

Cinnamyl alcohol: host-recognizing kairomone of the flower thrips *Frankliniella intonsa*

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Abstract

Flower-inhabiting thrips find hosts using olfactory and visual cues. In this study, we report the identification of a plant-produced kairomone of the flower thrips *Frankliniella intonsa* (Trybom), an important agricultural pest in northeast Asia. GC-MS analysis of solid-phase microextraction samples from blueberry flowers, *Vaccinium corymbosum* L., that mediate the attraction of adult *F. intonsa* revealed that the major component was cinnamyl alcohol, followed by cinnamyl acetate, cinnamaldehyde, germacrene D, β -bourbonene, β -caryophyllene, and benzyl benzoate. The biological activity of the floral compounds was investigated using commercial cinnamaldehyde, cinnamyl alcohol, β -caryophyllene, cinnamyl acetate, and benzyl benzoate in hot pepper (*Capsicum annuum* L.) fields. Significantly more *F. intonsa* males and females were caught in red delta traps with cinnamyl alcohol than in all other traps. Cinnamaldehyde and cinnamyl acetate attracted adult *F. intonsa* but were not as attractive as cinnamyl alcohol. β -Caryophyllene and benzyl benzoate were not attractive. Furthermore, the addition of four minor components to cinnamyl alcohol did not result in increased trap catches relative to cinnamyl alcohol alone, indicating that cinnamyl alcohol is responsible for attracting adult *F. intonsa* toward blueberry flowers. Therefore, this phenylpropanoid could be used as a selective, effective lure for monitoring and controlling *F. intonsa*.

Introduction

Thrips (Thysanoptera: Thripidae) are economically important pests of various agricultural crops in northeast Asia, causing damage by feeding and through the transmission of plant viruses (Okazaki and Sakurai, 2005; Moon et al., 2006; Wei et al., 2012). To control thrips, growers have depended exclusively on insecticide applications at regular intervals, but this can result in poorly timed or unnecessary insecticide applications. In addition, the heavy reliance on broad-spectrum insecticides to control these pests has resulted in insecticide resistance (Bielza, 2008; Cho et al., 2018). Consequently, alternative control methods are urgently needed. Volatile semiochemicals could be used to monitor the seasonal abundance of thrips and to control thrips by mass trapping and attract-and-kill tactics (Kirk et al., 2021).

There is increasing evidence that visual and olfactory cues are important for host-finding in flower-inhabiting thrips (Teulon et al., 1999; van Tol et al., 2020). The color preference of these thrips varies with species, and blue, yellow, and white are mainly effective for trapping (Kirk, 1984; Seo et al., 2006; Mao et al., 2018; van Tol et al., 2020). The behavioral activities triggered by olfactory attractants such as ethyl nicotinate (Penman et al., 1982; Teulon et al., 1993), p-anisaldehyde (Kirk, 1985; Teulon et al., 1993), methyl anthranilate (Murai et al., 2000), and methyl isonicotinate (van Tol et al., 2020), and host kairomones such as *cis*-jasmone (El-Sayed et al., 2009) and 6-pentyl-2H-pyran-2-one (El-Sayed et al., 2014) have been elucidated in several flower thrips.

In the course of a field study of crop damage caused by insect pests, we serendipitously observed the attraction of large numbers of the flower thrips *Frankliniella intonsa* (Trybom), an important pest of hot pepper in Korea (Moon et al., 2006), to highbush blueberry *Vaccinium corymbosum* L. (cultivar Darrow)

flowers, suggesting the existence of a host kairomone that mediates the attraction of adult *F. intonsa* to the flowers. Here, we report the results of chemical analyses of solid-phase microextraction samples collected from blueberry flowers and evaluate the biological activity of the identified compounds in hot pepper fields.

Materials And Methods

Chemicals

Five commercially available volatile compounds from blueberry flowers were used for field screening tests: cinnamaldehyde (95%, CAS no. 104-55-2), cinnamyl alcohol (98%, CAS no. 104-54-1), β -caryophyllene (80%, CAS no. 87-44-5), cinnamyl acetate (99%, CAS no. 103-54-8), and benzyl benzoate (99%, CAS no. 120-51-4) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Collection of headspace volatiles

Solid-phase microextraction (SPME) was used to collect headspace volatiles of highbush blueberry flowers. An SPME fiber (100- μ m polydimethylsiloxane coating; Supelco, Bellefonte, PA, USA) was conditioned before use for 30 min in a gas chromatography injector (250°C). The blueberry flowers were cut and immediately introduced into a 500-mL glass jar. The SPME fiber was then inserted into the jar through a small hole in the cap. After a 3-h exposure, the fiber was immediately subjected to gas chromatography–mass spectrometry (GC-MS).

Chemical analysis

The SPME-collected volatiles were analyzed on an Agilent 8890 GC system (Santa Clara, CA, USA) interfaced to an Agilent 5977B mass-selective detector. Samples were run on a DB-5MS column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m film; J&W Scientific, Folsom, CA, USA). The injector and transfer line temperatures were 250°C. To start the GC-MS analysis, the loaded SPME fibers were desorbed for 1 min in the injection port. The GC oven temperature was programmed from 80°C (1-min hold) to 200°C at 5°C/min and held for 10 min. Helium was the carrier gas (1 mL/min) and the injections were splitless. Electron impact mass spectra were monitored at 70 eV in a mass range of 40–300 amu, and the source was maintained at 230°C. Compounds were identified by matching mass spectra to the Wiley Mass Spectral Database; these identifications were confirmed by comparison of the retention times and mass spectra with those of authentic standards.

Field trials

Field trials were conducted in ‘Bangtanbog’ hot pepper (*Capsicum annuum* L.) fields in Kochang, South Korea (35.3°N, 126.4°E), during July and August 2021. Trial 1 investigated the attraction of adult *F. intonsa* to cinnamaldehyde, cinnamyl alcohol, β -caryophyllene, cinnamyl acetate, and benzyl benzoate singly. Trial 2 tested the attraction of adult *F. intonsa* to cinnamyl alcohol alone or in combination with cinnamaldehyde, β -caryophyllene, cinnamyl acetate, or benzyl benzoate in the ratio found in the blueberry

flower extract. Blank traps served as negative controls in all experiments and traps baited with a mixture of the five compounds were used as a positive control in Trial 2.

Test compounds were dissolved in 100 μ L diethyl ether and then stabilized by adding 1% butylated hydroxytoluene as an antioxidant. Candidate attractants were loaded into pink polyethylene sachets (2.5 $\times$ 6.0 cm, 100 μ m wall thickness; AD Corp., Andong, South Korea) and heat-sealed. Red delta traps containing a white adhesive-coated panel at the base (Green Agro Tech, Gyeongsan, South Korea) were baited with the sachets. Red delta traps were used because of their relative low catch of non-target species (El-Sayed et al., 2009). The delta traps were suspended from metal poles at the height of the red pepper canopy. The field test used a completely randomized block design with four replicates of each treatment. Traps were placed ~15 m apart within each block and the distance between blocks was approximately 50 m. Sticky bases were removed at 3- to 4-day intervals during each study. Thrips captured in the traps were identified and counted under a stereoscopic microscope.

Because the trap catch data were not normally distributed, differences were analyzed via nonparametric Kruskal–Wallis ANOVA followed by comparison of means using the Wilcoxon paired sample test (SAS Institute, 2014). In all analyses, α = 0.05. Males and females were analyzed separately.

Results

Chemical analysis

GC-MS analysis of the SPME samples from fresh blueberry flowers revealed one major compound and six minor compounds. No other component exceeded 5% of the major compound (Fig. 1). The mass spectrum of compound 1 (Rt 10.8 min) had fragment ions of m/z (abundance) 131 (100), 103 (55), 77 (44), and 51 (32), and matched that of cinnamaldehyde in a MS library search. The mass spectrum of compound 2 (Rt 11.3 min) had fragment ions of m/z 134 (66), 115 (51), 105 (53), 92 (100), 78 (61), and 51 (29), consistent with that of cinnamyl alcohol in the spectral library. Compound 3 (Rt 12.9 min) had a mass spectrum with fragment ions at m/z 204 (2), 161 (32), 123 (63), 81 (100), and 41 (20). This was tentatively identified as β -bourbonene by comparison with the mass spectral library data. The mass spectrum of compound 4 (Rt 13.6 min) had fragment ions of m/z 204 (6), 189 (14), 175 (8), 161 (29), 147 (27), 133 (75), 120 (38), 105 (52), 93 (94), 79 (74), 69 (82), 55 (40), and 41 (100), which matched β -caryophyllene in the spectral library. The mass spectrum of compound 5 (Rt 13.7 min) had fragment ions of m/z 176 (25), 134 (41), 115 (100), 105 (42), 92 (31), 77 (25), 51 (14), and 43 (94), and was virtually identical to that of cinnamyl acetate in the spectral library. Compound 6 (Rt 14.7 min) had a mass spectrum with fragment ions at m/z 204 (15), 161 (100), 147 (6), 133 (18), 119 (36), 105 (60), 91 (58), 81 (40), 67 (17), 55 (20), and 41 (38). This was tentatively identified as germacrene D on comparison with the mass spectral library data. The mass spectrum of compound 7 (Rt 20.3 min) had fragment ions at m/z 212 (21), 194 (8), 167 (6), 105 (100), 91 (50), 77 (34), 65 (12), and 51 (16), and matched that of benzyl benzoate in a library search.

Moreover, the retention times of compounds 1, 2, 4, 5, and 7 in the DB-5MS column matched those of authentic standards of cinnamaldehyde, cinnamyl alcohol, β -caryophyllene, cinnamyl acetate, and benzyl benzoate, respectively. The average ratio of compounds 1 to 7 in SPME samples of volatiles from blueberry flowers was 29:100:12:10:31:15:6 ($n = 3$).

Field trials

The field trial testing single compounds showed that significantly more females and males of *F. intonsa* were caught in traps with cinnamyl alcohol than in all other traps (females, $\chi^2 = 19.6703$, $P = 0.0014$; males, $\chi^2 = 15.5587$, $P = 0.0082$) (Fig. 1). Cinnamyl alcohol caught 42 times more females and 33 times more males than controls. Traps with cinnamaldehyde and cinnamyl acetate also caught more *F. intonsa* females than traps without volatiles (Fig. 1A). By contrast, there were no differences in the number of male thrips caught in traps baited with cinnamaldehyde or cinnamyl acetate and the unbaited control (Fig. 1B). This may be due to the relatively low numbers of males caught during the summer. The catches of male and female *F. intonsa* in traps baited with β -caryophyllene and benzyl benzoate did not differ from the catch in control traps.

Another field trial testing possible synergistic effects between cinnamyl alcohol and the other chemicals identified in blueberry flowers caught thousands of thrips, again indicating the strong attractiveness of cinnamyl alcohol (females, $\chi^2 = 13.6961$, $P = 0.0332$; males, $\chi^2 = 15.4339$, $P = 0.0171$) (Fig. 2). However, cinnamaldehyde, β -caryophyllene, cinnamyl acetate, and benzyl benzoate did not increase the trap catches when added to cinnamyl alcohol. Moreover, the full five-component blend did not increase the capture of adult male or female thrips relative to cinnamyl alcohol alone.

Discussion

Our data confirmed that cinnamyl alcohol is the major component of floral volatiles of highbush blueberry (Rodriguez-Saona et al., 2011). In the field, we found that traps with cinnamyl alcohol caught more adult *F. intonsa* than traps with the other compounds. Cinnamaldehyde and cinnamyl acetate also increased the trap capture of female *F. intonsa*, but not to the same extent as cinnamyl alcohol. In attractant field-screening experiments in the United States, Morgan and Crumb (1928) found that significantly more adults of *Frankliniella tritici* Fitch were caught in traps with cinnamaldehyde than in traps with cinnamyl alcohol in an oat field. The large difference between the two *Frankliniella* species in their responses to cinnamyl alcohol and cinnamaldehyde points to the functional and behavioral significance of floral scents in host location by flower-inhabiting thrips.

In the field trial testing chemical interactions between cinnamyl alcohol and the other compounds identified from blueberry flowers, the four minor compounds had no synergistic activity with cinnamyl alcohol alone. The lack of synergism from compound mixtures is similar to that observed for *Thrips obscuratus* (Crawford) (El-Sayed et al., 2009, 2014) and *Thrips tabaci* Lindeman (Teulon et al., 2007). This suggests relatively simple chemosensory input with single receptors for generic compounds or poor sensory integration between receptor neurons in thysanopteran insects.

It is unclear whether the response to floral volatiles differs between the sexes of flower-dwelling thrips. We observed that red delta traps with cinnamyl alcohol caught more male and female adult *F. intonsa* than delta traps without cinnamyl alcohol in hot pepper fields. Our result supports the finding by Teulon et al. (1993, 1999) that the addition of floral volatiles to traps significantly increases the capture of adult male and female thrips. Conversely, in a Y-tube olfactometer test, Cao et al. (2018) reported that female *Frankliniella occidentalis* preferred host plant floral volatiles, while male *F. occidentalis* did not show a preference for any floral odors. Thus, further tests with different volatile blends, and with different experimental conditions (e.g., distance to source and wind velocity) are required to clarify the sex-specific differences of the olfactory response to floral volatile components in thrips.

Many thrips species integrate visual information with olfactory information specific for host plant selection (Teulon et al., 1999; van Tol et al., 2020). Adult *F. intonsa* are predominantly attracted to blue and white over other colors (Seo et al., 2006; Mao et al., 2018). Besides cinnamyl alcohol identified here, the aldehydes *p*-anisaldehyde and benzaldehyde attracted *F. intonsa* during field screening tests (Kirk, 1985; Teulon et al., 1993). Consequently, it will be interesting to investigate the interaction between trap color and the presence of odors to obtain more effective monitoring and control agents for this thrips.

Declarations

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Author Contributions

CYY and MHS designed the experiments; CYY, MHS and JBY performed the experiments; CYY and MHS analyzed the data; CYY and JBY contributed reagents/materials/analysis tools; CYY wrote the paper.

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Code Availability: Not applicable.

Declarations

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

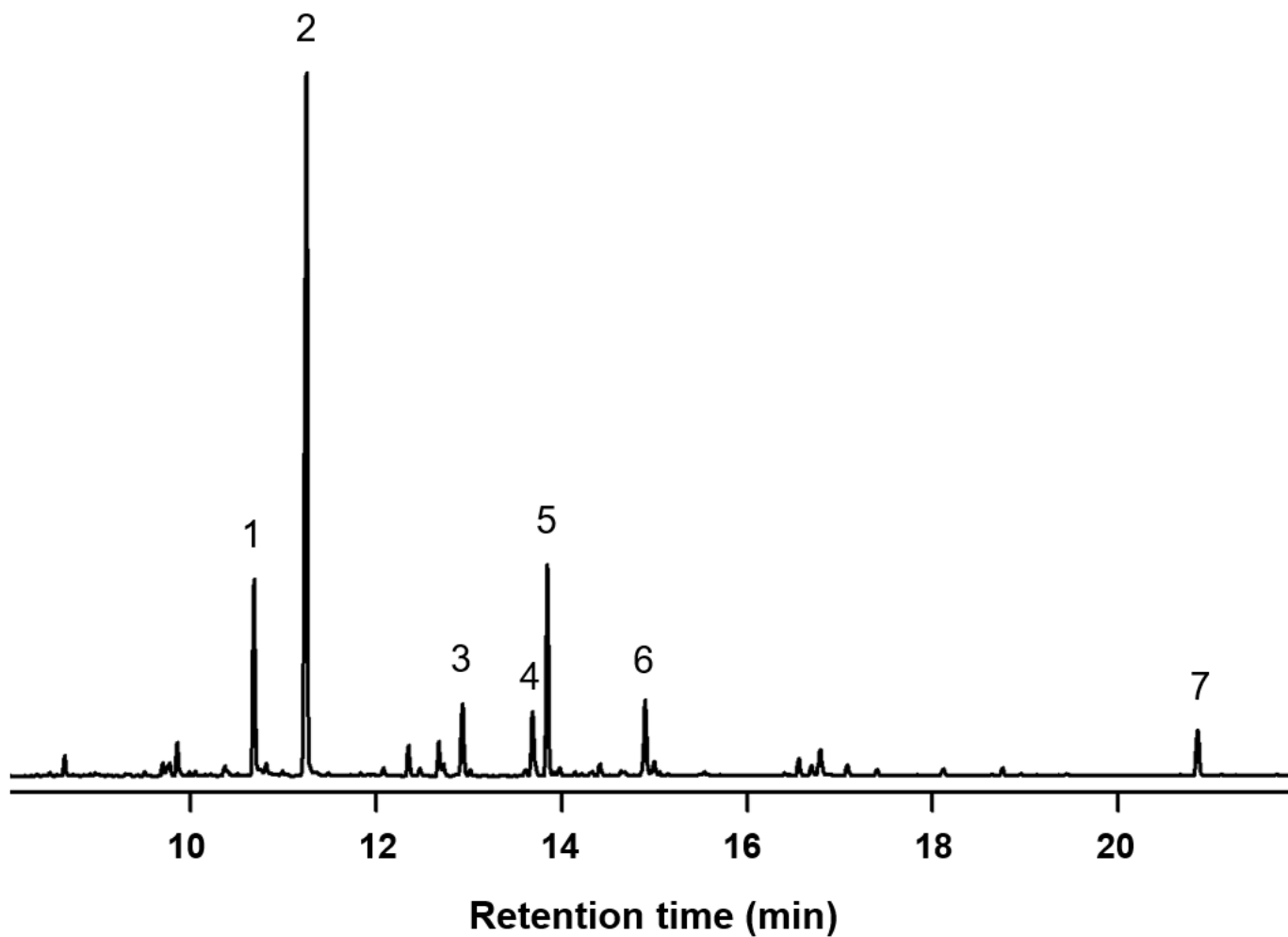


Figure 1

Gas chromatography–mass spectrometry (GC-MS) total ion chromatograms of SPME-collected volatiles from highbush blueberry flowers on a DB-5MS column.

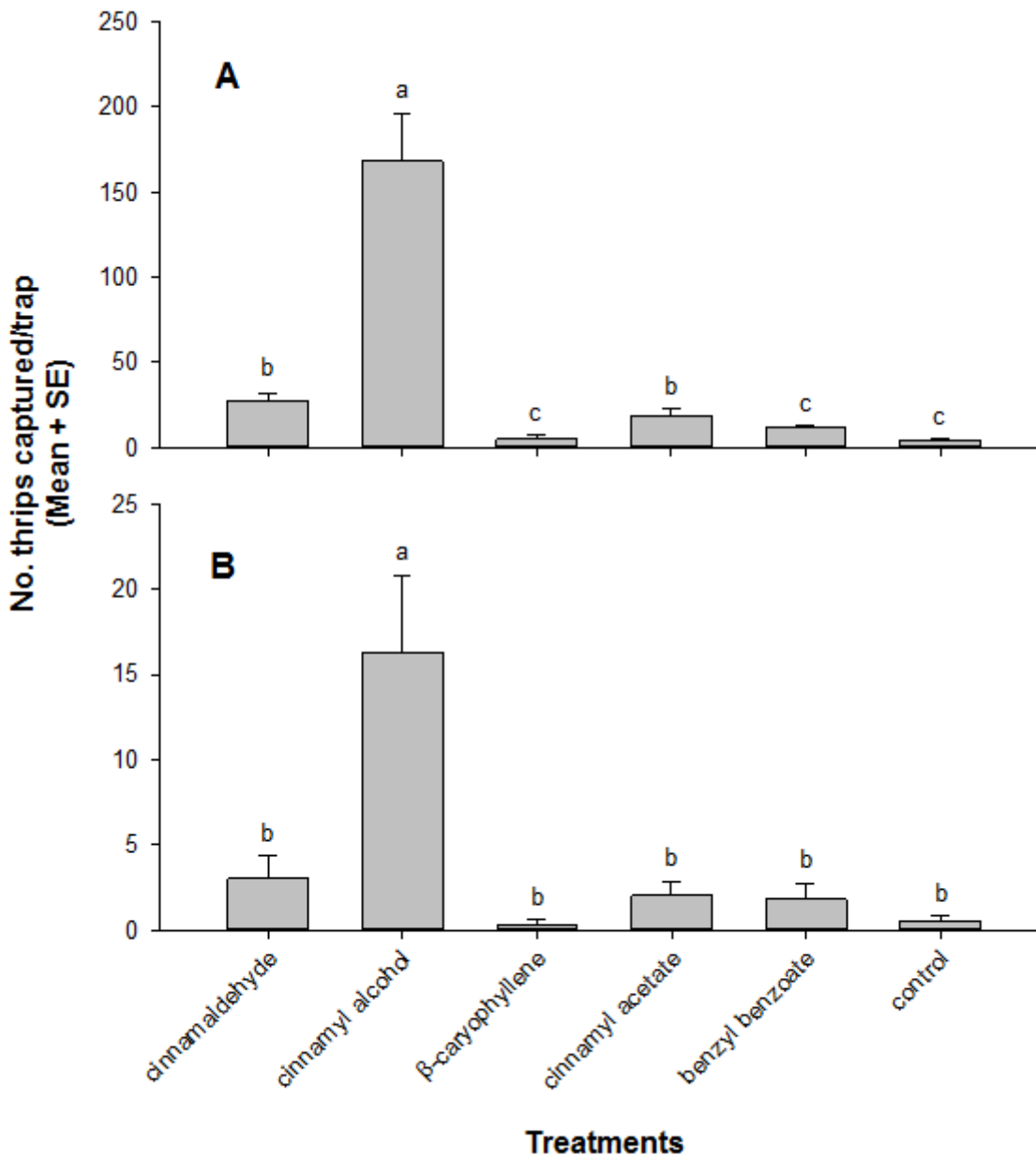


Figure 2

Catches of *Frankliniella intonsa* females (A) and males (B) in traps baited with five floral compounds (10 mg each) in red pepper fields in Kochang, South Korea, 16–22 July 2021 ($n = 5$). Bars with the same letter are not significantly different based on the Wilcoxon paired sample test ($P > 0.05$).

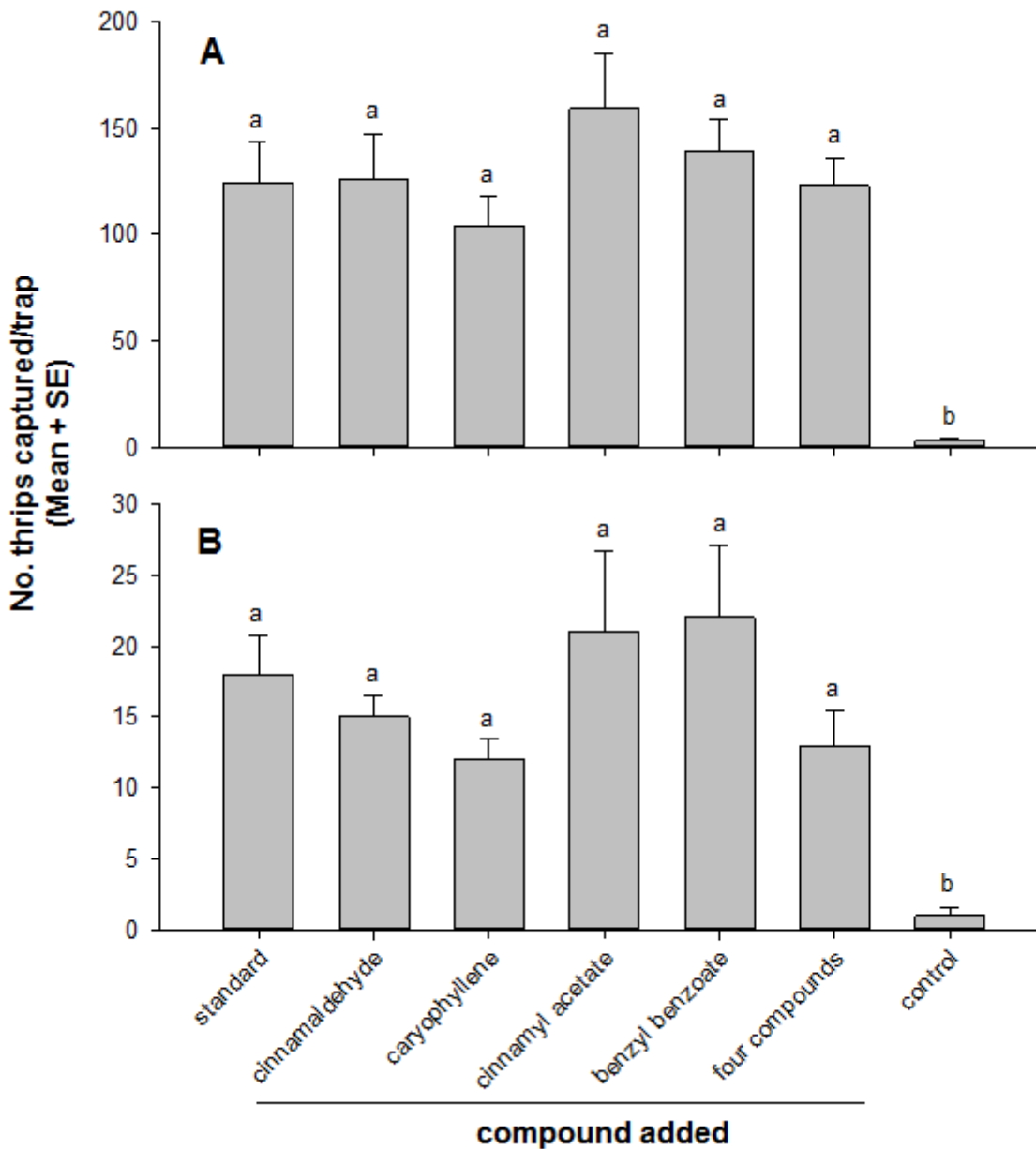


Figure 3

The effect of adding four minor compounds to the standard (cinnamyl alcohol; 10 mg) in the ratio found in blueberry flower extract on the capture of *Frankliniella intonsa* females (A) and males (B) in red pepper fields in Kochang, South Korea, 28 July to 3 August 2021 (n = 5). Bars with the same letter are not significantly different based on the Wilcoxon paired sample test ($P > 0.05$).