



Effect of pheromone blends, trap type and color on the capture of male clearwing moths, *Synanthedon bicingulata* (Lepidoptera: Sesiidae)

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Abstract

Two components of the *Synanthedon bicingulata* sex pheromone, (*E,Z*)-3,13-octadecadienyl acetate (*E3,Z13*-18:OAc) and (*Z,Z*)-3,13-octadecadienyl acetate (*Z3,Z13*-18:OAc), were synthesized to investigate the effect of pheromone blends, trap type and trap color on the capture of *S. bicingulata* males. The optimal sex pheromone ratio for *E3,Z13*-18:OAc and *Z3,Z13*-18:OAc was approximately 4.3:5.7 based on the purity of the two pheromone components in all test areas. A significant difference was observed in the number of *S. bicingulata* adult males caught in bucket and delta traps. The mean numbers of males caught in bucket and delta traps were 13.2 ± 2.2 and 7.6 ± 2.0 , respectively. Trap color affected the number of adult males caught in bucket traps. More adult males were attracted to a yellow bucket trap than to green, white, blue, black and red traps. An analysis of the relationship between trap capture and trap surface-color values ($L^*a^*b^*$) revealed a positive relationship between trap capture and b^* value.

Keywords Clearwing moth · *Synanthedon bicingulata* · Pheromone blends · Trap type · Trap color

Introduction

Twenty-five species of clearwing moths (Lepidoptera: Sesiidae) in nine genera are economically important pests of apple, peach, jujube, and persimmon trees and grape vines in Korea (Lee et al. 2004, 2017; Yang et al. 2012; Cho et al. 2016). Among them, *Synanthedon bicingulata* is one of the most important insect pests in fruit tree orchards (Yang et al. 2012; Cho et al. 2016; Lee et al. 2017).

Cherry trees are popular roadside trees in Korea due to their visually attractive early-spring blossoms. Most local governments in Korea have planted many cherry trees on roadsides, and damage from *S. bicingulata* has increased in recent years (Kim et al. 2019). Larvae of *S. bicingulata* bore into the trunk of a host tree and feed on the cambium layer (Kim et al. 2019). Controlling *S. bicingulata* larval infections with insecticides is difficult, creating a need for alternative control methods. Sex pheromones are promising candidates for this purpose. Traps baited with species-specific sex pheromones provide information on the timing of insecticide application. In addition, sex pheromones can directly control target insects through mass trapping (Naka et al. 2008; Leskey et al. 2009).

Following identification of the first sex pheromones in *Synanthedon pictipes* and *Synanthedon exitiosa* (Tumlinson et al. 1974), sex pheromones of several other *Synanthedon* species have been described (The Pherobase, 2020). Two 3,13-octadecadienyl compounds, (*E,Z*)-3,13-octadecadienyl acetate (*E3,Z13*-18:OAc) and (*Z,Z*)-3,13-octadecadienyl acetate (*Z3,Z13*-18:OAc), were identified as sex pheromones of *S. bicingulata* by Yang et al. (2011), but follow-up studies focused only on monitoring seasonal occurrence of *S. bicingulata* using pheromone traps in

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fruit orchards (Yang et al. 2012; Cho et al. 2016; Lee et al. 2017).

In this study, attraction of *S. bicingulata* males to various combinations of newly synthesized two sex pheromone components was evaluated to find out the optimal pheromone ratio in field. In addition, effect of trap type and color on the capture of *S. bicingulata* males was investigated to improve the pheromone-based monitoring of clearwing moth.

Materials and methods

Chemicals

1,8-Dibromooctane (98%), 1-hexyne (97%), n-BuLi (2.5 M in hexane), tetrahydrofuran (THF), palladium on barium sulfate (Pd/BaSO₄, 5% Pd basis), methanol (MeOH) (≥ 99.9%), 3-butyn-1-ol (97%), lithium aluminum hydride (LAH) (95%), and hexamethylphosphoramide (HMPA) (98%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA).

Synthesis of (E,Z)-3,13-octadecadienyl acetate and (Z,Z)-3,13-octadecadienyl acetate

Synthetic methods for *E*3,Z13-18:OAc and Z3,Z13-18:OAc were reported in several previous studies (Armstrong-Chong et al. 2004; Ebata et al. 1979; Naka et al. 2006; Uchida et al. 1978; Uchida et al. 1979; Yamamoto et al. 1989). In this study, we synthesized *E*3,Z13-18:OAc and Z3,Z13-18:OAc according to the method reported in our patent (Lee et al. 2018), and a synthetic scheme is shown in Fig. 1. Solvents were dried and purified by conventional methods prior to use. ¹H NMR (at 400 MHz or 600 MHz) and ¹³C NMR (at 150 MHz) spectroscopic data were recorded on an Advance 400 MHz and 600 MHz spectrometer (Bruker, Germany) in CDCl₃.

14-Bromotetradeca-5-yne (7) In a clean and dry 500 mL round-bottom flask, 5.8 g of 70.73 mmol 1-hexyne (**9**) was dissolved in 150 mL of THF and 20 mL of HMPA and then cooled to −78 °C. n-BuLi was added to this solution (29.7 mL, 74.27 mmol). The mixed solution was stirred for 1 h, and 1,8-dibromooctane (**8**) (25.0 g, 91.91 mmol) in 50 mL of THF was added slowly. Stirring was continued

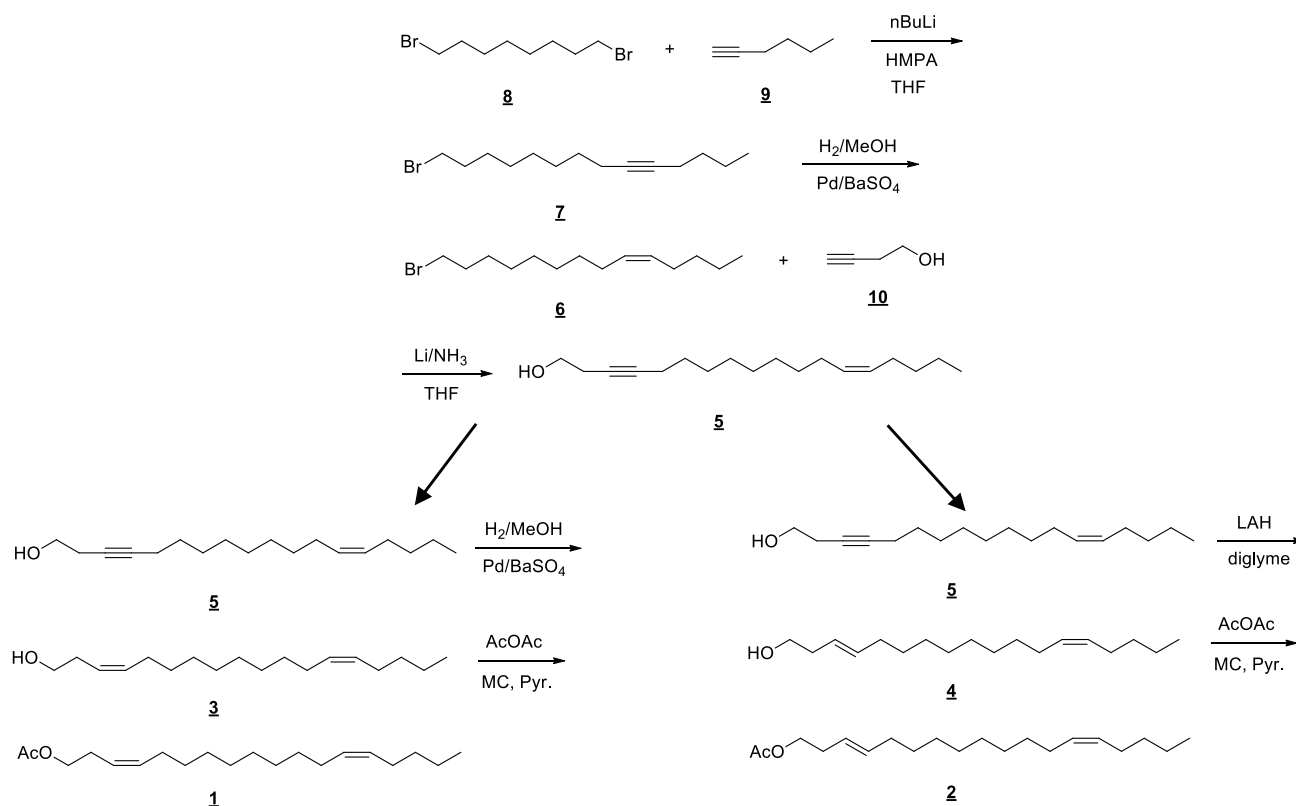


Fig. 1 Synthetic schemes for two sex pheromone of *Synanthedon bicingulata*. (3Z,13Z)-octadecadienyl acetate (**1**); (3E,13Z)-octadecadienyl acetate (**2**); (Z,Z)-3,13-Octadecadien-1-ol (**3**); (E,Z)-3,13-Octadecadien-1-ol (**4**); Z-13-Octadecen-3-yn-1-ol (**5**); (5Z)-14-Bromotetradeca-5-ene (**6**); 14-Bromotetradeca-5-yne (**7**); 1,8-Dibromooctane (**8**); 1-Hexyne (**9**); 3-Butyn-1-ol (**10**)

for 2 h at $-78\text{ }^{\circ}\text{C}$. The residue was treated cautiously with saturated 50 mL of NH_4Cl and the volatiles were then removed in a rotary evaporator under reduced pressure. The residue was extracted with EtOAc ($3 \times 150\text{ mL}$). The combined organic layers were washed with water and dried on anhydrous MgSO_4 . When crude residue was purified using column chromatography (EtOAc/Hexane, 1/10), 14-bromotetradeca-5-yne (**7**) (17.9 g, 92% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 3.37 (t, 2H), 2.10 (m, 2H), 1.84–1.80 (m, 2H), 1.52–1.27 (m, 16H), 0.87 (t, 3H).

(*5Z*)-14-Bromotetradeca-5-ene (**6**) Compound (**7**) (15.0 g, 63.22 mmol), 5% Pd/BaSO₄ (1.0 g), and quinoline (25 mg) in MeOH (250 mL) were placed in a 500 mL Parr reaction bottle and shaken in an H₂ atmosphere at 40 psi for 2 h using Parr hydrogenation apparatus. The reaction mixture was filtered through celite and volatiles were removed in a rotary evaporator under reduced pressure. The residue was extracted with EtOAc ($3 \times 150\text{ mL}$). The combined organic layers were washed with water and dried on anhydrous MgSO_4 . The crude residue was purified using column chromatography (EtOAc/Hexane, 1/10), and (*5Z*)-14-bromotetradeca-5-ene (**6**) (14.5 g, 83% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.37–5.31 (m, 2H), 3.38 (t, 2H), 2.00–1.99 (m, 4H), 1.85–1.81 (m, 2H), 1.53 (s, 1H), 1.42–1.28 (m, 13H), 0.87 (m, 3H).

Z-13-Octadecen-3-yn-1-ol (**5**) A 176 mg sample of 0.44 mmol $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ was added to a 3-necked 150 mL flask attached to a circulating condenser maintained at $-78\text{ }^{\circ}\text{C}$, and 150 mL of ammonia was condensed in this flask by stirring for 30 min. Lithium metal (1.1 g, 157 mmol) was added slowly to the mixture, which turned from a brown color to white–gray after 30 min of stirring. A solution of 3-butyn-1-ol (**10**) (3.67 g, 52.31 mmol) in 10 mL of dry THF was then added dropwise to the reaction mixture. The resulting solution was stirred for 1 h, and compound (**6**) (12.0 g, 43.59 mmol) in THF (25 mL) was added slowly. Stirring was continued for 2 h at $-78\text{ }^{\circ}\text{C}$, after which the reaction mixture was stirred overnight at ambient temperatures to evaporate the ammonia completely. The residue was cautiously treated with 50 mL of saturated NH_4Cl and volatiles were then removed in a rotary evaporator under reduced pressure. The residue was extracted with EtOAc ($3 \times 150\text{ mL}$). The combined organic layers were washed with water and dried on anhydrous MgSO_4 . The crude residue was purified using column chromatography (EtOAc/Hexane, 1/10), and *Z*-13-octadecen-3-yn-1-ol (**5**) (8.6 g, 74% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.32 (m, 2H), 3.70–3.63 (m, 2H), 2.41 (m, 2H), 2.13 (m, 2H), 2.00–1.96 (m, 4H), 1.55 (m, 1H), 1.46 (m, 2H), 1.26 (m, 14 H), 0.87 (m, 3H).

(*E,Z*)-3,13-Octadecadien-1-ol (**4**) Lithium aluminum hydride (LAH) (2.14 g, 56.47 mmol) and diethylene glycol

dimethyl ether (diglyme) (120 mL) were added to a 250 mL round-bottom flask, and compound (**5**) (4.0 g, 15.13 mmol) in 15 mL of dry THF was added slowly to this solution. The mixture was stirred at a reflux condition for 72 h. The reaction mixture was then cooled to $0\text{ }^{\circ}\text{C}$ and cautiously treated with saturated NH_4Cl (50 mL). The reaction mixture was extracted with EtOAc ($3 \times 150\text{ mL}$) and then washed sequentially with water and brine. The combined organic layers were dried over MgSO_4 . The crude residue was purified using column chromatography (EtOAc/Hexane, 1/9), and pure (*E,Z*)-3,13-octadecadien-1-ol (**4**) (2.9 g, 72% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.56–5.51 (m, 1H), 5.33 (m, 3H), 3.60 (m, 2H), 2.25–2.23 (m, 2H), 2.00 (m, 6H), 1.55–1.25 (m, 16H), 0.87 (m, 3H).

(*E,Z*)-3,13-Octadecadienyl acetate (**2**) Pyridine (1.12 g, 14.20 mmol) was added to compound (**4**) (2.5 g, 9.38 mmol) in 120 mL of dry dichloromethane at room temperature in a N₂ atmosphere. After stirring for 15 min, acetic anhydride (1.45 g, 14.20 mmol) was added dropwise at $0\text{ }^{\circ}\text{C}$. The reaction temperature was allowed to warm to room temperature and stirred for 15 h, and 100 mL of water was added to the reaction mixture and then extracted with dichloromethane ($2 \times 100\text{ mL}$). The combined organic layers were washed with water, dried over MgSO_4 , filtered, and concentrated under reduced pressure. Crude material was purified by column chromatography (EtOAc/Hexane, 1/9) and (*E,Z*)-3,3-octadecadienyl acetate (**2**) (2.49 g, 86% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ 5.50–5.48 (m, 1H), 5.37–5.31 (m, 3H), 4.07–4.04 (t, $J = 6.96\text{ Hz}$, 2H), 2.39–2.35 (m, 2H), 2.07–1.99 (m, 9H), 1.35–1.27 (m, 16H), 0.90–0.88 (m, 3H). $^{13}\text{C NMR}$ (150 MHz, CDCl_3): δ 171.09, 133.01, 129.87, 124.24, 64.00, 31.97, 29.76, 29.60, 29.51, 29.50, 29.29, 27.31, 27.19, 26.92, 26.83, 22.35, 20.98, 13.99.

(*Z,Z*)-3,13-Octadecadien-1-ol (**3**) Compound (**5**) (4.0 g, 63.22 mmol), 5% Pd/BaSO₄ (0.25 g), and quinoline (25 mg) in MeOH (50 mL) were placed in a 250 mL Parr reaction bottle and shaken in an H₂ atmosphere at 20 psi for 2 h using a Parr hydrogenation apparatus. The reaction mixture was filtered through celite, and volatiles were removed in a rotary evaporator under reduced pressure. The residue was extracted with EtOAc ($3 \times 150\text{ mL}$). The combined organic layers were washed with water and dried on anhydrous MgSO_4 . The crude residue was purified using column chromatography (EtOAc/Hexane, 1/9). Pure (*Z,Z*)-3,13-octadecadien-1-ol (**3**) (3.6 g, 90% yield) was obtained as a colorless liquid. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 5.56–5.50 (m, 1H), 5.35–5.30 (m, 3H), 3.61 (m, 2H), 2.31–2.29 (m, 2H), 2.04–1.94 (m, 6H), 1.29–1.22 (m, 16H), 0.85 (t, 3H).

(*Z,Z*)-3,13-Octadecadienyl acetate (**1**) Pyridine (1.12 g, 14.20 mmol) was added to 2.5 g of 9.38 mmol compound (**3**) in 120 mL of dry dichloromethane at room temperature under an N₂ atmosphere. After stirring for 15 min, 1.45 g of

14.20 mmol acetic anhydride was added dropwise at 0 °C. The reaction temperature was allowed to warm to room temperature, stirred for 15 h, 100 mL of water was added to mixture, and extraction was performed with dichloromethane (2×100 mL). The combined organic layers were washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure. Crude material was purified by column chromatography (EtOAc/Hexane, 1/9). (*Z,Z*)-3,13-Octadecadienyl acetate (**1**) (2.53 g, 87% yield) was obtained as a colorless liquid. ¹H NMR (600 MHz, CDCl₃): δ 5.52–5.48 (m, 1H), 5.37–5.31 (m, 3H), 4.07–4.04 (t, *J* = 6.96 Hz, 2H), 2.39–2.35 (m, 2H), 2.06–1.99 (m, 9H), 1.35–1.27 (m, 16H), 0.90–0.88 (m, 3H). ¹³C NMR (150 MHz, CDCl₃): δ 171.09, 133.62, 129.87, 124.96, 64.15, 32.60, 31.97, 31.96, 29.76, 29.50, 29.47, 29.40, 29.30, 29.12, 27.19, 26.92, 22.35, 20.98, 13.99.

Gas chromatography–mass spectrometry of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc*

The purity of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* was determined using a gas chromatograph (Agilent 7890A)–mass spectrometer (Agilent 5975C MSD, Agilent Technologies, Santa Clara, CA, USA) (GC–MS) equipped with a HP-5MS column (30 m×0.25 mm i.d., 0.25 μm film thickness, Agilent, CA, USA). The oven temperature for the HP-5MS column was initially set at 70 °C for 1 min, increased to 180 °C (10 °C/min), and increased to 220 °C (5 °C/min), and finally to 325 °C at a rate of 25 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Electron impact (70 eV, source temperature 230 °C) was used to obtain ionization, with a scan range of 41–400 amu.

Field experiment

Field trapping experiments were conducted on roadside cherry trees in several areas of South Korea from 2017 to 2019 (Fig. 2). Bucket [Korea Institute of Insect Pheromone (KIP), Daejeon, Republic of Korea] and delta traps (Green Agro Tech, Gyeongsan, Republic of Korea) were prepared using sleeve-stopper septa with a bottom internal diameter of 2.4 mm and an outer diameter of 5.3 mm (Sigma-Aldrich, Milwaukee, WI, USA) loaded with a hexane solution containing 1 mg of synthetic pheromones and an equivalent amount of 99.0% pure 2,6-di-*tert*-butyl-4-methylphenol (BHT) (Samchun Chemicals, Pyeongtaek, Republic of Korea). Traps baited with synthetic pheromones were hung on a cherry tree branch approximately 3 m above the ground. The distance between the traps within the same block was at least 15 m. Each block was at least 50 m from surrounding blocks. The number of males caught in traps was counted and the position of each trap was re-randomized within the

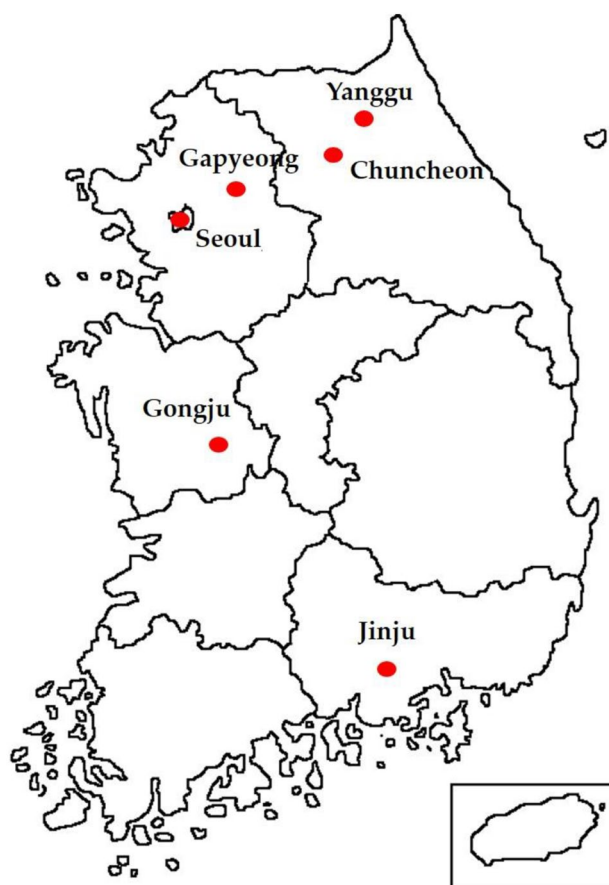


Fig. 2 Field experiment study sites

block every two weeks to minimize the effect of trap position. Pheromone lures were changed monthly.

Experiment 1 Attraction of male *S. bicingulata* to 1:9, 4:6, 6:4 and 9:1 (w:w) mixture of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* was tested in Gongju (N36°21'29.9", E127°14'52.0") from April 28 to June 13, 2017. Control traps received only hexane. In 2018, 1:9, 2:8, 3:7, 4:6 and 5:5 (w:w) mixture of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* was tested in Jinju (N35°11'58.7", E128°10'214"), Gongju (N36°21'29.9", E127°14'52.0"), and Gapyeong (N37°46'57.2", E127°28'07.3") from 27 August to 4 October. Green and yellow bucket traps were used in 2017 and 2018, respectively, and traps were supplied by the KIP. Traps were installed in a randomized complete-block design with four replicates.

Experiment 2 The efficacy of the two different types of traps (delta and bucket) was investigated in Chuncheon (N37°55'42.4", E127°46'27.3") from August 9 to September 25, 2017. Green bucket and white delta traps were purchased from KIP and Green Agro Tech, respectively. The ratio of *E3,Z13-18:OAc* to *Z3,Z13-18:OAc* was 4:6. Traps were installed in a randomized complete-block design with five replicates.

Experiment 3 The effect of two different colors of bucket traps on the capture of *S. bicingulata* males was investigated in Gongju (N36°21'29.9", E127°14'52.0") and Seoul (N37°31'57.8", E126°55'10.2") from April 26 to June 7, 2018. Green and yellow bucket traps (Fig. 3) were supplied by KIP. The ratio of E3,Z13-18:OAc to Z3,Z13-18:OAc was 4:6. Traps were installed in a randomized complete-block design with four replicates.

Experiment 4 The effect of six different bucket trap colors (black, blue, green, red, yellow, and white) (Fig. 3) on the capture of *S. bicingulata* males was investigated in Gapyeong (N37°46'57.2", E127°28'07.3") and Gongju (N36°21'29.9", E127°14'52.0") from August 27 to September 28, 2018, and in Yanggu (N38°05'33.1", E127°59'32.5") from August 19 to October 6, 2019. The ratio of E3,Z13-18:OAc to Z3,Z13-18:OAc was 4:6. Traps were installed in a randomized complete-block design with three replicates at Gongju and Gapyeong and four replicates at Yanggu.

Color spectrometry analysis

Bucket-trap surface-color values (L^*a^*b) and reflectance spectra at wavelengths between 360 and 750 nm were measured with a color spectrophotometer (ColorMate, SCINCO, Seoul, Republic of Korea). L^* indicates a measure of "lightness"; black (0) to white (100). Symbol a^* indicates a red shade when greater than zero (+) and a green shade when lower than zero (-). Symbol b^* indicates a yellow shade

when greater than zero (+) and a blue shade when lower than zero (-).

Statistical analysis

The mean numbers of male *S. bicingulata* caught in two different trap types (Experiment 2) and two different color bucket traps (Experiments 3) were compared using a *t* test. The results of experiments 1 and 3 were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's HSD test (IBM SPSS Statistics 23.0). Mean (\pm SE) values for untransformed data were reported. R (ver. 3.5.1. R Foundation for Statistical Computing, Austria) and R studio (ver. 1.1.456, R Studio Inc. MA, USA) were used for linear regression analysis of the effect of trap surface-color values on trap capture (Mendiburu 2020).

Results

GC-MS data analysis of E3,Z13-18:OAc and Z3,Z13-18:OAc

Total ion chromatograms of synthetic E3,Z13-18:OAc and Z3,Z13-18:OAc are shown in Fig. 4. The purities of E3,Z13-18:OAc and Z3,Z13-18:OAc were 94.18% and 82.8%, respectively, as shown in Fig. 4a, b. EI-MS m/z (RT 13.670): 308 (1), 248 (8), 233 (1), 219 (3), 205 (3), 191 (3), 177 (3), 163 (5), 149 (10), 135 (18), 121 (20), 109 (25), 95

Fig. 3 Colored bucket traps used in the field experiment



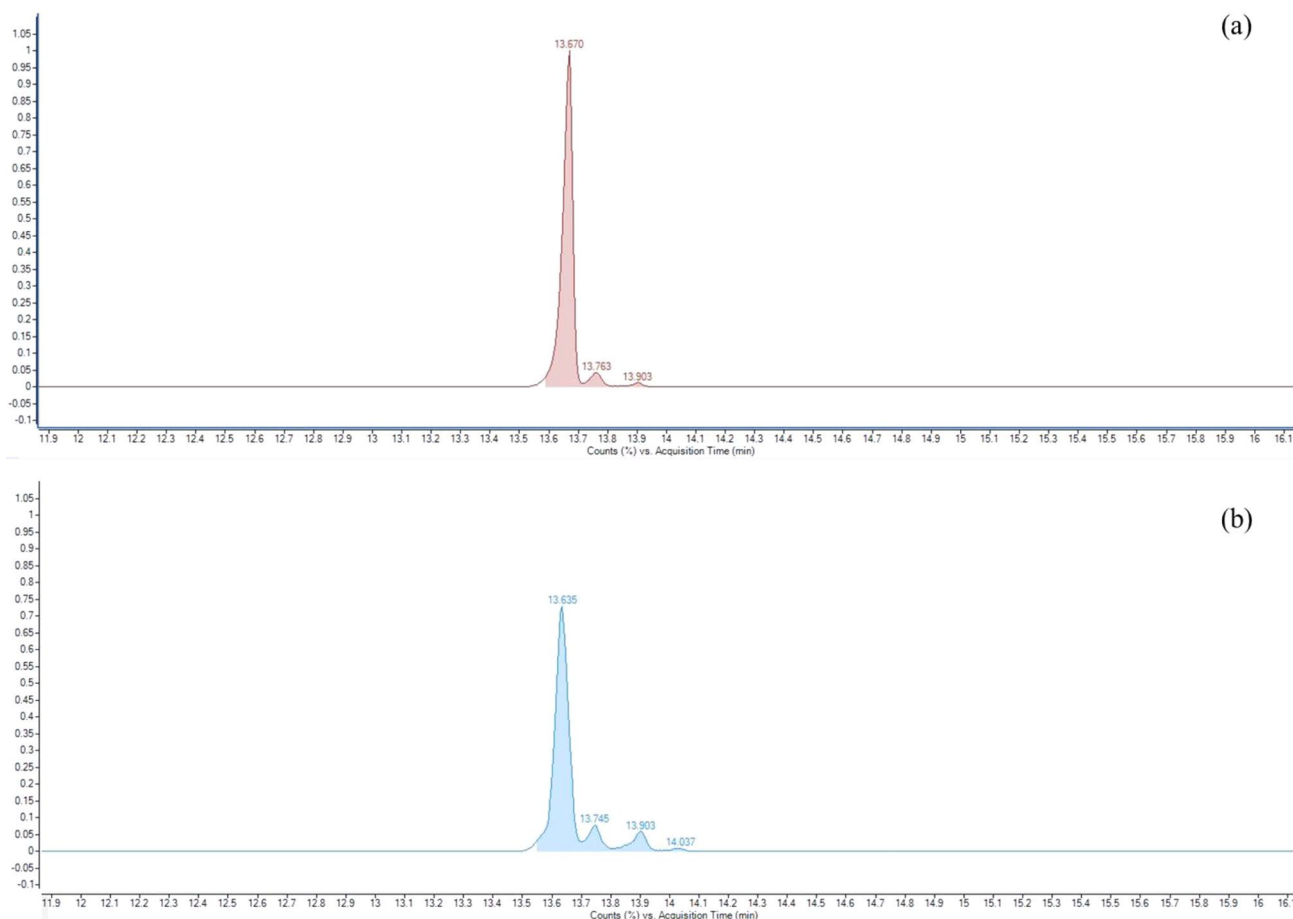


Fig. 4 Total ion chromatograms of *E3,Z13-18:OAc* (a) and *Z3,Z13-18:OAc* (b)

(47), 81 (69), 67 (86), 55 (78), 43 (100). EI-MS m/z (RT 13.635): 308 (1), 248 (8), 233 (1), 219 (2), 205 (2), 191 (2), 177 (3), 163 (5), 149 (10), 135 (18), 121 (20), 109 (25), 95 (47), 81 (70), 67 (84), 55 (79), 43 (100). Gas chromatography–mass spectrometry (GC–MS analysis) of synthetic *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* indicated that the two pheromone compounds did not contain isomeric compounds (Fig. 4).

Effect of pheromone blends on capture of *S. bicingulata* males

The number of the adult male moths caught in traps baited with ratios of 1:9, 4:6, 6:4, and 9:1 for *E3,Z13-18:OAc* to *Z3,Z13-18:OAc* is shown in Fig. 5a. Among test ratios of 1:9, 4:6, 6:4, and 9:1, a 4:6 mixture was the most attractive, followed by 1:9 and 6:4. No adult males were caught in traps baited with 9:1 or control traps. The number of the adult male moths caught in traps baited with ratios of 1:9, 2:8, 3:7, 4:6, and 5:5 for *E3,Z13-18:OAc* to *Z3,Z13-18:OAc* in Jinju, Gongju, and Gapyeong are shown in Fig. 5b–d. No regional differences in the response of *S. bicingulata* males

to sex pheromones were observed. A significant difference in the number of male adults caught in traps baited with 4:6 and 2:8 ratios was observed at all test areas in 2018. Significantly more males were attracted to traps baited with a 4:6 ratio of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* compared with traps baited with a 3:7 ratio of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* at Gonju in 2018.

Effect of trap type on capture of *S. bicingulata* males

The effect of the two trap types on the capture of *S. bicingulata* males is shown in Fig. 6. A significant difference was seen in the number of *S. bicingulata* males caught in bucket and delta traps ($t = 4.174$, $p < 0.01$, $df = 8$).

Color spectrometry analysis

Trap surface-color values ($L^*a^*b^*$) are shown in Table 1. The highest L^* value was obtained from a white bucket trap, followed by yellow, red, green, blue, and black bucket traps. Red and green bucket traps showed the highest and the lowest a^* values, respectively. The highest b^* values

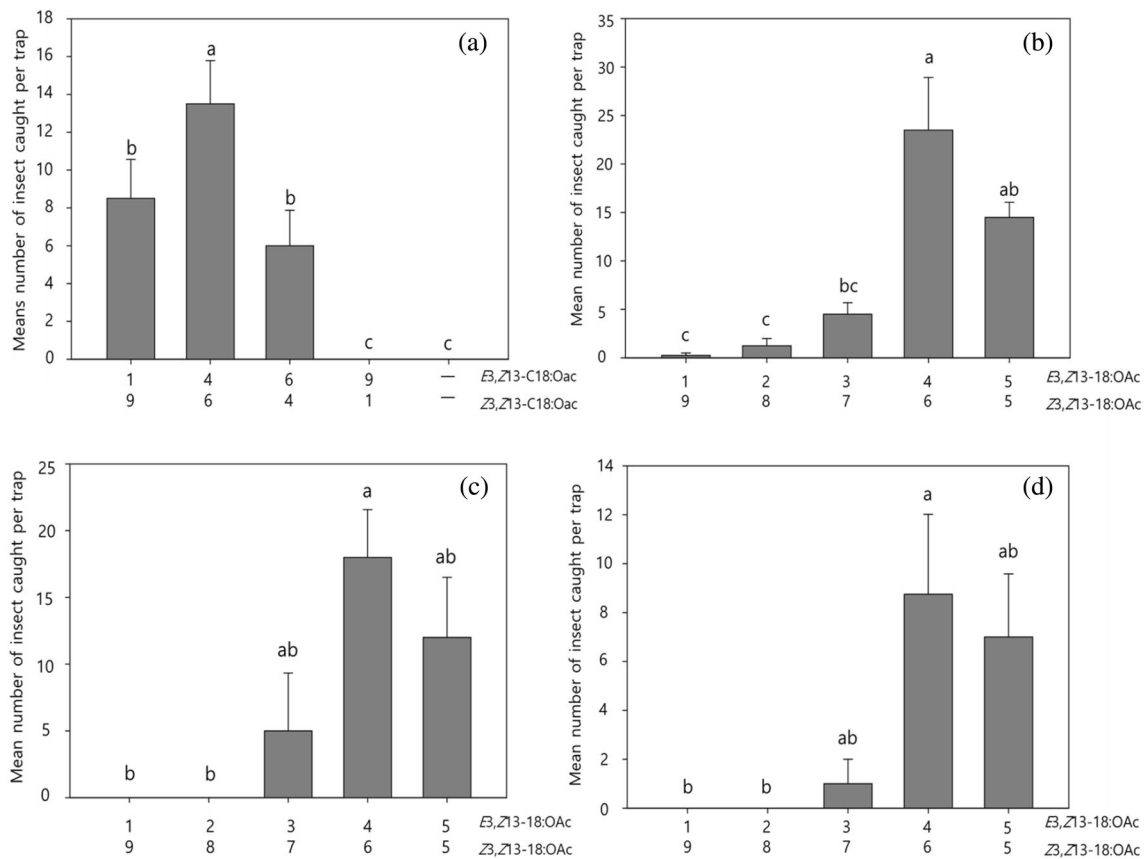


Fig. 5 Number of *S. bicingulata* adult males caught in traps baited with different ratios of E3,Z13-18:OAc and Z3,Z13-18:OAc in 2017 [Gongju (a), Tukey's HSD, $F_{4,15}=38.567$, $p<0.001$] and in 2018

[Jinju (b), $F_{4,15}=14.656$, $p<0.001$]; Gongju (c), $F_{4,15}=5.962$, $p=0.004$; Gapyeong (d), $F_{4,15}=4.787$, $p=0.011$]

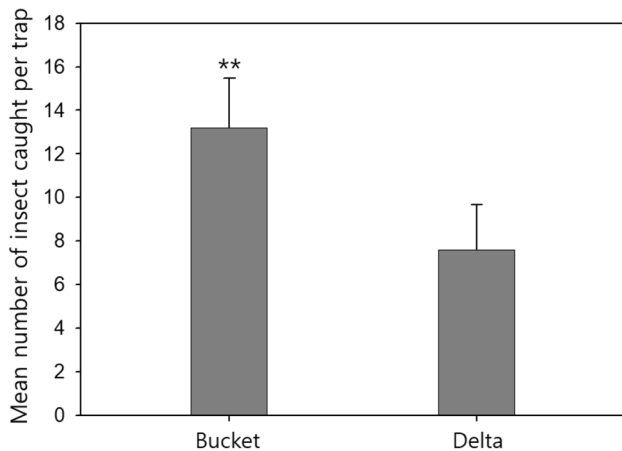


Fig. 6 Number of *S. bicingulata* adult males caught in bucket and delta traps (Chuncheon, $t=4.174$, $p<0.01$, $df=8$)

was obtained from a yellow bucket trap, followed by red, green, white, black and blue bucket traps. The reflectance rates of the six different colors of bucket traps in wavelength between 360 and 750 nm are provided in Fig. 7

Table 1 Trap surface-color value ($L^*a^*b^*$) measured by chromameter

Trap color	L^*1	a^*2	b^*3
White	94.89	-1.09	-1.86
Yellow	59.29	6.88	45.80
Red	41.25	46.70	21.48
Green	33.41	-11.28	0.46
Blue	27.60	3.81	-16.79
Black	26.03	1.49	-4.20

¹ L^* indicates a measure of "lightness"; black (0) to white (100)
² a^* indicates a red shade when greater than zero (+) and a green shade when lower than zero (-)
³ b^* indicates a yellow shade when greater than zero (+) and a blue shade when lower than zero (-)

and Table S1 (Online Resource 1). White bucket traps exhibited > 80% reflectance from 410 to 730 nm. Red and yellow bucket traps showed > 30% reflectance from 605 to 750 nm, and from 545 to 705 nm, respectively. Green, blue, and black bucket traps showed less than 14% reflectance at all test wavelengths.

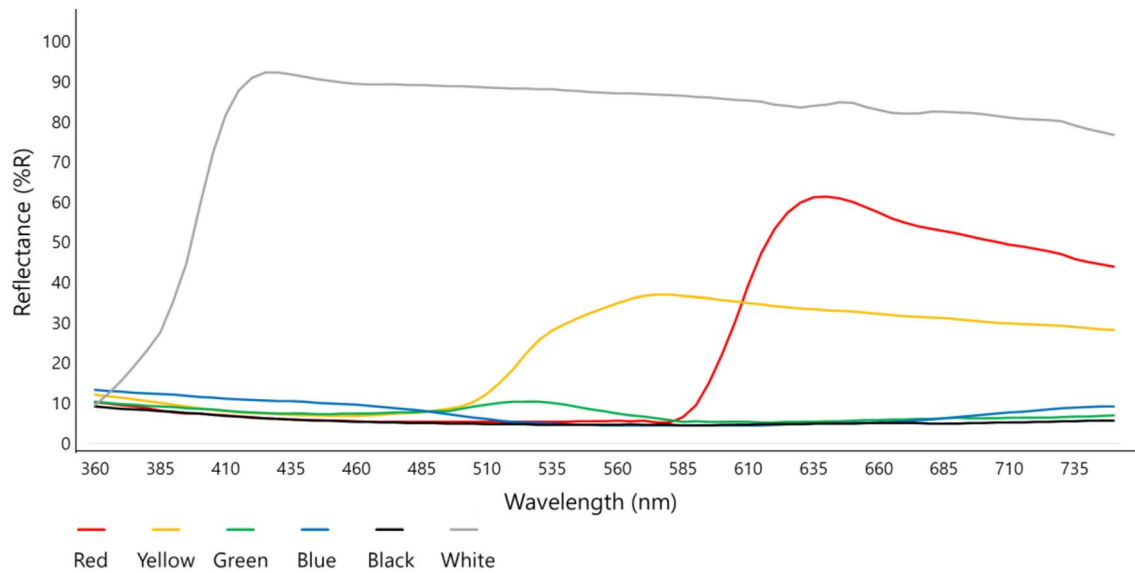


Fig. 7 Reflectance (%) of six colors of bucket traps at wavelengths between 360 and 750 nm

Effect of bucket trap color on capture of *S. bicingulata* males

The effect of yellow and green bucket traps on the capture of male *S. bicingulata* is shown in Fig. 8. A significant difference was evident in the number of male *S. bicingulata* caught in yellow and green bucket traps in Gongju ($t=6.983$, $p<0.001$, $df=6$) and Seoul ($t=3.174$, $p<0.05$, $df=6$). The effect of the six colors of bucket traps on the capture of male *S. bicingulata* are shown in Fig. 9. A significant difference was observed in the number of males caught in the six different colors of bucket traps. Yellow

bucket traps caught the largest number of male, and no adult male was caught in a blue bucket trap at Gongju. There was a significant difference in the number of *S. bicingulata* males caught in yellow bucket traps compared with the other colors of bucket traps at Yanggu. Linear regression analysis of the effect of trap surface-color value ($L^*a^*b^*$) on the capture of males is shown in Fig. 9d–f, respectively. A positive relationship was evident between b^* value and trap capture ($R^2=0.389$, $p<0.001$). A weak relationship was observed between L^* value and trap capture ($R^2=0.085$, $p=0.023$). No relationship was found between a^* value and trap capture ($R^2=0.005$, $p=0.567$).

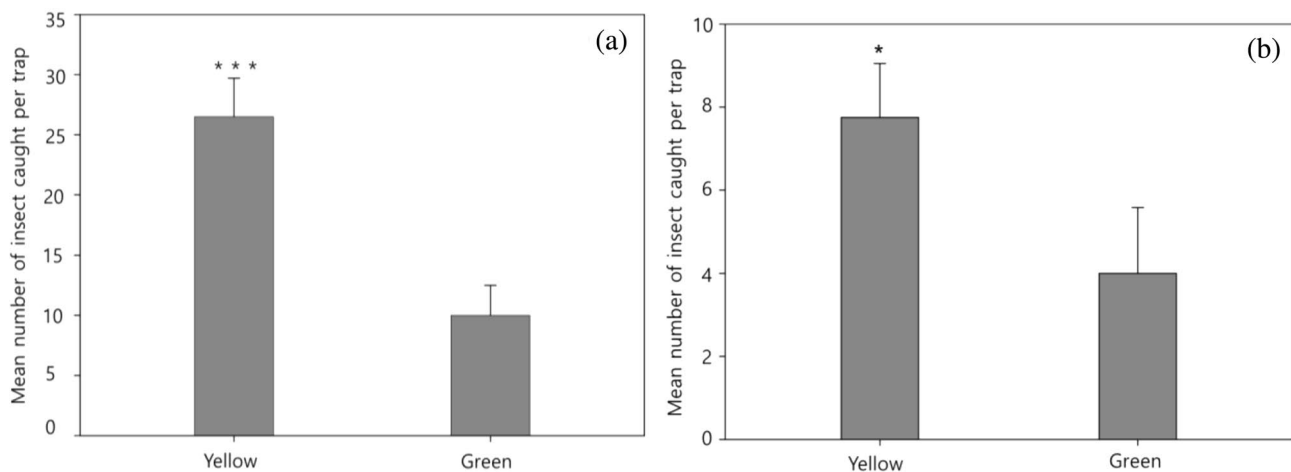


Fig. 8 Number of *S. bicingulata* adult males caught in two different colors of bucket traps [Gongju (a), $t=6.983$, $p<0.001$, $df=6$; Seoul (b), $t=3.174$, $p<0.05$, $df=6$]

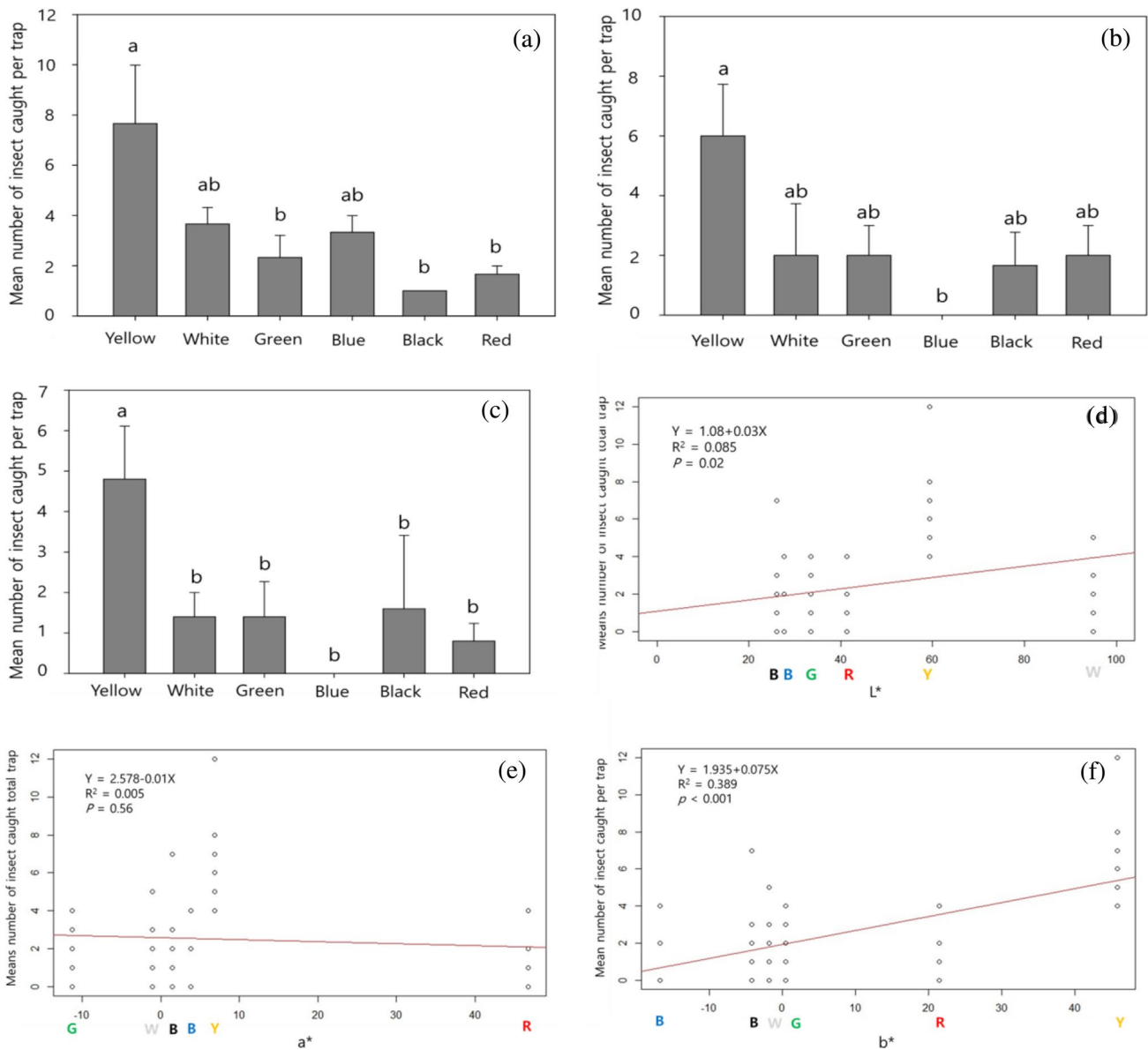


Fig. 9 Number of *S. bicingulata* adult males caught in bucket traps of six different colors [Gapyeong (a), Tukey's HSD, $F_{5,12}=4.668$, $p=0.0134$; Gongju (b), $F_{5,12}=5.305$, $p=0.0084$; Yanggu (c),

$F_{5,18}=5.470$, $p=0.0031$] and linear regression analysis of the effect of trap surface-color value [L* (d); a* (e); b* (f)] on trap capture

Discussion

In this study, we synthesized two sex pheromone components of *S. bicingulata*, $E3,Z13-18:OAc$ and $Z3,Z13-18:OAc$, and investigated the optimal sex pheromone ratio and the effect of trap type and color on the capture of *S. bicingulata* males. The largest number of *S. bicingulata* males was caught in traps baited with a 4:6 mixture of $E3,Z13-18:OAc$ and $Z3,Z13-18:OAc$ in this and previous study (Yang et al. 2011). However, a difference was evident in the interest shown by *S. bicingulata* males to other $E3,Z13-18:OAc$ -to- $Z3,Z13-18:OAc$ ratios between this study and the Yang et al.

(2011) study. Yang et al. (2011) reported no significant difference in the numbers of male adults caught in traps baited with 6:4, 5:5, 4:6, 3:7, and 2:8 ratios for $E3,Z13-18:OAc$ to $Z3,Z13-18:OAc$. The largest number of *S. bicingulata* males was caught in traps baited with a 4:6 ratio followed by 2:8, 3:7, 6:4, and 5:5 ratios. However, the largest number of *S. bicingulata* males was caught in traps baited with a 4:6 ratio, followed by 5:5, 3:7, 2:8, and 1:9 in this study. In addition, there was a significant difference in the numbers of male adults caught in traps baited with 4:6 and 2:8 ratio of $E3,Z13-18:OAc$ to $Z3,Z13-18:OAc$ in our study. This may be a product of differences in the purity of pheromone

components. The previous study by Yang et al. (2011) used *E3,Z13-18:OAc* and *Z3,Z13-18:OAc* containing an isomeric compound (a mixture of *E,Z* and *Z,Z*) for field experiments, and this may have caused the similar attractiveness of *S. bicingulata* to several ratios of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc*. However, the authors did not supply information on the isomeric ratio of *E3,Z13-18:OAc* and *Z3,Z13-18:OAc*, and the most attractive ratio could not be determined, whereas we synthesized and purified two pure components without a mixture of isomers in the laboratory. Based on the purity of the two pheromone components, we concluded that the optimal ratio of *E3,Z13-18:OAc* to *Z3,Z13-18:OAc* for *S. bicingulata* adult males was approximately 4.3:5.7.

Trap capture are greatly affected by pheromone trap design for many insect species (Malo et al. 2001; Athnaassiou et al. 2004, 2007; Strong et al. 2008; Kim and Park 2013). Various pheromone trap designs, such as delta traps, Jackson traps, wing-style, and bucket traps have been used for Sesiidae moths (Bakowski 2001; Adler 1983; Pfeiffer et al. 1999; Weihman and Liburd 2007). Zhang et al. (2013) compared the efficacy of delta, wing-style, and bucket traps for the dogwood borer *Synanthedon scitula* but could not identify a clearly superior single trap design. No reports have been published on the effect of trap design on the capture of *S. bicingulata* males, and only delta traps have been used for field experiments with the *S. bicingulata* sex pheromone (Yang et al. 2011, 2012; Cho et al. 2016; Lee et al. 2017). This study showed that bucket traps were superior to delta traps for capturing *S. bicingulata* males.

Colored traps baited with sex pheromones affected the capture of *S. bicingulata* males in this study. Previous studies have reported an effect of the color of traps baited with sex pheromones on the capture of clearwing moths (Suckling et al. 2005; Judd and Eby 2013; Karlius and Būda 2007) and other insect species (Athnaassiou et al. 2004, 2007; Gadi and Reddy 2014; Abuagla and Al-Deeb 2012). Athanassiou et al. (2004) reported that more *Palpita unionalis* males were caught in white bucket trap with strong light reflectance at 370–450 nm compared with brown bucket traps with weak light reflectance. Suckling et al. (2005) also reported that more *Synanthedon tipuliformis* males were caught in yellow and green delta traps compared with red, white, black, and blue delta traps. Judd and Eby (2013) investigated the effect of various solid and multicolored bucket traps on the capture of *Synanthedon myopaeformis* males; more were attracted to bucket traps with a yellow lid. Judd and Eby argued that color with reflectance in the green region of the light spectrum (500–550 nm) was an important factor affecting trap capture. However, white bucket traps with a high intensity of reflectance in the green region were associated with low capture of male *S. myopaeformis*. This suggests that *S. myopaeformis*, like *S. tipuliformis* (Karlius and Būda 2007), has blue-sensitive photoreceptors that enable discrimination of

white and yellow bucket traps using differences in their blue-green spectral compositions. Similar results were observed in this study. Yellow bucket traps with reflectance in the green region (Fig. 7, Table S1 in online Resource 1) were the most attractive, but capture with white bucket traps were lower than those with yellow bucket traps. *S. bicingulata* may be able to discriminate yellow and white bucket traps using differences in their blue–green spectral compositions, as is the case with *S. myopaeformis* (Judd and Eby 2013) and *S. tipuliformis* (Karlius and Būda 2007). Bucket traps of other colors with low reflectance in the green region were associated with low trap capture in this study. Analysis of trap surface-color values indicated that *S. bicingulata* males were most affected by b^* values rather than L^* and a^* values (Fig. 9). The results of this study suggest that male *S. bicingulata* uses both olfactory and visual cues to locate female adults. However, it is unclear why some clearwing moths are strongly attracted to patches of yellow. Based on the day-flying activity of *S. myopaeformis*, Judd and Eby (2013) hypothesized that a yellow bucket trap mimics patches of sunlit foliage of host trees and that males preferentially head toward to a yellow trap in search of females when baited with pheromones. This study showed that a yellow bucket trap baited with 4.3:5.7 ratio of *E3,Z13-18:OAc* to *Z3,Z13-18:OAc* could improve the efficacy of monitoring of *S. bicingulata*.

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Author contributions JHK and IKP designed the research and wrote the first draft of manuscript. JHK conducted field experiments. DHL conducted the chemical syntheses. JHK and MJH analyzed data. SMS conducted chemical analysis. All authors contributed to the final version of the manuscript.

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Declarations

Conflict of interest We declare we have no competing interests.

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