Optimization of blends of synthetic sex pheromone components for attraction of the sorghum plant bug *Stenotus rubrovittatus* (Matsumura) (Heteroptera: Miridae)

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(Received 5 March 2009; Accepted 22 July 2009)

Abstract

Three components, hexyl butyrate (=butanoate) (6:nBu), (*E*)-4-oxohex-2-enal (E2O4-6:Ald) and (*E*)-hex-2-en-1-yl butyrate (E2-6:nBu), were identified as sex attractant pheromone components of the sorghum plant bug *Stenotus rubrovittatus* (Matsumura) (Heteroptera: Miridae) in a previous study. The optimum ratio and amount of the three components loaded into rubber septa for male attraction were investigated. A 5:10:1 blend of 6:nBu, E2O4-6:Ald and E2-6:nBu at 64 µg per rubber septum was most effective for male attraction and regarded as an ‘optimized blend’. The attractiveness of the optimized blend lures did not differ between lures with and without a sunshade and was equal to that of 10 unmated females. The attractiveness of optimized blend lures after weathering for 14 d did not differ from fresh lures, whereas the attractiveness of optimized blend lures weathered for more than 21 d was lower than fresh lures. Female extracts of *S. rubrovittatus* contain 13 minor components; however, the attractiveness of lures impregnated with female extracts did not differ from optimized blend lures. This result suggests that minor components at the amounts contained in the female extract do not strongly enhance its attractiveness.

Key words: *Stenotus rubrovittatus*; sex attractant pheromone; hexyl butyrate; (*E*)-4-oxohex-2-enal; (*E*)-hex-2-en-1-yl butyrate

INTRODUCTION

The sorghum plant bug *Stenotus rubrovittatus* (Matsumura) (Heteroptera: Miridae) is a major pest of rice, *Oryza sativa* L., in Japan. *S. rubrovittatus* reproduces in poaceous plant fields and invades rice fields after rice plant heading, causing pecky rice (Hayashi and Nakazawa, 1988). *S. rubrovittatus* males are attracted to unmated females (Okutani-Akamatsu et al., 2007), and a three-component mixture of hexyl butyrate (6:nBu), (*E*)-4-oxohex-2-enal (E2O4-6:Ald) and (*E*)-hex-2-en-1-yl butyrate (E2-6:nBu) has been identified as a sex attractant

pheromone of *S. rubrovittatus* (Yasuda et al., 2008).

In several species of mirid bugs, the optimum ratios and amounts of pheromones were important factors contributing to the attractiveness of a lure (e.g., McBrien et al., 1994; Kakizaki and Sugie, 2001; Higuchi et al., 2004). In a previous study (Yasuda et al., 2008), an effective blend ratio of sex pheromone components for *S. rubrovittatus* was initially determined as 100 µg of 6:nBu, 200 µg E2O4-6:Ald and 40 µg E2-6:nBu. The full range of blend ratios and dosages of these components, however, were not tested and the optimum ratios

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DOI: 10.1303/aez.2009.611

and amounts of the three components were not fully examined.

In Miridae, pheromone lures usually have a relatively short lifetime in the field; for example, a synthetic pheromone lure developed for *Lygus rugulipennis*, using the same compounds as *S. rubrovittatus*, maintained lure effects for only 3 h after being set (Innocenzi et al., 2005); however, a synthetic lure developed for *S. rubrovittatus* is likely to have a longer effective lifetime (Yasuda et al., 2008) than *L. rugulipennis*. Moreover, an aluminum-foil cup was used as a sunshade for this synthetic lure (Yasuda et al., 2008) but it is uncertain whether a sunshade is effective for prolonging the lifetime of synthetic pheromone lures.

The objectives of this paper were to determine the optimum ratio and amount of these components in a synthetic pheromone lure for *S. rubrovittatus*. We also investigated whether the addition of a sunshade to lures would affect the attractiveness of the lure, and tested the lifetime attractiveness of a lure for use as a practical monitoring tool in the field.

Moreover, some minor peaks were detected in the whole-body extracts of unmated females in gas-chromatographic analysis (T. Yasuda, unpublished data). We also identified the minor components and tested the potential role of these minor components to establish the most effective lure blend.

MATERIALS AND METHODS

Insects. *S. rubrovittatus* adults and nymphs were collected from grassy fields in Tottori Prefecture and Ibaraki Prefecture, and reared on millet *Setaria italica* and/or wheat *Triticum aestivum* seedlings and water at 25°C with 16L8D in the laboratory (Ando and Watanabe, 2007). *S. rubrovittatus* adults and fifth instars were individually reared in glass tubes at 25°C and 16L8D.

Chemicals. Hexyl butyrate (6:nBu) (>98.0% chemical purity), (*E*)-hex-2-en-1-yl butyrate (E2-6:nBu) (>95.0%), hexyl acetate (>99.0%), pentyl butyrate (>98.0%), (*E*)-hex-2-en-1-yl acetate (>95.0%), (*E*)-hex-3-en-1-yl butyrate (>97.0%), hexyl hexanoate (>98.0%), hexan-1-ol (>98.0%), heptan-1-ol (>98.0%), octan-1-ol (>98.0%), 4-methylpentan-1-ol (>99.0%), propionic anhydride (>98.0%) and butyric anhydride (>98.0%) were purchased from Tokyo Chemical Industry Co., Ltd.,

Japan. (*E*)-4-Oxohept-2-enal (E2O4-6:Ald) (96.9%) was obtained from the Shin-Etsu Chemical Co. Ltd., Japan. (*E*)-But-2-enonyl chloride (90%) and tetradecanoyl chloride (97%) were purchased from Aldrich Chemical Company, Inc. (St. Louis, MO, USA). Methanol (99.8%) was purchased from the Nacalai Tesque (Kyoto, Japan). To prepare hexyl propionate, hexyl isovalerate and hexyl (*E*)-but-2-enoate, hexan-1-ol was reacted with propionic anhydride, isovaleric anhydride, (*E*)-but-2-enonyl chloride, respectively, in the presence of pyridine. To prepare 4-methylpentyl butyrate, heptyl butyrate and octyl butyrate, the corresponding alcohols were reacted with butyric anhydride in the presence of pyridine. Methyl tetradecanoate was prepared by the reaction of methanol with tetradecanoyl chloride in the presence of pyridine. These synthetic chemicals were purified by column chromatography on silica gel, and their purities were greater than 90% by gas chromatography-mass spectrometry (GC-MS) analyses.

Lure. For field experiments, gray septa made of halo-butyl isoprene blend elastomer (1F Sleeve Stopper 1888 Gray, 8 mm outside diameter; West Pharmaceutical Services Singapore Pte Ltd., Singapore) were used as dispensers for lures. A solution of synthetic chemicals with a chemical stabilizer 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.

Field experimental sites. Field experiments were conducted in a field (ca. 3500 m²; 36°01'N, 140°06'E) of NARC-Tsukuba in Ibaraki Prefecture, a field (ca. 3500 m²; 37°06'N, 138°16'E) of NARC-Hokuriku in Niigata Prefecture, a field (ca. 300 m²; 35°27'N, 134°13'E) of the Tottori Agricultural Experiment Station in Tottori Prefecture and a roadside field (ca. 200 m length; 35°10'N, 136°07'E) of Shiga Prefecture Agricultural Technology Promotion Center in Shiga Prefecture. These fields were planted and/or naturally established with poaceous plants, such as Japanese barnyard millet *Echinochloa esculenta* (A. Braun) H. Scholz and Italian ryegrass *Lolium multiflorum*.

Traps. Water pans (field experiments 1–3) and double-sided sticky boards (field experiments 4–6) were used as traps in this study. Traps were placed

at intervals of 8–10 m in the field.

A blue-colored water pan is often used as a trap (e.g., Higuchi et al., 2004; Okutani-Akamatsu et al., 2007; Yasuda et al., 2008). The water pan (40 cm diameter and 12 cm in depth) was filled with water and a small amount of benzalkonium chloride solution was added as a surfactant to prevent the trapped bugs from escaping. A green-colored arched wire frame (ca. 50 cm length) was bridged over the trap. Lures or cages (9 cm diameter×9 cm height) with unmated females were fixed at the center of an aluminum foil cup (5.0 cm inside diameter×1.2 cm height), and hung above the water pan from the top of the frame. The distance between the lure or bottom of the cage and the water surface was ca. 10 cm.

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). Trap height was adjusted so that the sticky boards were just above the canopy of the surrounding grass plants. The lure was hung at the top of the sticky boards without a sunshade.

General procedure of field experiments. The number of *S. rubrovittatus* males captured in each trap was counted at approximately 10:00 h every day. Captured insects were then removed or marked by an oil-based paint, and trap positions were altered in rotation during the period.

Field experiment 1: Ratio of components in synthetic pheromone lures. To determine the optimum ratio of three components for male attraction, a series of different amounts of one component and constant amounts of the other two components were impregnated into different septa as lures. The amounts of each component used in the trials were based on the results of a previous study (Yasuda et al., 2008) and experimental trials in the present study. For each component, two trials were conducted in 2006 and 2007.

The amount of 6:nBu was varied between 5–100 μg in 2006 and 10–80 μg in 2007. In the 2006 experiment, three traps for each lure (15 traps) were placed during 13–18 June in Tsukuba. In the 2007 experiment, six traps for each lure (24 traps) were placed during 22–26 May in Tsukuba and six traps for each lure (24 traps) were placed during 16–20 June in Hokuriku.

The amount of E2O4-6:Ald was varied between 10–200 μg in 2006 and 20–160 μg in 2007. In the 2006 experiment, two traps for each lure (10 traps)

were placed during 3–8 June in Tsukuba. In the 2007 experiment, six traps for each lure (24 traps) were placed twice during 28 May–1 June and 4–8 June in Tsukuba, and six traps for each lure (24 traps) were placed during 20–24 June in Hokuriku.

The amount of E2-6:nBu was varied between 2–40 μg in 2006 and 2–16 μg in 2007. In the 2006 experiment, three traps for each lure (15 traps) were placed during 8–13 June in Tsukuba. In the 2007 experiment, six traps for each lure (24 traps) were placed during 9–13 June in Tsukuba, and six traps for each lure (24 traps) were placed during 24–28 June in Hokuriku.

Field experiment 2: Total amounts of components in synthetic pheromone lures. To determine the optimum amount of each of the three components for male attraction, a series of different amounts of each component, but at a constant 5:10:1 ratio of 6:nBu, E2O4-6:Ald and E2-6:nBu as determined in field experiment 1, were impregnated into different septa as lures. Two trials were conducted in 2006 and 2007. In 2006, the total amount of the three components was divided into three levels: 3.2, 32 and 320 μg . One trap for each lure (four traps, including one blank trap) was placed three times during 16–20 October, 22–27 October and 29 October–3 November in Shiga. In 2007, the total amount of the three components was divided into five levels: 8, 16, 32, 64 and 128 μg . Four traps for each lure (20 traps) were set twice during 20–25 June and 26 June–1 July in Tsukuba and four traps for each lure (20 traps) were set during 28 June–3 July in Hokuriku.

Field experiment 3: Comparing the attractiveness of the synthetic pheromone lure with unmated females. The attractiveness of the synthetic lure was compared to unmated females to estimate the performance of synthetic pheromone blend lures. Ten unmated females (4 d old) were placed cages with wheat, *T. aestivum*, seedlings. Two traps baited with females and one trap baited with the lure containing 20 μg of 6:nBu, 40 μg E2O4-6:Ald and 4 μg E2-6:nBu (hereafter referred as 20:40:4 μg) were placed four times during 2–6, 9–13, 16–20 and 23–27 October in 2007 in Tottori.

Field experiment 4: Effects of sunshade on the attractiveness of synthetic pheromone lures. In a previous study (Yasuda et al., 2008) an aluminum foil cup (5.0 cm inside diameter×1.2 cm height) was used as a sunshade for the lure. To ex-

amine whether the sunshade was necessary for lure effectiveness, six traps for each lure (12 traps) with the synthetic pheromone blend lure (20:40:4 μg), with and without a sunshade, were placed twice during 29 May–2 June and 2–6 June in 2008 in Hokuriku.

Field experiment 5: Effects of weathering on the attractiveness of the synthetic pheromone lure. To examine whether the duration of exposure to the environment affected the attractiveness of the lures, synthetic pheromone blend lures (20:40:4 μg) weathered for various periods (0, 7, 14 and 21 d) were prepared. Ten traps for each lure (40 traps) were placed simultaneously during 30 September–3 October in 2008 in Tsukuba.

Field experiment 6: Comparing attractiveness of the synthetic pheromone lure with unmated female extracts. To obtain female whole-body extracts, unmated females (3 to 6 d old) were dipped in hexane for 30 min at room temperature. The extract was decanted from the female bodies into glass vials. In total, 168 unmated females were extracted and pooled. The extracts of hexane solution (ca. 60 ml) were concentrated to ca. 4 ml volumes under reduced pressure below 30°C. The extracts were stored below –20°C until GC-MS analyses and field experiments.

To identify minor components in female extracts, aliquots (50 μl ; ca. 2 female equivalents: FE) of female extracts were divided into glass vials and concentrated to ca. 10 μl volumes under reduced pressure below 30°C. One microliter of the concentrated extract was injected into the GC-MS injector. In certain analyses, straight-chained hydrocarbons (between 9 and 22) were added as internal standards to calculate the Kováts retention index (Kováts, 1965) using HP-INNOWax ($KI_{\text{HPINNOWax}}$) and HP-1 (KI_{HP1}) columns. Compounds were identified by comparing the Kováts retention index and mass spectra with those of standards. GC-MS analyses were performed on an Agilent 6890N GC with HP-INNOWax and HP-1 columns (30 m length $\times$ 0.25 mm inside diameter $\times$ 0.25 μm film thickness) by splitless injection combined with an Agilent 5975 Network Mass Selective Detector. Injection temperature was 230°C. Helium was used as the carrier gas and the flow rate was constant at 1.0 ml/min. The initial GC oven temperature was 50°C (2 min hold), increased to 240°C at 10°C/min, and then held for 5 min.

To determine the amounts of 6:nBu, E2O4-6:Ald and E2-6:nBu in female extracts, an aliquot (1 FE) of the female extract was divided into a glass vial and a hexane solution of heptadecane (10 μg) was added as an internal standard. Amounts of 6:nBu, E2O4-6:Ald and E2-6:nBu in female extracts were quantified by GC-MS analyses. Standard curves were obtained using known amounts of reference chemicals with the internal standard. The amounts of 6:nBu, E2O4-6:Ald and E2-6:nBu in female extracts were 3.9, 0.4 and 0.9 $\mu\text{g}/\text{FE}$, and therefore the amounts of 6:nBu and E2-6:nBu in 4 FE of female extracts were almost equal to those of a synthetic pheromone lure (20:40:4 μg). To adjust the amounts of components in female extracts to equal the amounts in the synthetic pheromone blend lure (20:40:4 μg), 175 μg synthetic 6:nBu, 1.6 mg synthetic E2O4-6:Ald and 17 μg synthetic E2-6:nBu were added to 160 FE of the female extract.

To estimate the potential role of minor components in the female extract, the attractiveness of a synthetic pheromone lure was compared to an unmated female extract. Four FE of the adjusted female extract was impregnated into gray septa. Twelve traps baited with the adjusted female extract lure and 12 traps baited with the synthetic pheromone blend lure (20:40:4 μg), 24 traps, were placed twice during 18–22 and 24–28 September in 2008 in Tsukuba.

Statistical analysis. For field experiments 1, 2 and 5, the numbers of insects caught in a trap (x) were log transformed ($x+0.5$) and subjected to one- or two-way ANOVA. For field experiments 1 and 2 in 2007, regression analysis was applied to the log-transformed dose ($\mu\text{g}/\text{septum}$) when ANOVA was significant at the 5% level. For field experiment 5, Dunnett's test was applied to compare the duration of exposure with the control (0-day exposure). For field experiments 3, 4 and 6, a paired t -test was used to compare the numbers of insects caught in traps. Statistical analyses were performed using JMP 7.0.2 (SAS Institute, 2007).

RESULTS

Ratios and amounts of components for a synthetic pheromone lure

In 2006, a blend with 100 μg of 6:nBu and 200 μg E2O4-6:Ald seemed to be a more effective

attractant relative to other blends, although there was no significant difference between treatments in these two experiments ($p>0.05$; Fig. 1A, B). When the amount of E2-6:nBu was 20 μg , more males were trapped relative to other blends ($p<0.01$; Fig. 1C). From these results, the optimum ratio was assessed as 5:10:1 of 6:nBu, E2O4-6:Ald and E2-6:nBu. When a blend consisted of 10 μg of 6:nBu, 20 μg E2O4-6:Ald and 2 μg E2-6:nBu, more males

were trapped relative to blends with different ratios ($p<0.05$; Fig. 1D). Because inconsistent results were obtained with the same composition (100:200:40 μg in Fig. 1B, C) and the total amount of lure was changed, they were reexamined in 2007.

In 2007, blends consisting of 10 μg of 6:nBu, 20–160 μg E2O4-6:Ald and 2 μg E2-6:nBu, trapped more males than other blends (Fig. 2A:

Fig. 1. Mean trap captures of *S. rubrovittatus* males with blends of synthetic 6:nBu, E2O4-6:Ald and E2-6:nBu in 2006 field experiments. A) 6:nBu (5–100 μg) ($n=15$). B) E2O4-6:Ald (10–200 μg) ($n=10$). C) E2-6:nBu (2–40 μg) ($n=15$). D) Different amounts of 6:nBu, E2O4-6:Ald and E2-6:nBu with a constant ratio (5:10:1) ($n=14$). Values are presented as the mean and SE (bar) of daily catches. Means accompanied by the same letter in C and D were not significantly different by the Tukey-Kramer test at $p=0.05$.

Fig. 2. Mean trap captures of *S. rubrovittatus* males with blends of synthetic 6:nBu, E2O4-6:Ald and E2-6:nBu in 2007 field experiments. A) 6:nBu (10–80 µg) ($n=12$). B) E2O4-6:Ald (20–160 µg) ($n=18$). C) E2-6:nBu (2–16 µg) ($n=12$). D) Different amounts of 6:nBu, E2O4-6:Ald and E2-6:nBu with constant ratio (5:10:1) ($n=16$). Values are presented as the mean and SE (bar) of 4 d catches. Means accompanied by the same letter in A and C were not significantly different by the Tukey-Kramer test at $p=0.05$.

two-way ANOVA $p<0.05$ for lures, $p>0.05$ for locations, Hokuriku and Tsukuba; Fig. 2B: $p>0.05$ for lures in each location, $p<0.05$ for locations; Fig. 2C: $p<0.01$ for lures, $p>0.05$ for locations). The number of trapped males decreased with an increase in dosage of both 6:nBu (Fig. 2A: linear regression $y=0.669-0.0109x$, where y is the log-transformed ($x+0.5$) data of the mean catch and x µg of the dosage, $r^2=0.136$, $F=7.24$, $p=0.0099$)

and E2-6:nBu (Fig. 2C: linear regression $y=0.592-0.0656x$, $r^2=0.176$, $F=9.84$, $p=0.0030$). Although the optimum ratio of E2O4-6:Ald was not critical in the range of 20–160 µg with 10 µg of 6:nBu and 2 µg E2-6:nBu (Fig. 2B), the addition of 20 µg E2O4-6:Ald was adequate to attract males. This 5:10:1 ratio of 6:nBu, E2O4-6:Ald and E2-6:nBu was consistent with the results from the 2006 experiments (Fig. 1). When the total amount

of the three compounds was varied between 8–128 μg , male catch increased when the amounts of E2-6:nBu varied between 8–64 μg . The number of trapped males peaked at a blend consisting of a 64 μg mixture ($p < 0.05$ for lures, $p > 0.05$ for locations; Fig. 2D). The relationship between the number of trapped males and total dosage of the three compounds was described well by a quadratic regression ($y = 0.000157x^2 - 0.00575x + 0.390$, $r^2 = 0.117$, $F = 5.093$, $p = 0.0021$), rather than a linear regression ($y = 0.00371x + 0.00657$, $r^2 = 0.041$, $F = 3.343$, $p = 0.0713$). These results suggested that the blend of 20 μg of 6:nBu, 40 μg E2O4-6:Ald and 4 μg E2-6:nBu is an optimized blend for attraction of *S. rubrovittatus* males.

Comparing attractiveness of the synthetic pheromone lure with unmated females

In traps baited with synthetic blend lures (20:40:4 μg), 13.5 ± 5.3 (mean $\pm$ SE; $n = 4$) males per trap were caught over 4 d, whereas 10.9 ± 3.5 ($n = 8$) males were captured in traps baited with unmated females. There was no significant difference between the synthetic lure and unmated females (t -test, $p > 0.05$).

Effectiveness of sunshades on optimized blend lures

In traps with a sunshade, 6.0 ± 0.8 (mean $\pm$ SE; $n = 12$) males per trap were caught over 4 d, whereas 6.9 ± 0.9 ($n = 12$) males were captured in traps without a sunshade. There was no significant difference between lures with or without a sunshade ($p > 0.05$).

Effect of exposure duration of optimized blend lures on male catch

A significant difference was detected between 0-day and 21-day-exposed lures ($p < 0.05$, Fig. 3); however, there was no significant difference between 0-day-exposed lures and 14-day-exposed lures ($p > 0.05$).

Comparison between the optimized blend lure and extracts of unmated females

Thirteen minor compounds, excluding 6:nBu, E2O4-6:Ald and E2-6:nBu, were identified (Table 1). In traps baited with synthetic blend lures (20:40:4 μg), 9.8 ± 1.0 (mean $\pm$ SE; $n = 24$) males per trap were caught over 4 d, whereas 11.3 ± 1.4

Fig. 3. Mean trap captures of *S. rubrovittatus* males after weathering the optimized blend lure. Exposure durations were 0, 7, 14 and 21 d ($n = 10$). Values are presented as the means and SE (bar) of 4 d catches. Asterisk indicates significant difference at $p < 0.05$ using Dunnett's test against the control (0-day exposed).

Table 1. Minor compounds in female extracts of *Stenotus rubrovittatus*

Minor compounds	$KI_{\text{HPINNOWax}}$	KI_{HP1}	Relative contents (%) ^a
Hexyl acetate	1276	996	0.8
Pentyl butyrate	1320	1076	0.5
(E)-Hex-2-en-1-yl acetate	1337	995	0.2
Hexyl propionate	1342	1091	0.2
4-Methylpentyl butyrate	1374	1142	0.8
Hexyl isovalerate	1447	1228	0.2
(Z)-Hex-3-en-1-yl butyrate	1466	1146	0.5
Hexyl valerate	1516	1274	0.2
Heptyl butyrate	1520	1276	0.1
Hexyl (E)-but-2-enoate	1562	1224	0.1
Hexyl hexanoate	1613	1370	0.1
Octyl butyrate	1620	1374	0.1
Methyl tetradecanoate	2014	1684	2.0

^a These values were relative ratios against the amounts of 6:nBu in female extracts.

($n = 24$) males were caught in traps baited with female extracts. There was no significant difference between the synthetic lure and the unmated female extract (t -test, $p > 0.05$).

DISCUSSION

The optimum ratio of three sex pheromone components to attract *S. rubrovittatus* males was determined in 2006 and 2007 field experiments. In 2006

experiments, inconsistent results were obtained with the same composition (100:200:40 μg) (Fig. 1). No males were captured in traps baited with a blend of 100:200:40 μg (Fig. 1C), whereas the results indicated that this blend was the most attractive (Fig. 1A, B). To clarify this unexpected result, similar experiments were conducted in 2007. More males were captured with a blend of 10:20:2 μg rather than 10:20:4 μg (Fig. 2C), which is just 1/10 of the amount used in 2006 experiments. This result suggested that a blend of 100:200:20 μg might be more attractive rather 100:200:40 μg ; therefore, the optimum ratio of E2-6:nBu and 6:nBu was 5:1 (Fig. 2A, C). On the other hand, the ratio of E2O4-6:Ald to 6:nBu and E2-6:nBu was not critical for attraction and blends from 2:1 to 16:1 of E2O4-6:Ald and 6:nBu showed similar attractiveness (Fig. 2B); therefore, the optimum ratio of the three components, 6:nBu, E2O4-6:Ald and E2-6:nBu, for practical purposes was determined to be 5:10:1.

The number of captured males differed when the amount of the three components varied (Fig. 2D). This result was described well by quadratic regression rather than linear regression. The peak amount, 64 μg , was chosen to represent the optimum amount of the three components; therefore, the blend of 20:40:4 μg was determined as an 'optimum blend' for male attraction and the attractiveness of the optimized blend lures was not inferior to ten unmated *S. rubrovittatus* females.

In mirid bugs, optimum amounts of compounds in lures seem to be dependent on the molecular weight of the compounds. In *Trigonotylus caelestialium*, pheromone components, such as hexyl hexanoate, have a relatively high molecular weight. The optimum amounts in lures were 10 μg in rubber septa (Higuchi et al., 2004). By contrast, when components had a low molecular weight, such as hexyl acetate, attraction of males increased with the dose within the range of 0.1–33 mg of pheromone loaded in the rubber septum for *P. relativus* (Millar et al., 1997) and *P. californicus* (Millar and Rice, 1998). The authors (Millar et al., 1997; Millar and Rice, 1998) did not test doses over 33 mg because it is difficult to load large amounts of chemicals into the septum. The molecular weight of the pheromone components of *S. rubrovittatus*, with the exception of E2O4-6:Ald, are intermediate for mirid bugs and the optimum total amount of the three compounds was also in-

termediate relative to assessments of other species.

In field experiments 1–3 and a previous study (Yasuda et al., 2008), an aluminum foil cup was used as a sunshade to protect the lures from direct sunlight. A similar sunshade for lures was used for pheromone lures baited with conjugated triene compounds, such as methyl (*E,E,Z*)-2,4,6-decatrienoate (Aldrich et al., 2006; Khirmian et al., 2008). Geometric isomers of methyl 2,4,6-decatrienoates are unstable under daylight, and (*E,E,Z*)-isomer decreased in purity under room conditions in the hexane and rubber septa formulations (Khirmian, 2005; Khirmian et al., 2008). However, the sunshade did not affect the attractiveness of the lures in the present study; therefore, we decided to use lures without the sunshade after field experiment 5.

Although 13 minor-content compounds were identified in the extracts of unmated *S. rubrovittatus* females (Table 1), the minor components did not seem to enhance male attraction with the amounts in the female extract. Hexyl acetate, (*E*)-hex-2-en-1-yl acetate, hexyl hexanoate and octyl butyrate are known as pheromone components in heteropteran insects, including mirid bugs (e.g., Millar et al., 1997; Kakizaki and Sugie, 2001; Zhang and Aldrich, 2008), whereas methyl tetradecanoate was reported in Hymenoptera (e.g., Krieger et al., 2006), not in Heteroptera; however, other components have not been reported as insect pheromones.

In the Miridae, synthetic pheromone lures usually have a relatively short lifetime in the field. In *P. relativus* and *P. californicus*, lures aged two weeks old were significantly less attractive than fresh lures (Millar et al., 1997; Millar and Rice, 1998). In *T. caelestialium*, the attraction period of a lure was 30 d for a rubber septa (Higuchi et al., 2004). In *L. rugulipennis*, the lifetime of a synthetic lure, using the same compounds as *S. rubrovittatus*, is only 3 h (Innocenzi et al., 2005); however, the effective period of synthetic pheromone lures for *S. rubrovittatus* males seemed to be 14 d (Fig. 3). This effective period of *S. rubrovittatus* synthetic pheromone lures would be considered to be relatively long as compared with other Miridae pheromone lures, and therefore this lure could be used for practical purposes, such as monitoring.

ACKNOWLEDGMENTS

We thank Takayuki Mitsunaga of NARC-Tsukuba for invaluable suggestions for statistical analysis, and Ken Tabuchi and Noriko Ozeki of NARC-Tsukuba for assistance with the experiments. This study was partly supported by a Grant-in-Aid for Research Projects Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries from the Ministry of Agriculture, Forestry and Fisheries of Japan. The authors appreciate the comments given by two anonymous referees and the editor who greatly improved the manuscript.

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