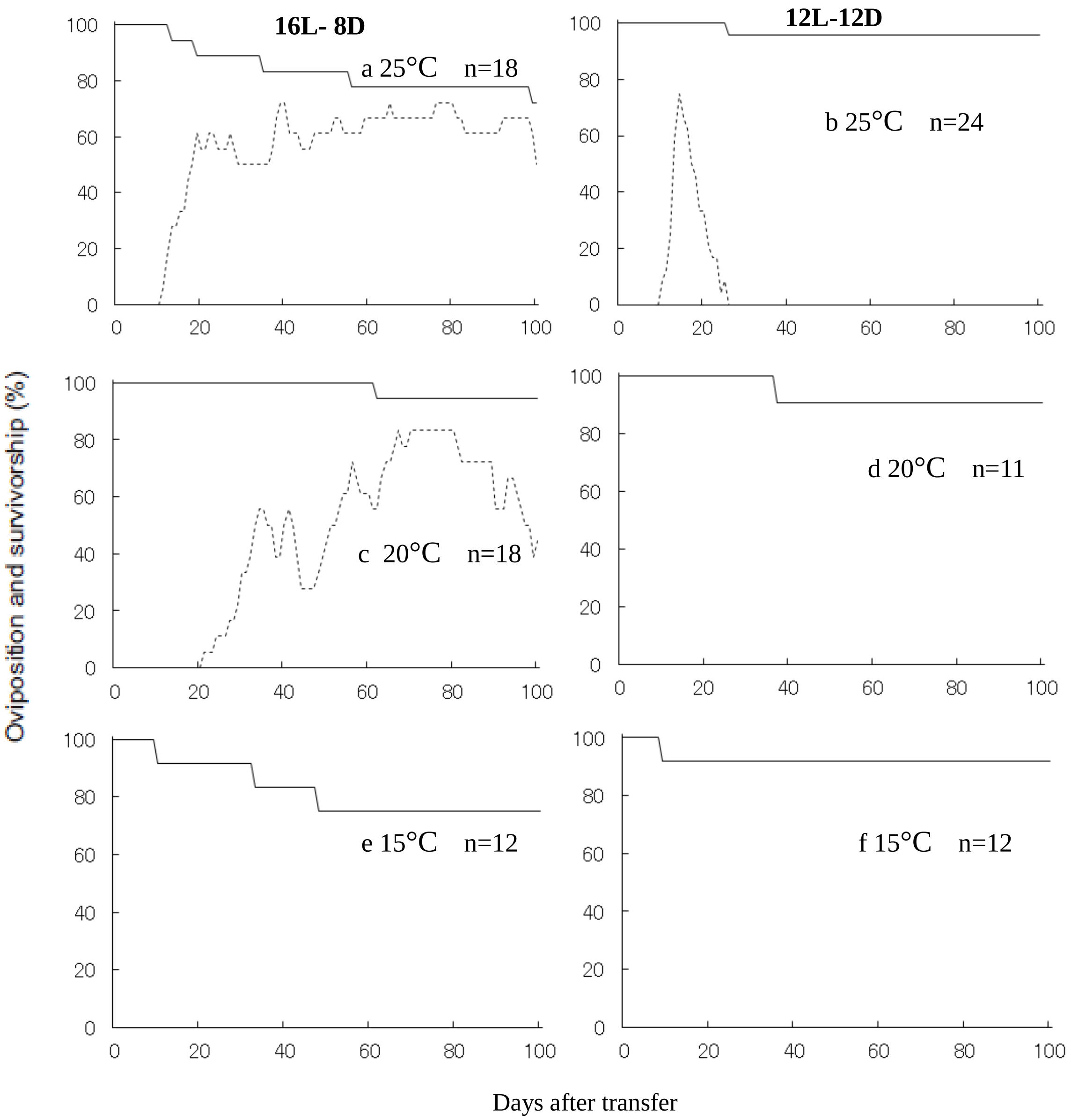

Fig. 2-1. Effects of temperature and photoperiod on survival and oviposition of adult female *L. chinensis* reared as nymphs under a long-day photoperiod of 16:8 (L:D) h under 25°C (a and b) , 20°C (c and d), and15°C (e and f). Solid and broken lines represent the percentage of adult female surviving and ovipositing, respectively, The initial number of newly emerged females (<1 d old) was 11-24 (n).

The effects of temperature and photoperiod on survival and oviposition of adults that were reared as nymphs under a short-day photoperiod (12:12 [L:D] h) are shown in Fig. 2-2. Females under a long-day photoperiod (16:8 [L:D] h) at 25°C started ovipositing on day 40 after the start of the experiment and the number of ovipositing females increased gradually (Fig. 2-2a). Under a long-day photoperiod at 20°C, a small number of females oviposited (Fig. 2-2c). However, no females oviposited under any other conditions (Fig. 2-2e and f).

There were no significant differences in the survival rate at day 100 between the females in each photoperiodic condition in the same temperature in Figs. 2-1 and 2 ($P >$ 0.05; Fisher exact probability test).

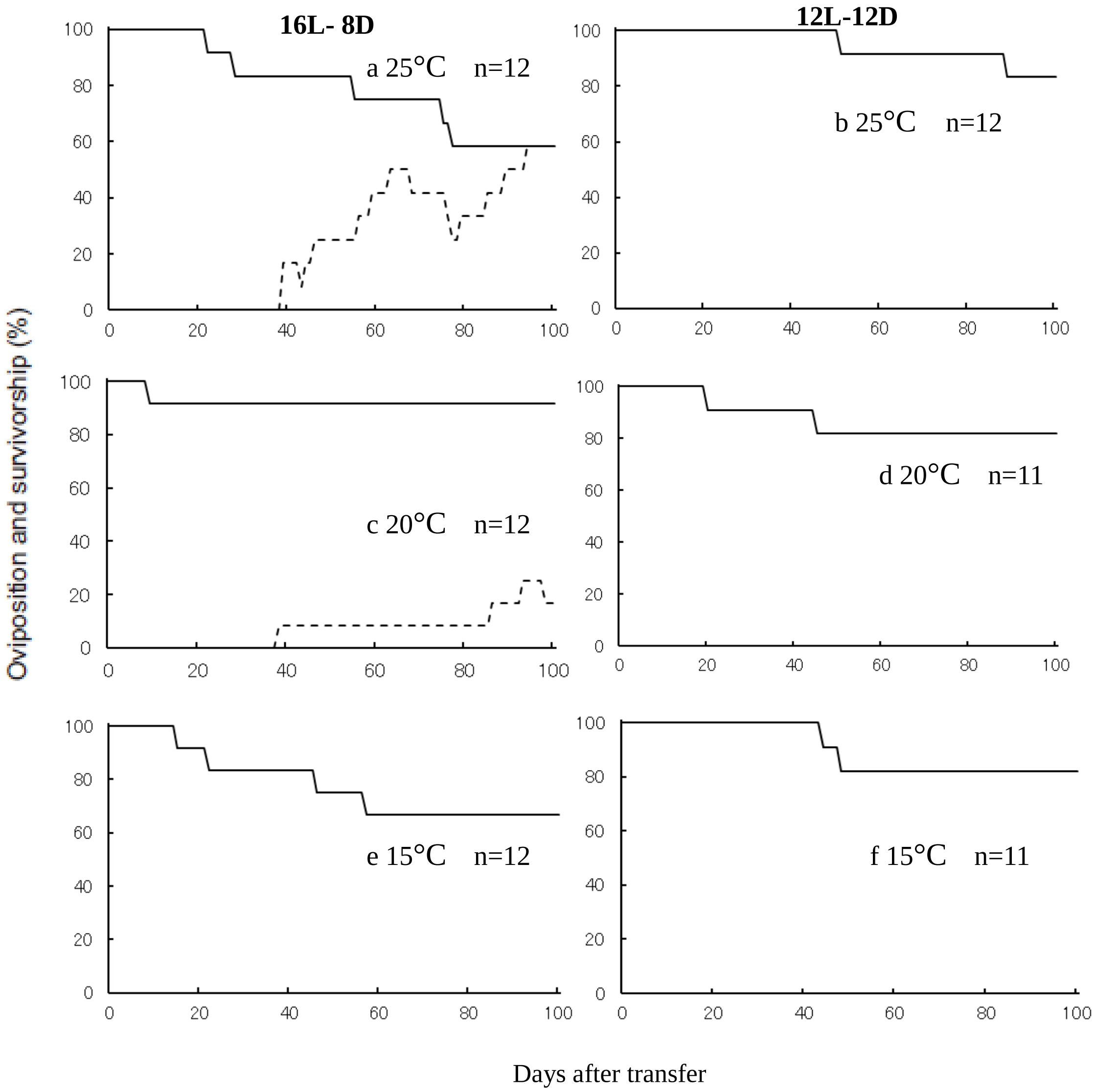

Fig. 2-2. Effects of temperature and photoperiod on survival and oviposition of adult female *L. chinensis* reared as nymphs under a short-day photoperiod of 12:12 (L:D) h under 25°C (a and b), 20°C (c and d), and 15°C (e and f). Solid and broken lines represent the percentage of adult female surviving and ovipositing, respectively. The initial number of newly emerged females (< 1 d old) was 11-24 (n).

Photoperiodic effects on survival and postdiapause reproductive recovery of female *L. chinensis*

Adult females transferred from the overwintering cage in the field to the long-day photoperiod at 25°C in the laboratory on both 1 February and 29 March commenced ovipositing day ≈40 after transfer (Fig. 2-3a and c). However, no adult females that were transferred on either date to the short-day photoperiod at 25°C oviposited for the duration of the experiment (150 d; Fig. 2-3b and d). Field-collected adult females that were transferred to a long-day photoperiod at 25°C in the laboratory on 10 June commenced oviposition almost immediately and continued to lay eggs for the duration of the experiment (Fig. 2-3e). Similarly, collected adult females transferred to a short-day photoperiod at 25°C in the laboratory on the same day commenced ovipositing immediately. However, after 14 d the number of ovipositing females gradually decreased (Fig. 2-3f).

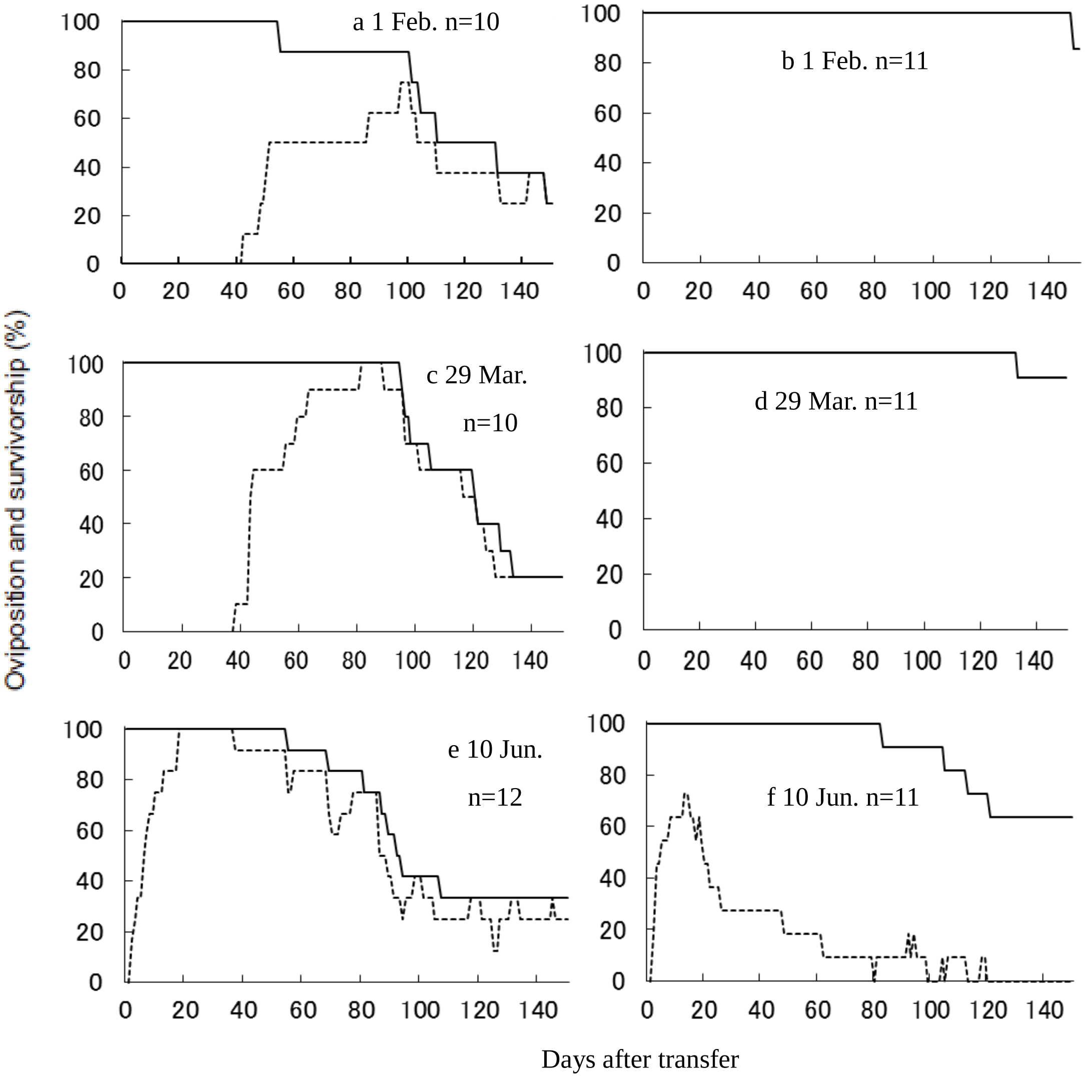

Fig. 2-3. Survival and oviposition of adult female *L. chinensis* transferred from field conditions to the laboratory and maintained at 25°C and either a 16:8 (L:D) h (left) or 12:12 (L:D) h (right) photoperiod. Solid and broken lines represent the percentage of adult female surviving and ovipositing, respectively. The initial number females was 10-12 (n). Survivorship and oviposition data reported in a-d are for adults collected on 8 December 2005 and in e and f for adults collected on 10 June 2005. Dates when adults were transferred to the laboratory conditions are shown.

Discussion

Nymphal and adult stages are the most sensitive to photoperiod and temperature in many insects that undergo reproductive diapause (de Wilde 1954, Tauber and Tauber 1976, Numata 2004, Danks 2007). In *L. chinensis,* oviposition curves differed between similarly maintained adults that had been reared as nymphs under differing photoperiod conditions (Figs. 2-1 and 2). Likewise, oviposition was influenced by differing photoperiod conditions during the adult stage of nymphs that were reared under the same conditions (Figs. 2-1 and 2). More specifically, adult females that had been reared as nymphs under the long-day photoperiod oviposited for only a short time under short-day conditions at 25°C (Fig. 2-1b). Females did not oviposit under a short-day photoperiod at 20°C, even when they were reared as nymphs under the long-day photoperiod (Fig. 2-1d). These findings suggest that short-day photoperiod conditions during the adult stage induce diapause in *L. chinensis* females. This is consistent with the findings of Tachibana and Watanabe (2007).

There were also differences in oviposition patterns between the adult stages of *L. chinensis* nymphs reared under the long-day and short-day photoperiods (Fig. 2-1a and c , Fig. 2-2a and c). Adults began ovipositing after ≈40 d under a long-day photoperiod, when they had been reared as nymphs under a short-day photoperiod (Fig. 2-2a and c).

Females required constant long-day photoperiod conditions to initiate oviposition, indicating that in *L. chinensis*, not only adult but also nymphal stages are sensitive in the process of reproductive diapause induction. Thus, both adults and nymphs can undergo a reproductive diapause. This is further supported by the fact that in Japan, both nymphs and adults can be found in the field when the daylength is shorter than the critical photoperiod of 13.75 h/d. (Takeuchi et al. 2005, Yamashita et al. 2005, Tachibana and Watanabe 2007). Thus, it was concluded that *L. chinensis* females exhibit a facultative adult diapause and that photoperiod controls its' induction and termination, confirming the results of Tachibana and Watanabe (2007).

There were also differences in oviposition patterns between the adult stages of *L. chinensis* nymphs reared under the long-day and short-day photoperiods (Figs.2-1a and c and 2a and c). Adults began ovipositing after ≈40 d under a long-day photoperiod, when they had been reared as nymphs under a short-day photoperiod (Fig. 2-2a and c).

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critical photoperiod of 13.75 h/d. (Takeuchi et al. 2005, Yamashita et al. 2005, Tachibana and Watanabe 2007). Thus, it was concluded that *L. chinensis* females exhibit a facultative adult diapause and that photoperiod controls its' induction and termination, confirming the results of Tachibana and Watanabe (2007).

The results of the transfer experiments also indicate *L. chinensis* females exhibit a facultative adult diapause. For example, when transferred to 25°C in the laboratory, field overwintering adult females did not commence oviposition when maintained under short-day conditions but commenced ovipositing after ≈40 d under long-day photoperiod conditions (Fig. 2-3). Moreover, field-collected, post overwintering (June) female adults maintained under short-day conditions did not oviposit as readily or as consistently as those maintained under the long-day photoperiod at 25°C (Fig. 2-3).

The termination of diapause in *L. chinensis* might also be infuenced by temperature. When females were reared as nymphs under a short-day photoperiod, and maintained as adults under a long-day photoperiod, oviposition began after ≈40 d, but the percentage that oviposited over the experimental period of 100 d was lower at 20°C than at 25°C (Fig. 2-2a and c). Females did not lay any eggs at 15°C regardless of the photoperiod conditions. Tachibana and Watanabe (2007) assumed that reproductive development after diapause in female *L. chinensi* would resume under photoperiods longer than the critical photoperiod and when temperatures exceeded a given high, although females never began oviposition during the 30-d experiments. In our study, field overwintering adult females transferred to 25°C in the laboratory did not commence oviposition when maintained under short-day conditions but did so under long-day conditions after ≈40 d (Fig. 2-3a and c). These results suggest that, to commence oviposition after winter, female *L. chinensis* require a long-day photoperiod and ≈40 d at temperatures >20°C.

Oviposition patterns for the 1 February cohort were basically the same as those for the 29 March cohort when they were transferred to both short-and long-day photoperiod conditions (Fig. 2-3a and d). These results indicate that changes in photoperiod or temperature did not terminate diapause in *L. chinensis* females by 29 March. However, by 10 June, adult female *L. chinensis* were able to oviposit readily when transferred to long-day photoperiod conditions and had thus recovered their reproductive ability after diapause. The females also oviposited for a short period when they were transferred to short-day photoperiod conditions, but the number of ovipositing females gradually decreased (Fig. 2-3f). This pattern was similar to that of adult females reared as nymphs under a long-day photoperiod and then held under short-day photoperiod at 25°C (Fig. 2-1b). These findings indicate that *L. chinensis* females remain sensitive to photoperiod, even after winter.

Additional studies are needed to evaluate the developmental parameters for field populations of *L. chinensis* in various climatic regions. Furthermore, in the current study, the diapause of *L. chinensis* was evaluated using oviposition and survival data without examining the condition of the ovaries and fat body. To get more information about overwintering of *L. chinensis*, condition of these organs also should be studied.

Chapter 3

A pecky rice-causing stink bug *Leptocorisa chinensis* escapes from volatiles emitted by excited conspecifics

Introduction

Disturbed stink bugs emit pungent volatile compounds that could have several ecological functions including behavior regulation and defense against predators (Blum 1985; Aldrich 1995). The behavior regulator function might be used for the behavioral control of stink bugs in agroecosystems. The volatiles from smashed bugs likely repel other stink bugs in the area. We hypothesized that the volatiles emitted from disturbed *L. chinensis* could be used to repel conspecifics in rice fields and reduce the number of pecky rice grains.

Leal et al. (1996) identified the volatiles emitted from *L. chinensis* anaesthetized with CO_2 to minimize the release of defensive secretions, and found that a 5:1 mixture of (*E*)-2-octenyl acetate and octanol, major components of the emissions of undisturbed *L. chinensis* was an attractant pheromone. However, the chemistry and ecological functions of the volatiles from disturbed *L. chinensis* individuals have not yet been studied.

The objective of this study was to clarify whether the volatiles from disturbed *L. chinensis* elicit excitement and escape behavior in conspecifics as a first step to test the above hypothesis. We first investigated whether *L. chinensis* escaped from disturbed conspecifics in an observation arena under laboratory conditions. We then analyzed the volatiles emitted by both disturbed and undisturbed *L. chinensis* and observed the responses of undisturbed *L. chinensis* to the components in the volatiles from disturbed conspecifics.

Materials and methods

Insects

A colony of *L. chinensis* was established from adults collected from paddy fields in Kasai, Hyogo Prefecture, Japan, in October 2005, and maintained for two generations prior to the experiments. Males and females were kept in a plastic cage (9 cm dia.×5 cm height) in a climate controlled room at 25 ± 0.5°C under a photoperiod of 16L:8D, with distilled water in a small Petri dish and rice panicles (ca. 50 mm) in the milk-ripe stage as food. After mating occurred in the cage, I used them for the experiments. For chemical analyses and bioassays, an individual bug 3–15 days old was used only once.

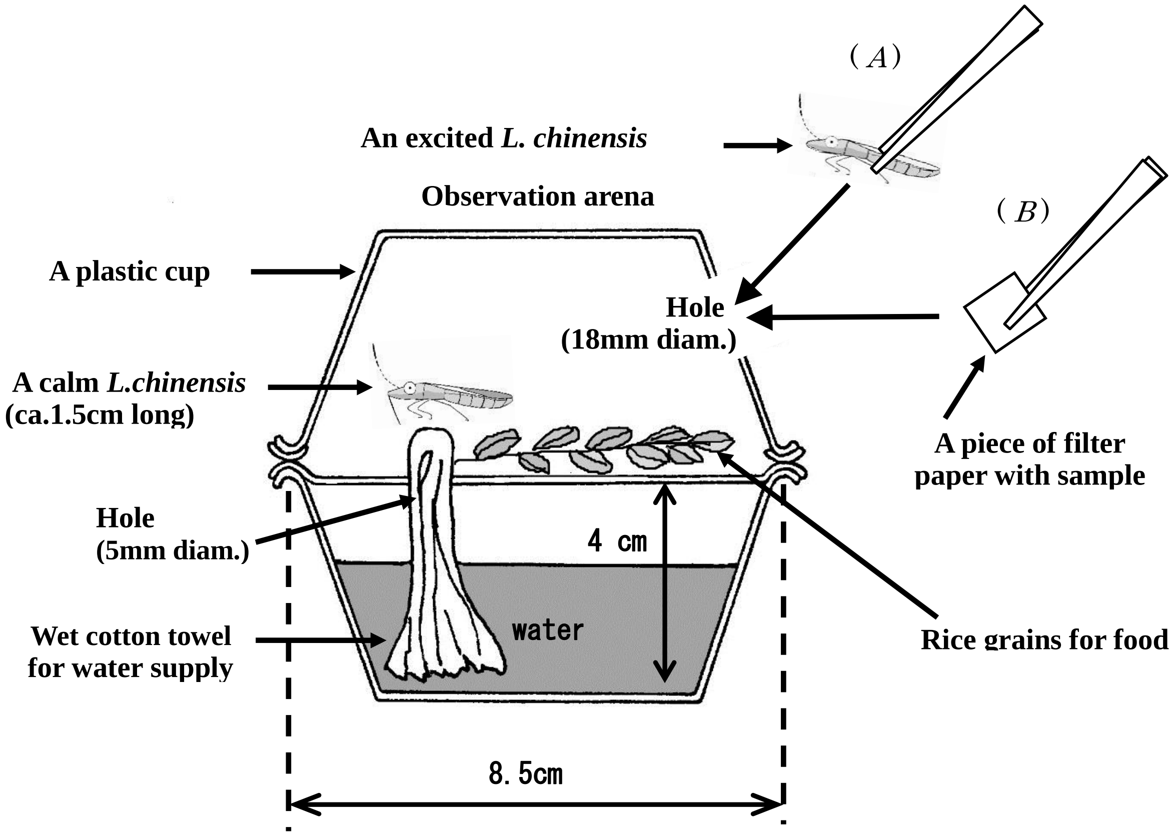

Fig. 3-1. Bioassay set-up to evaluate the responses of *Leptocorisa chinensis* to alarm odours . An undiaturbed adult was introduced into an observation arena made from two plastic cups (85mm diameter 40mm height), and then either (*A*) an *L.chinensis* or (*B*) a piece of filter paper imoregnated with a synthetic chemical was introduced through the hole (18mm diameter).

Bioassays

The bioassay set-up was constructed to evaluate the excitement and escape behaviour of undisturbed *L. chinensis* to cues from excited conspecifics (Fig. 3-1). Water (60 cc) was provided in a lidded plastic cup (85 mm diameter, 40 mm height). The lid had a 5-mm-diameter opening, into which a piece of wet cotton towel was inserted. I placed 10 rice seeds at the milk-ripe stage on top of part of the lid as food. I covered the lid with an inverted plastic cup of the same size with one escape hole (18 mm diameter) at the side. I called the inside of the inverted plastic cup the observation arena.

To introduce an undisturbed individual into the arena, we carefully transferred one *L. chinensis* from a rearing cage to a test tube (18 mm diameter, 120 mm length), and the opening of the test tube was connected to the opening in the inverted plastic cup to allow the bug (initial occupant) to enter the arena. When the bug stopped moving, it was considered undisturbed. For the control experiment, we introduced an undisturbed bug into the arena in the same way. For experimental treatments, we introduced an individual of the same gender using forceps with gentle nipping to disturb it and cause it to emit the pungent odour. The two individuals were distinguishable because the second, newly introduced one was already excited. After the introduction, the behavior of the initial occupant was observed for 3 min. When the occupant raised its antennae to scan the air and started walking actively, it was judged to be excited. When it left the arena through the hole, we judged that it had escaped. We measured the duration of time until it showed excitement and escape behaviour. The introduced disturbed individual was actively walking in a circle on the ceiling of the arena. During the observation, the two individuals did not interact in the arena.

To further test the ecological functions of the pungent volatiles from *L. chinensis*, I conducted the same bioassays using the dominant chemical components. A pure compound (1 or 10 μg) was applied to a piece of filter paper (10×10 mm^2) and introduced into the observation arena containing an undisturbed *L. chinensis* through the opening (Fig. 1-1). The amounts of pure compound [(*E*)-2- octenal] were determined based on chemical analyses: the amounts released by one disturbed female per minute were ca. 4.5–14μg . We measured the duration of time until the bug showed excitement and escape behaviour. The bioassays were conducted in a climate-controlled room (25 ± 3°C, 50–60% relative humidity; during 10:00–16:00). I repeated each experiment, 5–10 times per day on 2–4 experimental days.

Then the responses of males and females of *L. chinensis* were tested to (*E*)-2-octenal at different concentrations to identify the optimal airborne levels needed to excite the bugs. In this study, we needed (*E*)-2-octenal to volatilise slowly to measure the air concentrations, so we dissolved (*E*)-2-octenal in methanol (10% v/v) and then diluted with distilled water to make 0.1, 0.01, and 0.001% solutions. For each bioassay, we used 1 mL solution impregnated into a piece of moist cotton wool (2 cm×2 cm×0.5 cm) on the same size of aluminium foil. We conducted experiments in a mesh cage (30 ×30 × 50 cm^3: 2 mm mesh) with an electric fan (flow rate 50 cm/s; SY124010L, 40 mm × 30 mm × 10 mm thickness; Size Corporation, Tokyo, Japan) in the centre of the cage. We carefully introduced 4–5 undisturbed *L. chinensis* (either males or females) into the mesh cage as described above. When the bugs stopped moving, they were considered undisturbed. We then placed a piece of impregnated cotton wool on the fan. We observed the flight behavior for 5 min. In our preliminary behavioural observations of both sexes in the mesh cage, we confirmed that flying individuals did not elicit any behavioural responses in undisturbed individuals. For both males and females, we conducted the experiments 4 times (0.1% solution and 0.01% solution) and 3 times (0.001% solution) on 1–3 experimental days in a climate-controlled room (25 ± 3°C, 50–60 % relative humidity; during 10:00–16:00).

Volatile collection and chemical analysis

Either a male or a female *L. chinensis* was used for volatile sampling. For collection from a disturbed individual, it was nipped with forceps. Immediately afterwards, it was put in a 200-mL glass bottle with an air inlet and outlet (55 mm diameter, 110 mm height). For the collection of volatiles from an undisturbed individual, it was allowed to walk into the volatile collection vial from the rearing cage. An undisturbed individual was motionless, walking, or flying in the bottle. Volatile collections were done on four individuals per gender and treatment during daytime (25±3°C, 50–60 % relative humidity; during 10:00–15:00).

The headspace volatiles in the glass bottles were collected for 1 min (disturbed adult) or 10 min (undisturbed) at flow rate of 100 mL/min using Tenax adsorbent in a glass tube (Tenax TA 20/35 100 mg; 3-mm inner diameter (ID), 160 mm long; GL Science, Tokyo, Japan). The volatile collection time was determined based on preliminary chemical analyses. The trapped compounds from disturbed adults were eluted with 2 mL *n*-hexane (Wako Pure Chemical Industries Ltd.) containing *n*-eicosane (0.5 μg; Wako Pure Chemical Industries Ltd.) as internal standard for the recovery rate. The eluate was concentrated by nitrogen gas flow to 10 μl. One microliter of concentrated eluate was injected into the injection port (250°C) of a gas chromatograph–mass spectrometer (GC–MS; GC: Agilent 6890 with HP-5MS capillary column: 30 m long, 0.25 mm i.d. and 0.25 μm film thickness; MS: Agilent 5973 mass selective detector, 70 eV with He as carrier gas; Agilent, Santa Clara, CA, USA). The GC oven temperature was programmed to rise from 40°C (5 min hold) to 280°C at 15°C/min. The compounds were identified by comparing their mass spectra and retention times with those of authentic compounds and quantified using a calibration curve of the respective compound.

Air in the flight cage with volatilised (*E*)-2-octenal was collected for 3 min at flow rate of 100 mL/min using Tenax adsorbent in a glass tube [Tenax TA 20/35 100 mg; 3-mm inner diameter (ID), 160 mm long]. The collections were repeated four times for each of the four concentrations. The volatile collection time was determined based on preliminary chemical analyses. The collected volatile compounds were analysed by GC–MS as described above, except for the injection method. The GC–MS was equipped with a thermal desorption cold trap injector (TCT; CP4010, Chrompack, The Netherlands). Headspace volatiles collected on Tenax-TA were released in the TCT thermal desorption unit at 220°C for 8 min in He flow. The desorbed compounds were collected in the TCT cold trap unit (SIL5CB-coated fused silica capillary) at -130°C. Flash heating of the cold trap unit provided sharp injection of the compounds into the capillary column of the GC.

Chemicals

Hexyl acetate, octyl acetate, octanal, (*E*)-2-octenal and octanol were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. (*E*)-2-Octenyl acetate and (Z)-2-octenyl acetate were synthesised by Sumika Technoservice Corporation (Hyogo, Japan).

Statistical analyses

The amounts of each volatile compound emitted from males and females were compared by t test. The time durations needed for excitation and escape were tested by the Kaplan–Meier time-to-event model using a log-rank test statistic. All statistical tests, except multiple-comparison tests, had a significance of 0.05. For multiple comparisons of duration, we adjusted the significance according to Holm's sequentially rejective Bonferroni test to reduce type I errors. The multiple comparisons involved three log-rank tests, therefore the lowest of the three P values was compared with a = 0.0167 (0.05/3), the second lowest with $\alpha = 0.025$ (0.05/2), and the third lowest with $\alpha = 0.05$ (0.05/1). The proportions of flight responses of *L. chinensis* to various concentrations of

(*E*)-2-octenal were analysed using two-way analysis of variance (ANOVA) with factors concentration and sex, and their interaction. The data of the flight proportion were arcsine square root transformed before two-way ANOVA, and were weighted in the analysis by the number of individuals released into the mesh cage. All statistical analyses were conducted using the JMP software package (version 9.0.2; SAS Institute, Cary, NC, USA).

Results

Response of *L. chinensis* to volatiles emitted from conspecifics

Following the introduction of a disturbed same-sex conspecific, all undisturbed females (n = 19) and males (n = 16) exhibited excitement within 40 s (Fig. 3-2a) and escaped the arena within 3 min (Fig. 3-2b), with no differences between the sexes (P = 0.14 and P = 0.72 log-rank test, respectively). When an excited individual touched the hole (18 mm diameter) with antennae, the individual immediately walked out of the arena through the hole and flew to the fluorescent lights on the ceiling. In contrast, addition of an undisturbed same-sex conspecific elicited no excitement or escape behaviours in the first adult (Fig. 3-2a, b).

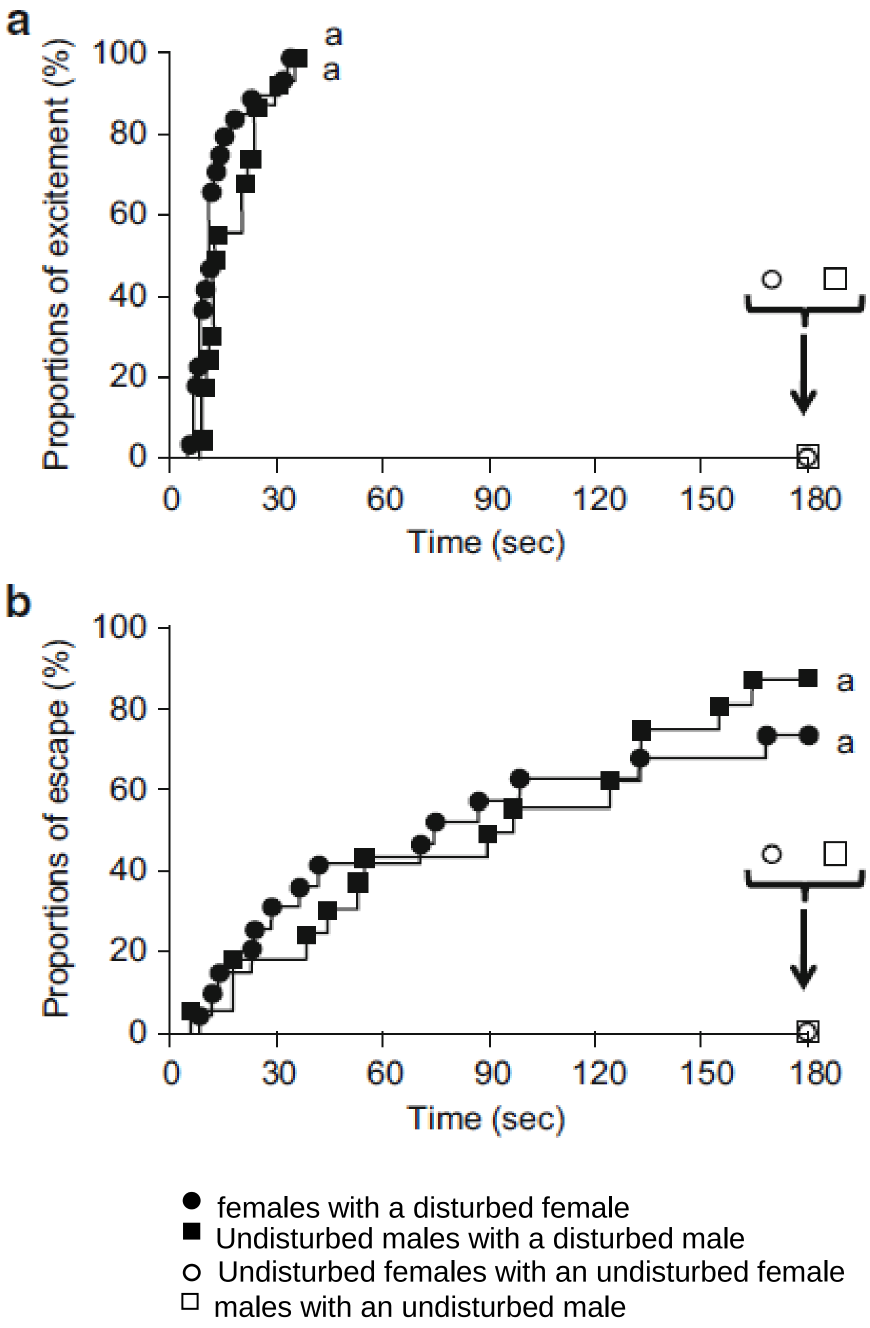

Fig. 3-2. Proportions of excitement and escape of undisturbed *Leptocorisa chinensis* females (*n* = 19) and males (*n* = 16) when exposed to undisturbed or disturbed conspecifics of the same gender. *Lines* with the same *lower-case letter* are not significantly different (Holm’s sequentially rejective Bonferromi test after Kaplan-Meier time-to-event model using a log-rank test statistic, P<0.05). Females (*n* = 19) and males (*n* = 16) exposed to an undisturbed individual showed no excitement/escape behavior.

Chemical analyses of volatiles emitted from *L. chinensis*

Volatiles from undisturbed males and females were below detectable levels (data not shown). When disturbed, both male and female *L. chinensis* adults (n = 4) emitted hexyl acetate, octyl acetate, (*Z*)-3-octenyl acetate, (*E*)-2-octenyl acetate, octanal, (*E*)-2-octenal and octanol (Table 3-1). Females emitted significantly higher amounts of hexyl acetate, octyl acetate, (*E*)-2-octenyl acetate, (*E*)-2-octenal and octanol than males (*t* test) (Table 3-1).

Table 3-1. Amounts of volatile compounds recorded in headspace of disturbed male and female *Leptocorisa chinensis* (*n* = 4)

Compound	ng (relative amounts %)		P value(*t* test)
	Male	Female	
Hexyl acetate	27±14 (0.5)	193±40 (1.2)	0.007
Octyl acetate	273±86 (5.5)	1170±347 (7.1)	0.046
(Z)-3-octenyl acetate	23±12 (0.4)	82±27 (0.5)	0.09
(*E*)-2-octenyl acetate	41±31 (0.8)	590±198 (3.6)	0.03
Octanal	22± 6 (0.4)	29± 3 (0.2)	0.31
(*E*)-2-octenal	4536±1191 (92.0)	14341±2208 (87.0)	0.008
Octanol	10±10 (0.2)	72±16(0.4)	0.02

Response of *L. chinensis* to synthetic compounds of volatiles emitted from a disturbed individual

I studied the responses of *L. chinensis* females to three volatile compounds, i.e. octyl acetate, (*E*)-2-octenyl acetate and (*E*)-2-octenal, which were predominant in volatiles emitted from a disturbed male and/or female (Table 3-1). The numbers of individuals tested for each compound is shown in Fig. 3-3. Octyl acetate was the least active in eliciting excitement/escape behaviour at two doses (Holm’s sequentially rejective Bonferroni test after logrank test) (Fig. 3-3a, b). At 1-μg dose, no individuals escaped from the arena when offered octyl acetate, while 60–90% of individuals escaped when offered (*E*)-2-octenyl acetate or (*E*)-2-octenal with no significant differences between the compounds (P = 0.29, log-rank test) (Fig. 3-3c). At the 10-μg dose, the proportion of escape for octyl acetate was significantly lower than for the other two compounds, while there were no significant differences between (*E*)-2-octenyl acetate and (*E*)-2-octenal (Holm’s sequentially rejective Bonferroni test after log-rank test) (Fig. 3-3d).

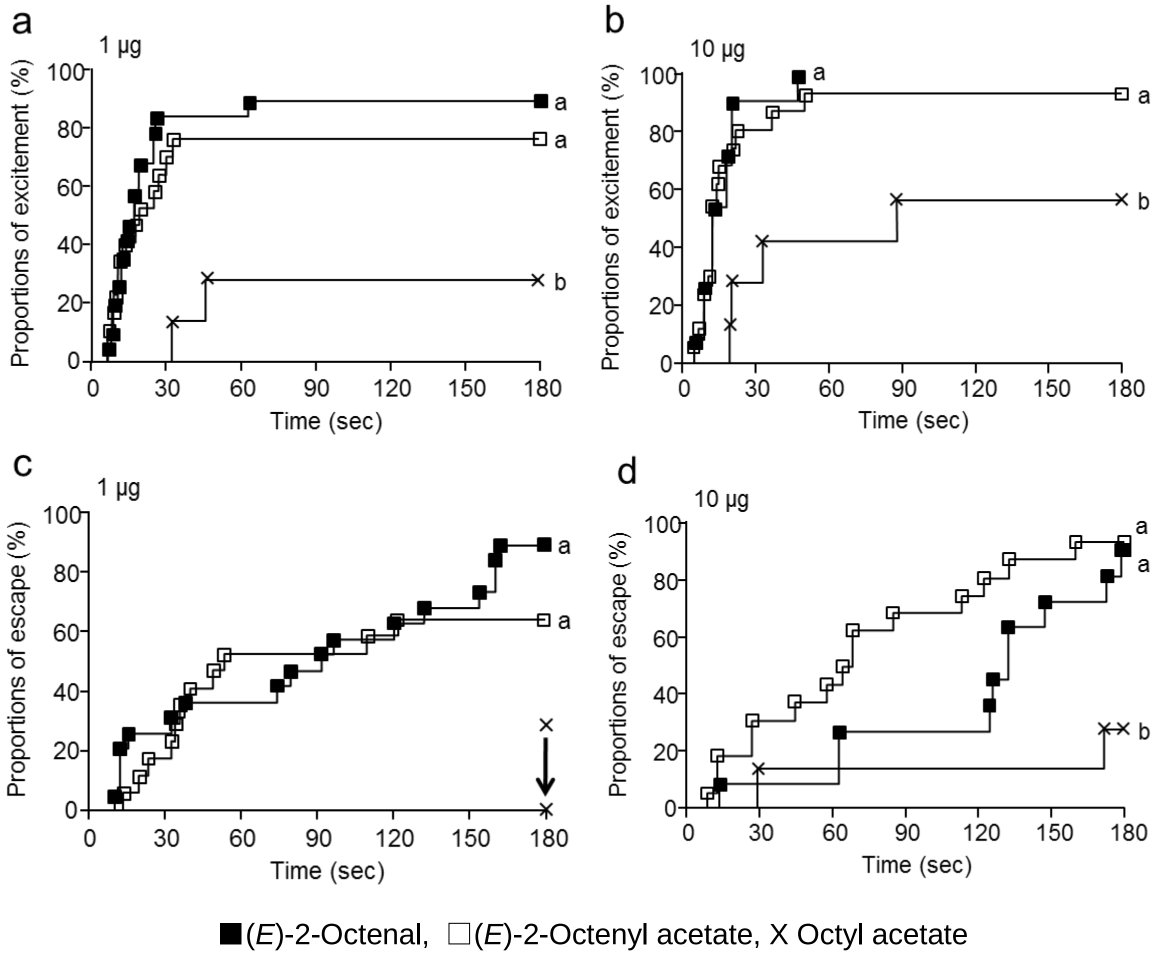

Fig. 3-3. Proportions of excitement (**a, b**) and escape (**c, d**) of undisturbed *Leptocorisa chinensis* female when exposed to synthetic chemicals at different concentrations of (**a, c**) 1 µg and (**b, d**) 10 µg. *Lines* with the same *lower-case letter* are not significantly different (Holm's sequentially rejective Bonferroni test after Kaplan–Meier time-to-event model using a log-rank test statistic, P<0.05). n = 19 for (*E*)-2-octenal, 17 for (*E*)-2-octenyl acetate, 7 for octyl acetate

Concentration of (*E*)-2-octenal in air needed to induce escape behaviour in *L. chinensis*

Males started showing flight behaviour when exposed to 0.001% solution of (*E*)-2-octenal, while females started showing flight behaviour when exposed to 0.01% solution (Fig. 3-4). The solution concentration significantly affected the flight behaviour ($F_{2,16}$ = 10.19, P = 0.001). However, effects of sex and the interaction (concentration 9 sex) were not significant (sex: $F_{1,16}$ = 10.13, P = 0.72; interaction: $F_{2,16}$ = 2.44, P = 0.30). The headspace analyses of air in the cage showed that 0.01 and 0.1% solutions of (*E*)-2-octenal resulted in 1.5 ppbV and 9.3 ppbV, respectively. The concentration of (*E*)-2-octenal with the 0.001% solution was below the detectable level.

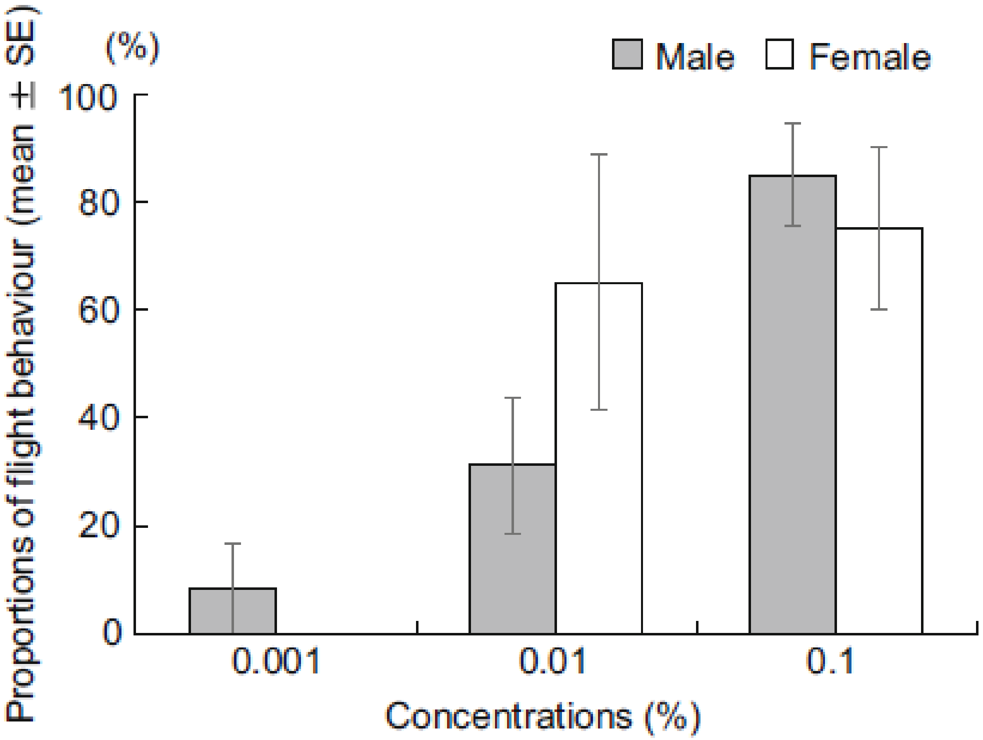

Fig. 3-4. Proportions of flight behavior of undisturbed *Leptocorisa chinensis* males and female in mesh cage when offered emulsified (*E*)-2-octenal at different concentrations

Discussion

Undisturbed males and females of *L. chinensis* became excited and escaped from the arena when exposed to previously excited individuals of the same gender. Their behaviours suggested that they responded to volatiles emitted from disturbed conspecifics. Alternatively, visual/physical cues, such as walking and sounds associated with movement, might have affected the excitation and escape behaviour. To clarify the effects of the volatiles, we conducted chemical analyses and tested the effects of the volatile components on the behaviour of *L. chinensis.*

Most of the volatiles found from the disrupted individuals were C8 aldehydes, alcohol and acetates. The major compounds found in the headspace of disturbed males and females were (*E*)-2-octenal and octyl acetate. (*E*)-2-Octenyl acetate was also one of the major compounds in the headspace of disrupted females. Gunawardena and Bandumathie (1993) reported that the chemical compositions of defensive secretions produced by disturbed *Leptocorisa oratorius* males and females were similar; the two major components in both were (*E*)-2-octenal (76% v/v) and octyl acetate (16%w/w). In this study, in total, females emitted～3 times more volatiles than males. A similar trend was reported in disturbed and undisturbed *Lygus lineolaris* (Wardle et al. 2003).

The relative amounts of (*E*)-2-octenal and (*E*)-2-octenyl acetate were 87–92% and 0.8–3.6%, respectively. Thus, we concluded that (*E*)-2-octenal was one of the major factors eliciting the excitement and escape behaviour in *L. chinensis* females in the arena when a disturbed individual of the same gender was introduced. No significant differences in escape proportions were observed between males and females (Fig. 3-2b), so we hypothesised that (*E*)-2-octenal was also a major factor eliciting excitement and escape behaviour in *L. chinensis* males. This conclusion was supported by the bioassays using emulsified (*E*)-2-octenal in the mesh cage.

The proportions of escape in response to a disturbed female and to synthetic volatile compounds [(*E*)-2-octenal and (*E*)-2-octenyl acetate (1-μg dose)] were not significantly different ($P = 0.23$, log-rank test). The low activity of hexyl acetate suggested that the location of the double bond at the (*E*)-2 position was more important than the presence of the aldehyde group. Further studies are needed to evaluate this idea. Further, we did not test the effects of minor compounds (less than ca. 1% in the blends from both genders: hexyl acetate, (*Z*)-3-octenyl acetate, octanal and octanol) on excitement and escape behaviours. Studies on the ecological functions of such volatiles are needed as well.

Leptocorisa chinensis males are strongly attracted to a 5:1 mixture of (*E*)-2-octenyl acetate and octanol (Leal et al. 1996; Watanabe et al. 2009; Fukatsu et al. 2012). These compounds were also detected from males and females at ratios of approximately 2:1 and 20:1, respectively, in our study. Leal et al. (1996) also reported that a whole blend did not attract males and the attractiveness of a binary mixture decreased with addition of (*Z*)-3-octenyl acetate. In this study, we also detected (*Z*)-3-octenyl acetate as one of the minor compounds. The attractiveness of

(*E*)-2-octenyl acetate and octanol emitted from a disrupted adult, if any, would have been hampered by the different ratios and/or by the presence of other compounds, such as (*Z*)-3-octenyl acetate.

Leal et al. (1996) identified compounds found in this study as well as nonanal and (*E*)-2-octenol in the headspace and hexane extracts of both male and female *L. chinensis* that were anaesthetised with CO_2 to minimize the release of defensive secretion. CO_2-induced anaesthesia might have resulted in production of small amounts of volatile compounds. Our inability to detect volatiles from undisturbed males and females was probably due to differences in collection methods.

As mentioned in the ‘‘Introduction,’’ in Sri Lanka, farmers protect rice plants by putting smashed adult stink bugs, including *Leptocorisa* species, around their fields (Yamashita, personal communication). Our data suggest that the volatiles from these smashed bugs would have repelled stink bugs from the agricultural fields. In this study, we clarified that (*E*)-2-octenal and (*E*)-2-octenyl acetate are involved in the excitement/escape behavior of *L. chinensis.* Continual release of synthetic (*E*)-2- octenal in paddy fields at concentration of ca. 2 ppbV or higher is expected to protect rice grains from *L. chinensis* damage during critical stages of the growing season. I in chapter 4 will report on field experiments.

Chapter 4

Evaluation of the behavior regulator of *Leptocorisa chinensis* to control this species in paddy fields

Introduction

The previous chapter outlines the possible use of volatile compounds eliciting excitement/escape behavior in *L. chinensis* to control this species in paddy fields (Chapter 3). The life history of *L. chinensis* has also been estimated to predict and control *L. chinensis* invasion during the rice heading stage (Chapters 1 and 2).

However, to the best of our knowledge, the efficacy of the behavior regulator of *L. chinensis* against the control of this species has not yet been investigated in paddy fields. The aim of the present study was to establish a program for the pest control of *L. chinensis* in paddy fields using a behavior regulator of *L. chinensis*. To achieve this goal, we investigated the effectiveness of the behavior regulator of *L. chinensis* in paddy fields.

Materials and Methods

We conducted behavior regulator release experiments in two open paddy fields at Aogaki (35.3° N, 135.0° E) and Kasai (34.9° N, 134.8° E), Hyogo, Japan, during

August and September 2006. The experimental details are summarized in Fig.4-1, We conducted two release experiments, trials 1 and 2. Each experiment involved two plots, one in which behavior regulators were released and a control plot that received no treatment (Fig. 4-1). Two paddy fields were transplanted with seedlings at intervals of 18 to 20 days from mid-May. Each field had an area of 350–600 m^2 and adjoined the other, and the rice variety was Koshihikari. No insecticides were used in any of the fields throughout the study. A fixed plot within each field of about 288 m^2 (12 m × 24 m) was used for a routine population census. The routine population census was conducted from August to September. The number of *L. chinensis* and peck rice were systematically counted in 49 hills per plot on each census date. The concentration of the behavior regulator was also estimated using Twister (Gerstel, M¨ulheim and der Ruhr, Germany; 1 mm film thickness × 10 mm length), which uses magnetic stir bars coated with polydimethylsiloxane, in each plot on each census date. The Twister was set as shown in Fig.4-1. After one day, the Twister was taken to the laboratory and analyzed by gas chromatograph–mass spectrometer (GC-MS).

To test for the effects of the number of *L. chiensis* and pecky rice on the behavior regulator, the data were analyzed by t-test and one-way analysis of variance (ANOVA) with a Bonferroni correction. R version 2.0.1 was used for these analyses.

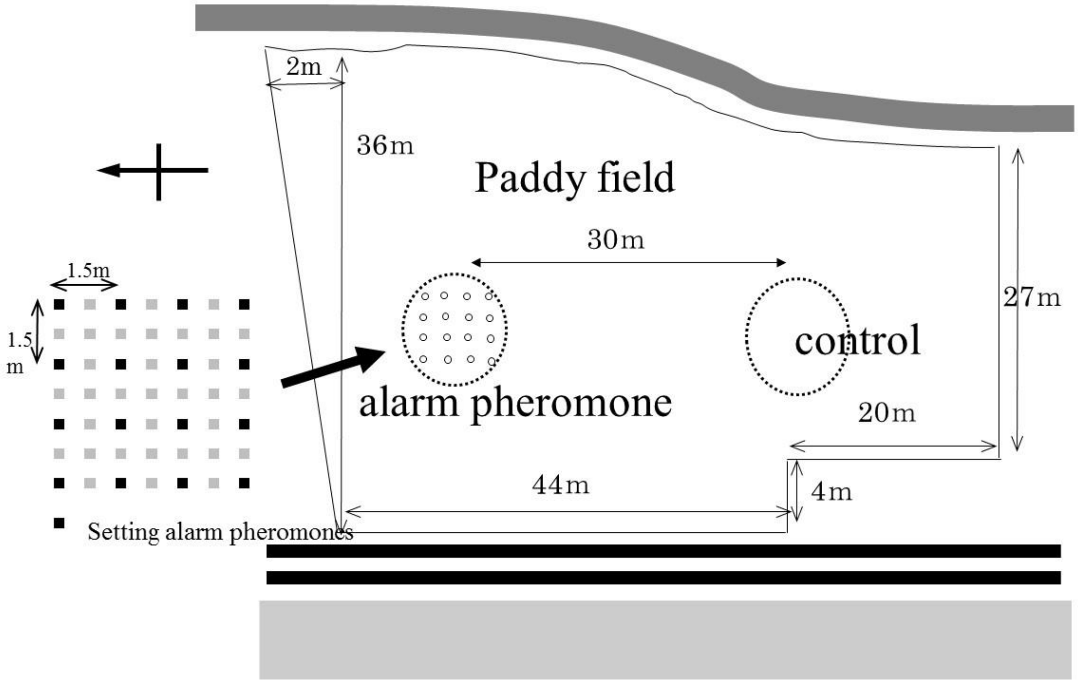

Fig. 4-1. Location of experiment in Aogaki.

Results and Discussion

The number of *L. chinensis* and the percentage of pecky rice in the plots with the behavior regulator were lower than those in control plots (Figs. 4-2 and 4-3). In particular, the number of pecky rice in each plot with the behavior regulator was surprising less than 0.1, which is an excellent value for the investigation of rice grade (Fig. 4-4). The concentrate of behavior regulator was recognized to be higher than the threshold to release excitement/escape behavior in *L. chinensis*.

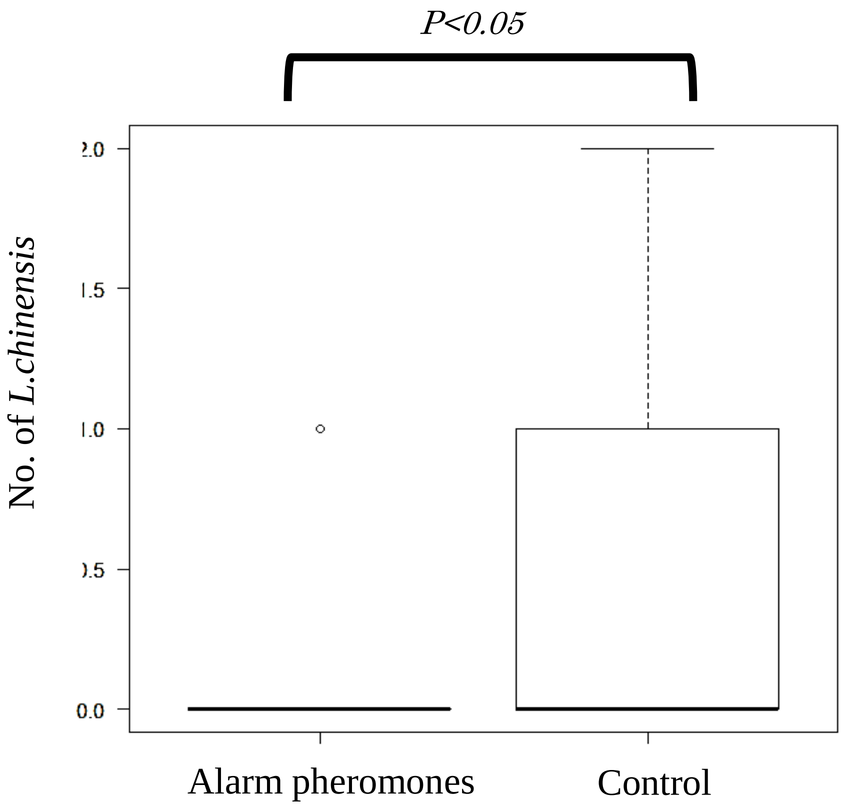

Fig. 4-2 Number of *N. chinensis* in alarm pheromones and control sites.

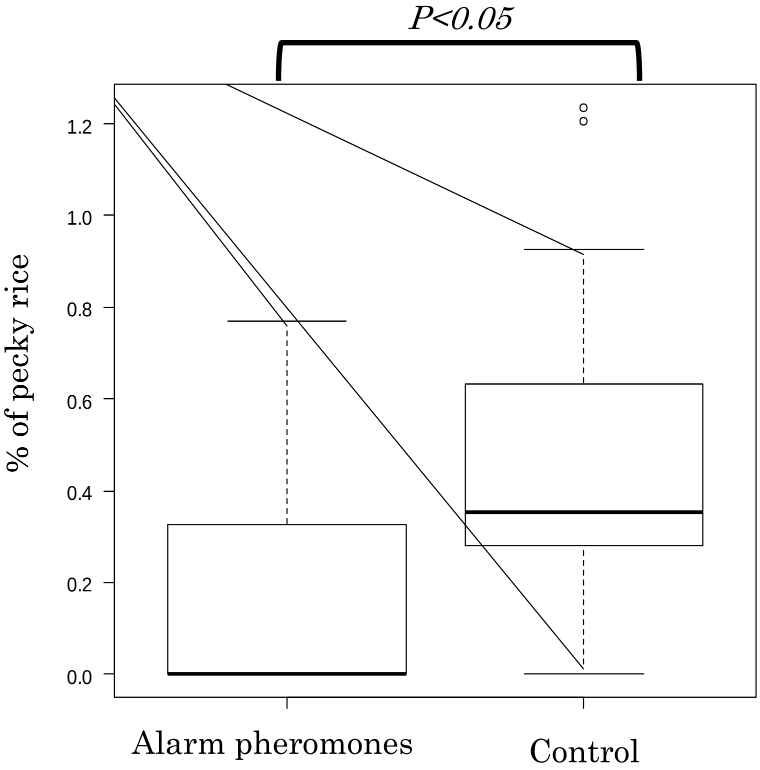

Fig. 4-3 Percentage of pecky rice in alarm pheromones and control sites.
Vales translate to arcsince.

Based on these results for the open paddy fields, the behavior regulator would be effective for the control of *L. chinensis* to reduce pecky rice. Thus, the utilization of the behavior regulator can be considered a new technique for pest control. The behavior regulator does not harm beneficial species and could become a key technique for efficient and sustainable insect management strategies in the future.

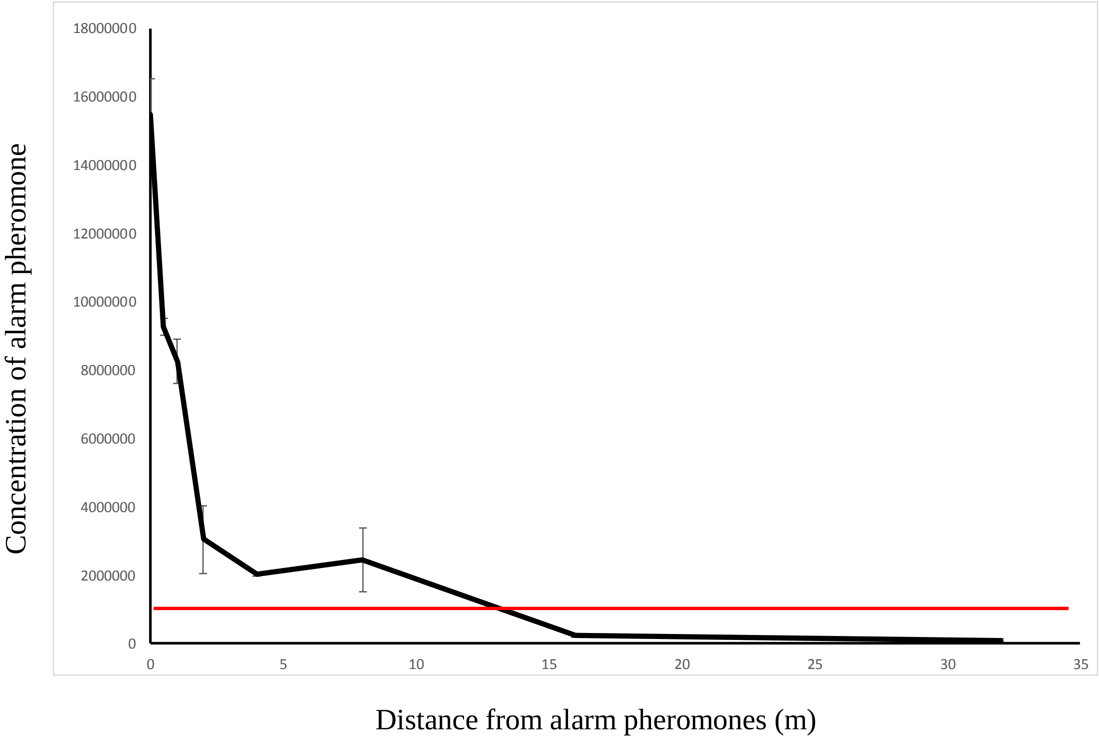

Fig. 4-4 Relationship between distance from alarm pheromones and concentration of them in a paddy field. Different letters indicate significant differences (P<0.05 Bonferroni corrections after ANOVA). Red line indicates the threshold.

Summary

The rice stink bug *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae) is a major cosmetic pest, and is one of the main causes of pecky rice (Suzuki, 2001). Thus, the effective and environmentally benign control of this species in paddy fields is essential, without the need for chemical insecticides. The aim of the present study was to develop a new method of control using a behavior regulator found in *L. chinensis.*

In Capters 1 and 2, I examined the life history of *L. chinensis* to predict and control *L. chinensis* invasion during the rice heading stage. First, I investigated the effects of a range of constant temperatures (18°C–30°C) on the development of eggs, nymphs and the pre-oviposition period of adult females in *L. chinensis*. The duration of all stages decreased as the temperature increased from 18°C to 30°C. Developmental thresholds of 8.1°C, 10.1°C and 9.6°C were estimated for the eggs, nymphs and pre-oviposition period, respectively. Thermal constants of 147 and 370 degree-days above the thresholds of 8.1 degree-days and 10.1°C were required for the development of the eggs and nymphs, respectively. The pre-oviposition period required 256 degree-days above the developmental threshold of 9.6°C. After placing individuals collected in November and February in an incubator in the laboratory, 469.7 degree-days above 9.6°C, a threshold for nondiapause female adults that was assumed

to be equal to that of diapause female adults, were estimated to be required for the pre-oviposition period of individuals that had overwintered. The present results were useful to predict the field population phenology of *L. chinensis* in Japan.

Next, the effects of temperature and photoperiod on diapause induction and termination in *L. chinensis* were studied under constant conditions or by using transfer experimental protocols. Nymphs were reared either under a long-day (16:8 [L:D] h) or a short-day (12:12 [L:D] h) photoperiod at 25°C in the laboratory. Females oviposited at 25°C and 20°C under the long-day photoperiod. However, females did not lay eggs within 100 d at 15°C under the long-day photoperiod or at 20°C and 15°C under short-day conditions. At 25°C, when nymphs were reared under a long-day photoperiod and transferred to a short-day photoperiod on the day of adult emergence, females started oviposition in 10 d but stopped shortly thereafter. When nymphs were reared under a short-day photoperiod and transferred to a long-day photoperiod on the day of adult emergence, females started oviposition in 40 d. Females that had been transferred from the field on 1 February and 29 March to long-day laboratory conditions at 25°C started oviposition in 40 d. However, females that had overwintered in the field were transferred to a short-day photoperiod at 25°C on 1 February and 29 March did not start oviposition in the laboratory. Both the nymphal and adult stages of *L. chinensis* are considered to be sensitive to reproductive diapause induction and termination signals both before and after overwintering. Female *L. chinensis* require not only a long-day photoperiod and 40 d at a temperature of 20°C or higher but also threshold temperatures for the start of oviposition even after the winter. From the results of Chapters 1 and 2, I was able to predict *L. chinensis* invasions during the rice heading stage.

In Chapter 3, I examined whether *L. chinensis* escaped from disturbed conspecifics in an observation arena under laboratory conditions. When an undisturbed individual of the same gender was introduced into the arena, the initial occupying *L. chinensis* did not show any behavioral responses. However, when a disturbed conspecific of the same gender was introduced, the initial occupant was immediately excited and escaped from the arena through a hole, suggesting that the pungent volatiles from the disturbed conspecific caused excitement/escape behavior.

Next in Chapter 3, I analyzed the volatiles emitted by both disturbed and undisturbed *L. chinensis* and observed the responses of the undisturbed *L. chinensis* to the components in the volatiles from disturbed conspecifics. Chemical analyses using a GC–MS showed that disturbed adults of both sexes emitted octanal, (*E*)-2-octenal, octanol, hexyl acetate, (*Z*)-3-octenyl acetate, octyl acetate and (*E*)-2-octenyl acetate. (*E*)-2-Octenal was the major compound. When exposed to (*E*)-2-octenal and

(*E*)-2-octenyl acetate, undisturbed females were excited and escaped from the observation arena with a similar proportional response as the disturbed females. Males and females escaped from (*E*)-2-octenal at ca. 2–10 ppbV in a mesh cage. Thus, the volatile compounds eliciting excitement/escape behavior in *L. chinensis* could be used for the control of this species in paddy fields.

In Chapter 4, I tried to control *L. chinensis* in the paddy fields using a behavior regulator in *L. chinensis*. The number of *L. chinensis* and pecky rice in plots where the behavior regulator was used was lower than that in the control plots. In particular, the number of pecky rice in each plot with the behavior regulator was less than 0.1, which represents excellent value for the investigation of rice grade. The concentrate of the behavior regulator was recognized to be higher than threshold to induce excitement/escape behavior by *L. chinensis*.

The behavior regulator would be effective for the control of *L. chinensis* for peck rice. In particular, the utilization of the behavior regulator can be considered a new technique for pest control. The behavior regulator does not harm beneficial species and could become an established technique for efficient and sustainable insect management strategies in the future.

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Yamashita K., Sudo K., Adachi T., Miura K. (2005) Estimation of number of annual generations using effective heat unit of development for the rice bug *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae). *Appl. Entomol. Zool.* 40: 621-624.

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Publications

1. Main Publications

Yamashita K., Sudo K., Adachi T., Miura K. (2005) Estimation of number of annual generations using effective heat unit of development for the rice bug, *Leptocorisa chinensis* (Dallas) (Hemiptera: Alydidae). *Appl. Entomol. Zool.*, 40: 621-624.

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Yamashita K., Isayama S., Ozawa R., Uefune M., Takabayashi J., Miura K. (2015) A pecky rice-causing stink bug *Leptocorisa chin*ensis escapes from volatiles emitted by excited conspecifics. *J. Ethol.* 34: 1-7.

2. Related Publications

Shiojiri K., Ozawa R., Yamashita K., Uefune M., Matsui K., Tsukamoto C., Tokumaru S., Takabayashi J. (2017) Weeding volatiles reduce leaf and seed damage to field-grown soybeans and increase seed isoflavones, *Sci. Rep.* 7: 41508.

3. Patent

Repellent for the *Leptocorsia chinensis*
Patent application No. 2003-208794.
Patent publication No. 2005-68022.
Patent No. 4404579.

Promoter of the eating action of bugs
Patent application No. 2005-97479.
Patent publication No. 2006-273778.