A multi-species pheromone lure: A blend of synthetic sex pheromone components for two mirid species, *Stenotus rubrovittatus* (Matsumura) and *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae)

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Abstract

Two mirid bugs, *Stenotus rubrovittatus* (Matsumura) and *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae), are sympatric pests that infest rice in many regions of Japan. Three components, hexyl butyrate, (*E*)-4-oxohex-2-enal and (*E*)-hex-2-en-1-yl butyrate, in *S. rubrovittatus* and three other components, hexyl hexanoate, (*E*)-hex-2-en-1-yl hexanoate and octyl butyrate, in *T. caelestialium*, were identified as sex attractant pheromone components in these species. The synthetic pheromone lures for *S. rubrovittatus* do not attract *T. caelestialium* and vice versa. The attractiveness of the combination of two separate lures for the two mirid bugs within one trap (combination lures) and the attractiveness of a mixed lure, in which all six synthetic-pheromone components of both species were loaded onto a single septum, were compared with that of separate lures for each species in field experiments. Both the combination lures and the mixed lure were as attractive to males of both mirid bugs as the separate lures for each species in fields with gramineous plants. In paddy fields, the mixed lure was also as attractive to males of both species of mirid bugs as the separate lures for each species. These results suggest that the mixed lure and the combination lures could be useful as simultaneous monitoring tools for two sympatric major rice pests using a single trap.

Key words: *Stenotus rubrovittatus*; *Trigonotylus caelestialium*; sex attractant pheromone; pheromone combination; monitoring

INTRODUCTION

A multi-species monitoring system that can be housed in one trap is a candidate for use in the detection of sympatric pest species. In several groups of sympatric pests, multi-species pheromone-based approaches have been used to monitor the populations of multiple species simultaneously. In Coleopteran species, a single lure containing aggregation pheromone components of several

species has been successful in detecting the pests (Bartelt et al., 1995; James et al., 2000; Miller et al., 2005; Wakefield et al., 2005). In Lepidoptera and Hymenoptera, the attractiveness of combination lures (two or more lures within one trap) containing sex pheromones has been examined. For examples, lures for *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), *Grapholita molesta* and *Archips breviplicanus* Walsingham (Lepidoptera: Tortricidae) were as attractive to males of

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these species as traps baited with each species' pheromone alone, although the addition of a lure for *Adoxophyes orana fasciata* Walsingham (Lepidoptera: Tortricidae) to them caused a significant reduction in the attraction of males (Sato, 1992). A mixed lure for *Malacosoma disstria* (Lepidoptera: Lasiocampidae) and *Choristoneura conflictana* (Lepidoptera: Tortricidae) using a single lure has been as attractive to the males of each species as traps baited with each species' pheromone alone (Evenden, 2005; Jones et al., 2009); however, other combinations of species have not been successful (Lopez et al., 1990; Johansson et al., 2002). Thus, a multi-species pheromone lure is useful in some species, but not in others.

Two mirid bugs, the sorghum plant bug *Stenotus rubrovittatus* (Matsumura) and the rice leaf bug *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae), are major pests infesting rice, *Oryza sativa* L., in Japan (Ito, 1989; Watanabe and Higuchi, 2006). They reproduce in gramineous plant fields and invade rice fields after rice plant heading, causing pecky rice (Hayashi and Nakazawa, 1988; Ito, 2004). The distribution area of *S. rubrovittatus* has been spreading since the 1990s in Japan (Hayashi, 1997), and this bug is now distributed from the southern part of Hokkaido to Kyushu in Japan (Watanabe and Higuchi, 2006). On the other hand, *T. caelestialium* is distributed in most parts of the eastern region of Japan, and its damage to rice occurs mainly in the northern part of Japan; therefore, *S. rubrovittatus* and *T. caelestialium* are sympatric species in the central regions of Japan.

In both species, males are attracted to conspecific unmated females (Kakizaki and Sugie, 1997; Okutani-Akamatsu et al., 2007). The sex attractant pheromones have been identified to be a mixture of hexyl butyrate, (*E*)-4-oxohex-2-enal and (*E*)-hex-2-en-1-yl butyrate for *S. rubrovittatus* (Yasuda et al., 2008) and a mixture of hexyl hexanoate, (*E*)-hex-2-en-1-yl hexanoate, and octyl butyrate for *T. caelestialium* (Kakizaki and Sugie, 2001). Synthetic pheromone lures of *S. rubrovittatus* and *T. caelestialium* were separately developed by determining the optimal ratios and/or amounts of the components for male attraction (Higuchi et al., 2004; Yasuda et al., 2009). Traps baited the synthetic pheromone lures are useful for monitoring the occurrence and/or population of bugs (Higuchi et al., 2004; Ishimoto et al., 2006). At present, how-

Fig. 1. Schematic representation of combination lures and mixed lure used in the present study.

ever, we have to set separate traps to monitor *S. rubrovittatus* and *T. caelestialium* simultaneously. If a sex pheromone-based trap could detect both species simultaneously, it would be of great practical and economic benefit.

The objectives of this paper were to reveal the attractiveness of the combination of two separate lures for the two mirid bugs, *S. rubrovittatus* and *T. caelestialium*, within one trap (combination lures) and the attractiveness of a lure, in which all pheromone components of both species were loaded onto a single septum (mixed lure) (Fig. 1) for use as a practical monitoring tool in the paddy field.

MATERIALS AND METHODS

Chemicals. Hexyl butyrate (>98.0% chemical purity), (*E*)-hex-2-en-1-yl butyrate (>95.0%), hexyl hexanoate (>98.0%) and (*E*)-hex-2-en-1-yl hexanoate (>95.0%) were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Octyl butyrate (>95.0%) was purchased from Wako Pure Chemical Industries, Ltd., Japan. (*E*)-4-Oxohex-2-enal (96.9%) was obtained from Shin-Etsu Chemical Co. Ltd, Japan.

Lure. For field experiments, gray septa made of a halo-butyl isoprene blend elastomer (1F Sleeve Stopper 1888 Gray, 8 mm outside diameter; West Pharmaceutical Services Singapore Pte Ltd., Sin-

gapore) were used as dispensers for lures. A solution of synthetic chemicals was impregnated into the depression in the gray septa. Each septum was placed in a draft chamber for ca. 1 h at room temperature to allow the solvents to evaporate and was then packed into an aluminum foil-laminated package and stored in a refrigerator until use.

The lures for *S. rubrovittatus* contained 20.0 μg hexyl butyrate, 40.0 μg (*E*)-4-oxohex-2-enal and 4.0 μg (*E*)-hex-2-en-1-yl butyrate per rubber septum (Yasuda et al., 2009), while the lures for *T. caelestialium* contained 7.0 μg hexyl hexanoate, 2.8 μg (*E*)-hex-2-en-1-yl hexanoate and 0.2 μg octyl butyrate per rubber septum (Higuchi et al., 2004). The lures for the two mirid bugs, *S. rubrovittatus* and *T. caelestialium*, contained 20.0 μg hexyl butyrate, 40.0 μg (*E*)-4-oxohex-2-enal, 4.0 μg (*E*)-hex-2-en-1-yl butyrate, 7.0 μg hexyl hexanoate, 2.8 μg (*E*)-hex-2-en-1-yl hexanoate and 0.2 μg octyl butyrate per rubber septum (mixed lure).

Field experimental sites. Field experiments were conducted in a field (Tsukuba; ca. 3,500 m²; 36°01'N, 140°06'E) at NARC-Tsukuba in Ibaraki Prefecture, a field (Hokuriku; ca. 3,500 m²; 37°06'N, 138°16'E) at NARC-Hokuriku in Niigata Prefecture, two fields (Toyama 1 and 2; ca. 3,000 m² each; 36°36'N, 137°14'E) at the Toyama Prefectural Agricultural, Forestry & Fisheries Research Center, a field at Toyama (Toyama 3; ca. 3,000 m²; 36°38'N, 137°07'E) in Toyama Prefecture and four fields (Furukawa 1: ca. 200 m²; Furukawa 2: ca. 210 m²; Furukawa 3: ca. 200 m²; Furukawa 4: ca. 260 m²) at the Miyagi Prefectural Furukawa Agricultural Experiment Station (38°35'N, 140°54'E) in Miyagi Prefecture. These fields were planted and/or naturally established with gramineous plants, such as Japanese barnyard millet *Echinochloa esculenta* (A. Braun) H. Scholz and Italian ryegrass *Lolium multiflorum*.

Additional field experiments were conducted in two paddy fields in Katori (ca. 4,600 m²; 35°53'N, 140°35'E) and Kozaki (ca. 3,100 m²; 35°54'N, 140°23'E) in Chiba Prefecture, a paddy field in Toyama City (ca. 3,000 m²; 36°36'N, 137°14'E) in Toyama Prefecture and a paddy field in Furukawa (ca. 500 m²; 38°35'N, 140°54'E) in Miyagi Prefecture. The cultivars of rice, *Oryza sativa*, planted in the paddy fields of Katori in Chiba and Miyagi were cv. "Akitakomachi" and cv. "Hitomebore",

respectively, and the cultivar in the other paddy fields was cv. "Koshihikari".

Traps. A double-sided sticky trap combined with two sticky boards (24 cm×30 cm; Sankei Chemical Co., Ltd., Kagoshima, Japan) was placed vertically (Ishimoto et al., 2006). Traps were placed at intervals of 8–10 m in the field, and the trap height was adjusted so that the sticky boards were just above the canopy of the surrounding plants.

Field experiment 1: Attractiveness of the combination lures and the mixed lure in the fields with gramineous plants. The attractiveness of two separate lures of two mirid bugs placed within one trap (combination lures) and that of the mixed lure was compared to the two exclusive lures for *S. rubrovittatus* males and for *T. caelestialium* males to estimate the performance of the combination and mixed lures in the fields with gramineous plants. A naïve rubber septum was used as a control lure.

In the experiment, six traps for each lure (30 traps) were placed five times over the periods 25–29 May and 1–5, 6–12, 14–18 and 18–22 June 2009 in the Tsukuba field. One trap for each lure (5 traps) was placed twice for the periods 18–25 June and 29 June–6 July in Toyama field 1, three times during the periods 17–24 July, 30 July–6 August and 6–13 August in Toyama field 2, and six times every 7 days from 3 July to 14 August 2009 in Toyama field 3. One trap for each lure (5 traps) was placed four times every 7 days from 16 June–14 July in Furukawa 1 and 2, and 13 times every 7 days from 14 July–15 September 2009 in Furukawa 3 and 4.

Field experiment 1-2: Reconfirmation of the attractiveness of the combination lures and the mixed lure in the fields with gramineous plants. The attractiveness of the combination lures and the mixed lure was compared to examine whether the activities of these lures were equivalent. A naïve rubber septum was used as a control lure.

For each mirid bug, two trials were conducted in Hokuriku, because the two species of mirid bugs were not present in the same experimental periods. In each experiment, three traps for each lure (9 traps) were placed five times every 4 days from 19 June to 8 July 2009 for *T. caelestialium*, and from 10 to 29 September 2009 for *S. rubrovittatus*.

Field experiment 2: Attractiveness of the

mixed lure in the paddy fields. The attractiveness of the mixed lure was compared to that of the two exclusive lures for *S. rubrovittatus* males and for *T. caelestialium* males to estimate the performance of the mixed lure in the paddy fields. A naïve rubber septum was used as a control lure.

The experimental trials were conducted during the heading and flowering stages of rice in each location, because mirid bugs invade rice fields after rice plant heading from gramineous plant fields (Hayashi and Nakazawa, 1988). Two traps for each lure (8 traps) were placed three times every 7 days from 13 July–3 August 2009 in Katori and 30 July–20 August 2009 in Kozaki in the Chiba Prefecture. One trap for each lure (4 traps) was placed twice every 7 days from 6–20 August 2009 in Toyama Prefecture. Two traps for each lure (8 traps) were placed three times every 7 days from 28 July–18 August 2009 in Miyagi Prefecture.

Statistical analysis. For the field experiments, the numbers of insects caught in a trap (x) were log transformed ($x+0.5$) and subjected to one- or two-way ANOVA. Statistical analyses were performed using JMP 7.0.2 (SAS Institute, 2007).

RESULTS

Attractiveness of the combination lures and the mixed lure in fields with gramineous plants

Because the experimental durations of the trap-catches were different between Tsukuba (4 days) and Toyama and Miyagi (7 days), the trap-catches in Tsukuba were analyzed separately from those in Toyama and Miyagi. Both the combination lures and the mixed lure were as attractive to *S. rubrovittatus* males as the *S. rubrovittatus* lure in both a) Tsukuba (ANOVA: $p>0.05$), and b) Toyama and Miyagi ($p>0.05$) (Fig. 2A). The *T. caelestialium* lure was not attractive to *S. rubrovittatus* males in any location.

For the trap-catches of *T. caelestialium* males in Toyama and Miyagi, an interaction between lures and location was observed in the results of two-way ANOVA; therefore, the trap-catches were analyzed separately in Toyama and Miyagi (Fig. 2B). In the Tsukuba and Toyama experiments, both the combination lures and the mixed lure were as attractive to *T. caelestialium* males as the *T. caelestialium* lure in both a) Tsukuba ($p>0.05$) and b) Toyama ($p>0.05$). In the Miyagi experiment, there

was no significant difference in the attractiveness of the combination lures, the mixed lure and the *T. caelestialium* lure ($p>0.05$), although there was also no significant difference between the attractiveness of the combination lures and the *T. caelestialium* lures and that of the control ($p>0.05$) (Fig. 2B-c). The *S. rubrovittatus* lure was not attractive to *T. caelestialium* males in any location.

To examine whether the attractiveness of the combination lures and the mixed lure was different, re-confirmative field experiments were conducted in the fields of Hokuriku. For both *S. rubrovittatus* and *T. caelestialium*, there was no significant difference between the attractiveness of the combination lures and that of the mixed lure ($p>0.05$) (Fig. 3); therefore, the mixed lure was used in the following experiments in the paddy fields.

Attractiveness of the mixed lure in the paddy fields

The mixed lure was as attractive to *S. rubrovittatus* males as its own lure of *S. rubrovittatus* in the paddy fields ($p>0.05$) (Fig. 4A). The *T. caelestialium* lure was not attractive to *S. rubrovittatus* males.

The mixed lure was also as attractive to *T. caelestialium* males as its own *T. caelestialium* lure in the paddy fields ($p>0.05$) (Fig. 4B). The *S. rubrovittatus* lure was not attractive to *T. caelestialium* males.

DISCUSSION

A multi-species monitoring system that can be housed in one trap is a candidate for use in the detection of sympatric pest species. The present study showed that mixed or combination lures are useful for detecting two sympatric mirid pests, *S. rubrovittatus* and *T. caelestialium*. The mixed or combination lures were as attractive to both *S. rubrovittatus* and *T. caelestialium* males as traps baited with each species' pheromone alone both in fields planted with gramineous plants and in paddy fields. No agonistic or antagonistic effects of heterospecific pheromone components on the males' response were observed. These results suggested that a mixed or combination pheromone-based approach to monitoring two sympatric mirid pests, *S. rubrovittatus* and *T. caelestialium*, simultaneously is feasible. For practical use, mixed lures are

A) *S. rubrovittatus* male B) *T. caelestialium* male

Fig. 2. Mean trap captures of *S. rubrovittatus* A) and *T. caelestialium* B) males when using two separate lures for the two mirid bugs housed within one trap (combination lures), the mixed lure, and two separate species-specific lures in fields with gramineous plants (Tsukuba, Toyama, and Miyagi in 2009). Values are presented as the means and SE (bar) of 4 d catches at Tsukuba and 7 d catches in Toyama and Miyagi. Means accompanied by the same letter in each experiment were not significantly different according to the Tukey-Kramer test at $p=0.05$.

likely to be more suitable monitoring tools than combination lures, because using one septum would be more economical and easier to manipulate than two septa.

The components identified as sex attractant pheromones of *S. rubrovittatus* (Yasuda et al., 2008) and *T. caelestialium* (Kakizaki and Sugie, 2001) were different; however, four of these components were detected in the solvent extracts of females in both species. Furthermore, hexyl butyrate and (*E*)-hex-2-en-1-yl butyrate were also detected in *T. caelestialium* females (Kakizaki and Sugie, 2001), and hexyl hexanoate and octyl butyrate

were also detected in *S. rubrovittatus* females (Yasuda et al., 2009). In *T. caelestialium* (Kakizaki and Sugie, 2001), the attractiveness of the pheromone lure to *T. caelestialium* males was not affected by the addition of 1.15 μg hexyl butyrate and/or 450 ng (*E*)-hex-2-en-1-yl butyrate to a blend of hexyl hexanoate, (*E*)-hex-2-en-1-yl hexanoate and octyl butyrate. In that experiment, the amount of hexyl butyrate and (*E*)-hex-2-en-1-yl butyrate added was almost 1/20 the dose of the *S. rubrovittatus* lure (Yasuda et al., 2009). In the present study, although the doses of hexyl butyrate, (*E*)-4-oxohex-2-enal and (*E*)-hex-2-en-1-yl butyrate were

Fig. 3. Mean trap captures of *S. rubrovittatus* A) and *T. caelestialium* B) males when using two separate lures for the two mirid bugs housed within one trap and the mixed lure in fields with gramineous plants (Hokuriku in 2009). Values are presented as the means and SE (bar) of 4 d catches. Means accompanied by the same letter in the results for each bug were not significantly different according to the Tukey-Kramer test at $p=0.05$.

Fig. 4. Mean trap captures of *S. rubrovittatus* A) and *T. caelestialium* B) males when using the mixed lure and two separate, species-specific lures in the paddy fields (Chiba, Toyama, and Miyagi in 2009). Values are presented as the means and SE (bar) of 7 d catches. Means accompanied by the same letter in the results for each bug were not significantly different according to the Tukey-Kramer test at $p=0.05$.

higher than those used by Kakizaki and Sugie (2001), this difference did not affect the attraction of *T. caelestialium* males. The opposite condition produced similar results. In the preliminary field experiment on *S. rubrovittatus*, when $0.1 \mu\text{g}$ octyl butyrate, $0.6 \mu\text{g}$ (*E*)-hex-2-en-1-yl acetate, $1.3 \mu\text{g}$ hexyl hexanoate and $1.8 \mu\text{g}$ hexyl acetate were added to a blend of hexyl butyrate, (*E*)-4-oxohex-2-enal and (*E*)-hex-2-en-1-yl butyrate, no additional effects on the male attraction of *S. rubrovittatus* were observed (Yasuda, unpublished data).

The merit of pheromone-baited traps is that the results provide data on the population of specific species. If the species caught had morphological similarity using a multi-species monitoring system and the trap-catches were not distinguished by species, a multi-species monitoring system would not offer the above-mentioned advantage. In *S. rubrovittatus* males, the femur of the hind legs is bright red, and there is a red stripe on the dorsal thorax and abdomen, while these parts are light green in *T. caelestialium*; therefore, it would be

easy to distinguish *S. rubrovittatus* from *T. caelestialium*, even if the observers were not experts.

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