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Recruitment of Predator *Cheilomenes sexmaculata* by Active Volatiles from Lemon Plants Infested with *Frankliniella intonsa*

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Abstract

The flower thrips, *Frankliniella intonsa*, is a major pest threatening citrus production. However, chemical control remains the primary management measure, which poses significant risks on ecosystems. Hence, it is urgent to prioritize more eco-friendly measures to efficiently control thrips. The ladybird, *Cheilomenes sexmaculata*, is a predominant natural enemy in the local citrus agroecosystem and could play a key role in suppressing thrips in agricultural landscapes. Although some ladybirds are known to be attracted to herbivore-induced plant volatiles (HIPVs), little is known about the specific attractive compounds and the effect of *F. intonsa*-infested lemon plants on the predatory response of *C. sexmaculata*. Here, we studied the chemical interaction between *F. intonsa*, *C. sexmaculata,* and lemon plants. In dual-choice behavioral assays, *C. sexmaculata* adults significantly preferred volatiles from *F. intonsa*-infested plants over those from healthy plants. Volatile collection and analysis identified six monoterpenes, five of which (α-pinene, β-pinene, sabinene, myrcene, and eucalyptol) individually attracted *C. sexmaculata* at specific concentrations. Moreover, a blend of these five compounds, formulated at their optimal attractive concentrations, elicited a stronger attraction in *C. sexmaculata* than individual compounds, indicating a synergistic interaction. This attractive blend can thus be used to develop a kairomone-based lure to enhance biological control and to complement existing integrated pest management approaches against thrips in lemon agroecosystems.

Keywords: HIPVs; citrus plants; attractant; terpene; biological control; integrated pest management

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1. Introduction

Thrips are one of the primary threats to global citrus production. Their nymphs and adults cause damage to flower buds and young fruits (Figure 1), thereby reducing the economic value of fruits [1,2]. The flower thrips, *Frankliniella intonsa* (Trybom) (Thysanoptera: Thripidae), is one of the dominant pests in citrus planting area, affecting pomelo, lemon, grapefruit, and navel orange [3,4]. It is difficult to control them due to their complex life cycle (e.g., eggs concealed within plant tissues, pupae dwelling in the soil, a broad host range, and high reproductive rates) and rapid adaptation to pesticides. Additionally, they are highly polyphagous and act as a vector for destructive plant viruses [5]. The virus is acquired by thrips larvae when they feed on infected plants, and is subsequently

transmitted to healthy plants. To date, *F. intonsa*, *Thrips palmi*, and *Megalurothrips usitatus* have been reported to act as vectors for the transmission of more than 19 distinct viruses, such as tobacco streak virus (TSV) and tomato spotted wilt virus (TSWV). Consequently, these factors together seriously diminish both the quality and yield of citrus fruits, making effective management measures essential for growers.

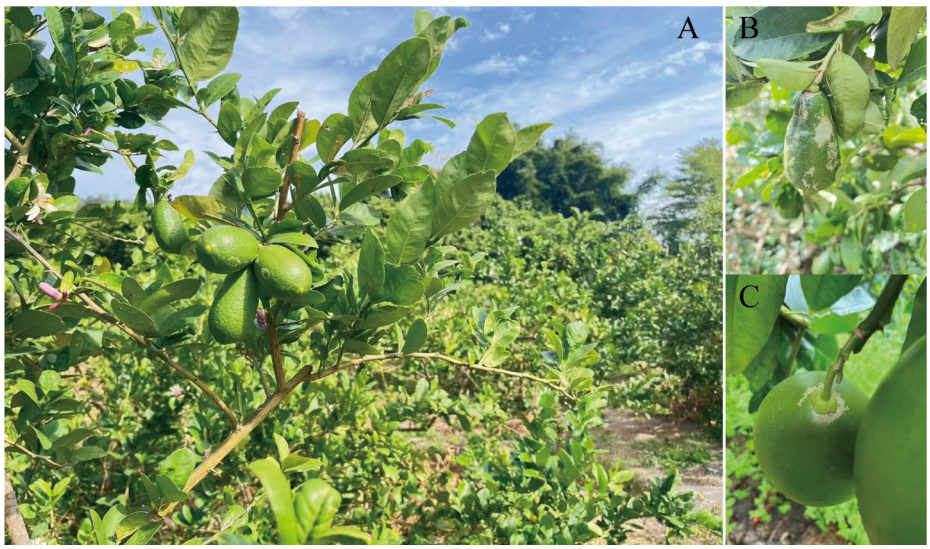


Figure 1. Infestation of thrips on lemon (**A**,**B**) and pomelo (**C**).

Although certain measures, including employing biopesticides, implementing classical biological control, and planting wildflower strips, have been adopted to enhance biological control of citrus pests [6,7] thrips management still mainly relies on chemical control [5,8]. However, long-term application of pesticides has resulted in a range of negative side effects, including increased thrips resistance [5], elevated pesticide residues in soil [9], and harm to non-target organisms, especially natural enemies [10,11]. The decline of natural enemies can trigger surges in pest populations and disrupt ecosystem services [12–14]. Hence, it is urgent to prioritize more sustainable, safer, and healthier production strategies for the effective management of citrus thrips.

Conservation biological control is a potential approach in pest management owing to its safety for humans, ecosystems, and the environment. There are plenty of natural enemies in citrus orchards, such as ladybirds, parasitic wasps, and lacewings [15]. Additionally, spiders and predatory mites also prey on a wide range of pests in citrus orchards [16,17]. Among these natural enemies, ladybirds play a pivotal role in controlling pests in citrus orchards [15]. The ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae), is one of the locally dominant natural enemies in the citrus ecosystem due to its wide feeding range, strong predation ability, and high reproductive ability [18,19], which may contribute greatly to suppressing multiple pests in both natural and agricultural ecosystems. Thus, utilizing *C. sexmaculata* for citrus thrips control not only helps mitigate insecticide resistance, but also aligns with eco-friendly pest management principles, offering an alternative strategy for integrated pest management in citrus orchard. However, the practical application of *C. sexmaculata* for biological control in open-field agroecosystems still faces several inherent challenges. The major limitation is their high dispersal capability; released ladybirds often rapidly disperse from the target orchards, failing to establish a stable natural enemy population where needed. To mitigate dispersal, herbivore-induced plant volatiles (HIPVs) provide a chemical retention cue.

Herbivore-inducible plant defenses are elicited following the detection of specific cues from damage caused by herbivory [5]. Numerous studies have shown that plants

emit specific HIPVs upon infestation of their leaves, or flowers by pests [20,21]. HIPVs play a vital role in regulating various behaviors of natural enemies, including foraging for prey, locating potential hosts, and selecting suitable habitats [22–24]. Moreover, these volatiles are key mediators in tritrophic interactions between plants, herbivores, and natural enemies [21]. For instance, infested plants emit HIPVs to anticipate herbivore attack and trigger their defensive systems [21,25]. Herbivores, in turn, may use HIPVs to avoid plants already infested by competitors, whether conspecific or heterospecific [26,27]. Meanwhile, natural enemies, including predators and parasitoids, can exploit HIPVs to locate their prey. For example, volatiles from tomato *Solanum lycopersicum* L. (Solanales: Solanaceae) infested by the whitefly *Trialeurodes vaporariorum* (Westwood) attracted the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) [28]. Similarly, the predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) was significantly attracted to the annual plant *Cnidium monnieri* (L.) Gusson flower when it was infested by the aphid *Semiaphis heraclei* (Takahashi) (Hemiptera: Aphididae) [29]. Hence, exploring the use of HIPVs to enhance the localization and retention efficiency of *C. sexmaculata* within target citrus orchard areas may offer an ecologically compatible and innovative direction to overcome the limitation of their high dispersal tendency. However, HIPV emissions vary depending on the specific plant–herbivore combination. Both biotic factors, including plant traits (e.g., host species, cultivar, and age) [30,31], pest traits (e.g., species, developmental stage, and mouthpart type) [28,32,33], and abiotic factors (e.g., temperature, moisture, and light) [30] can shape the volatile profiles and subsequently influence natural enemy responses. Consequently, the application of HIPVs to diverse farmland ecosystems is conditional on further testing, due to the high specificity of their emissions.

In this study, we hypothesized that thrips infestation alters the emitted volatile compounds of lemon plants and these alterations would influence the predatory behavior of *C. sexmaculata*. To test this hypothesis and investigate the tritrophic interactions between *C. sexmaculata*, *F. intonsa*, and lemon plants, we conducted the following experiments: (1) dual-choice behavioral assay to evaluate the attraction of *C. sexmaculata* adults to *F. intonsa*-infested versus healthy lemon plants; (2) volatile profile analysis to compare the volatile composition of *F. intonsa*-infested and healthy lemon plants; (3) Y-tube olfactometer assay to evaluate the behavioral responses of *C. sexmaculata* adults to the individual volatile compounds identified in step 2. Through this approach, we intended to clarify how changes in plant volatiles induced by *F. intonsa* infestation affect the predatory behavior of *C. sexmaculata*. Ultimately, this study is expected to provide practical insights for optimizing the deployment of the natural enemy *C. sexmaculata* against citrus thrips *F. intonsa*. Incorporating specific HIPVs into management strategies could offer a sustainable alternative to chemical insecticides and support integrated pest management (IPM) practices.

2. Materials and Methods

2.1. Insects and Plants

Laboratory colonies of the ladybird *C. sexmaculata* and the thrips *F. intonsa* were collected from a lemon orchard (24.76° N, 118.05° E) in Xiamen City, Fujian Province, China in 2023. Adults of *C. sexmaculata* were subsequently maintained in fine mesh cages (50 × 30 × 50 cm) on a diet of the cowpea aphids *Aphis craccivora* Koch (Hemiptera: Aphididae) for over two years (>20 estimated generations). Prior to the experiments, the newly emerged (unmated) female *C. sexmaculata* beetles were starved for 24 h in Petri dishes (5 cm diameter). The *A. craccivora* population was maintained in the same size cages with seedlings of cowpea *Vigna unguiculata* (L.) Walp (Fabales: Fabaceae). *F. intonsa* colonies were reared on pre-soaked kidney beans. All insect colonies were reared in a climate chamber under the following conditions: 25 ± 1 °C, 60 ± 5% RH, and a photoperiod of

16 L: 8 D. Two-year-old plants of lemon cultivar 'Xiangshui' (*Citrus limon* (L.) *Burm* f.) were used for both the dual-choice behavioral assay and the volatile components analysis. The selected seedlings plants had similar architecture and size, with a height of approximately 30 cm and between 3 and 4 branches. All healthy plants selected for testing were confirmed to be free of pest damage and disease prior to the experiment.

2.2. Dual-Choice Behavioral Assay

To evaluate the olfactory preference of *C. sexmaculata*, we conducted dual-choice behavioral assays using a Y-tube olfactometer (stem length: 25 cm; arm length: 20 cm; arm angle: 60°; internal diameter: 10 mm) following an established method [34]. Three pairwise treatments were tested to assess the response of *C. sexmaculata* to volatiles emitted from different sources: (1) *F. intonsa*-infested lemon plant versus clean air; (2) *F. intonsa*-infested lemon plant versus healthy lemon plant; and (3) healthy lemon plant versus clean air. *F. intonsa*-infested lemon plants were prepared by exposing a single plant to 100 thrips of mixed ages and sexes in a mesh bag (20 × 20 × 35 cm) for 48 h. Thrips were subsequently removed carefully using a fine brush to avoid mechanical damage to the plant. Healthy plants, grown under identical conditions but without thrips exposure, served as untreated controls.

Each tested plant was placed in a separate dome-shaped volatile collection chamber (35 × 25 cm), and purified air was blown into each chamber at a flow rate of 200 ± 10 mL/min. Airflow was delivered by a vacuum pump through desiccant and activated charcoal filters, with the entire system temperature maintained at 25 ± 1 °C. Experimental ladybirds were individually released at the base of the Y-tube stem and allowed 300 s to make their first choice. A choice was recorded when an adult ladybird reached more than 1/2 of the length of the arm; otherwise, no response was recorded. For each of the three pairwise treatments, 70 adult female ladybirds were tested, with each individual used only once. To minimize contamination and positional bias, the Y-tube was thoroughly cleaned with dichloromethane, and the volatile source was changed between the two arms after every five individuals. Overall, 70 female ladybirds were individually tested per choice test. The olfactory preference was calculated using the following Equation:

$$\text{Olfactory preference (\%)} = T/(T + H) \times 100$$

where H is the number of female ladybirds that chose the healthy lemon plant or clean air side, and T is the number of female ladybirds that chose the thrip-infested lemon plant side.

2.3. Collection and Analysis of Headspace Plant Volatiles

Volatiles emitted from healthy and thrips-infested lemon plants were collected using headspace solid-phase microextraction (HS-SPME). For *F. intonsa*-infested lemon plant treatment, each single lemon plant was exposed to 100 thrips of mixed ages and sexes in a mesh bag (20 × 20 × 35 cm) for 48 h prior to the collection. The pot of each lemon plant was fully enclosed with aluminum foil. Each treated plant was enclosed in a dome-shaped volatile collection chamber (35 × 25 cm). Then volatiles collection was conducted in a separate greenhouse (25 ± 2 °C; 60 ± 5% RH; 16L: 8D photoperiod) for 72 h that contained only the treated plants to minimize cross-contamination. Subsequently, volatiles were collected for 0.5 h by inserting an SPME fiber (50/30 μm DVB/CAR/PDMS) through the cap into the collection chamber. After sampling, the SPME fiber was retracted and immediately inserted into the inlet (250 °C) of a gas chromatography–mass spectrometry system (GC-MS, GCMS-TQ8040, Shimadzu Corporation, Kyoto, Japan) equipped with a DB-5 column (Rxi-5Sil MS, Restek Corporation, Bellefonte, PA, USA; 30 m × 0.25 mm × 0.25 μm). The column temperature was initially held at 40 °C for 60 s, then increased to 260 °C at a rate

of 10 °C/min and maintained for 120 s. Electron impact (EI) ionization was performed at 70 eV, with a mass scan range of 35–550 m/z. Volatile compounds were matched to the NIST 17 library (Scientific Instrument Services, Inc, Ringoes, NJ, USA) based on their mass spectra and retention time of authentic standards. Relative quantification of each volatile compound was based on their peak area. For each treatment, one plant per chamber constituted one biological replicate, with five replicates per treatment. A total of 10 plants were sampled: 5 plants infested with *F. intonsa*, and 5 healthy plants.

2.4. Behavioral Responses to Individual HIPVs and Synthetic Blends

The behavioral responses of ladybird *C. sexmaculata* to individual volatile compounds were evaluated using the Y-tube olfactometer setup in the previous section. Each compound was individually dissolved in n-Hexane to obtain three test concentrations (0.1, 1, and 10 μg/μL). All of the tested volatile compounds (purity ≥ 95%) were purchased from Macklin Biochemical Co. Ltd. (Shanghai, China). For each trial, a 20 μL aliquot of the test solution was applied to a filter paper strip (50 × 5 mm), which was then promptly placed in one of the treatment flasks. A filter paper strip treated with 20 μL of pure n-Hexane served as the control. Purified air was delivered into the system at a flow rate of 200 ± 10 mL/min. Individual ladybirds were released at the entrance of the Y-tube and observed for up to 300 s. A choice was counted only if the ladybird moved beyond more than 1/2 of the arm's length; otherwise, the trial was recorded as no response. For each compound, 60 adult female ladybirds were tested. All behavioral assays were performed from 9:00 am to 5:00 pm under the same environmental conditions maintained for the insect colony.

Based on the initial screening, 5 compounds were found to elicit attraction: α-pinene, sabinene, β-pinene, myrcene, and eucalyptol. These compounds were therefore selected for further evaluation in synthetic blends. A baseline blend (Blend B) was prepared by mixing these five compounds at their respective optimal attractive concentrations, which were determined from the single-compound assays (0.1 μg/μL for α-pinene, 1 μg/μL for sabinene and eucalyptol, 10 μg/μL for β-pinene and myrcene). To assess the concentration–response relationship, two additional blends were tested: Blend A (10 × the concentration of Blend B: 1 μg/μL for α-pinene, 10 μg/μL for sabinene and eucalyptol, 100 μg/μL for β-pinene and myrcene), and Blend C (0.1 × the concentration of Blend B: 0.01 μg/μL for α-pinene, 0.1 μg/μL for sabinene and eucalyptol, 1 μg/μL for β-pinene and myrcene). All behavioral tests were performed using the Y-tube olfactometer method described previously.

2.5. Statistical Analysis

The number of ladybird *C. sexmaculata* responding to each odor source was recorded. In the dual-choice behavioral assay, adult ladybird preference for each pairwise comparison was analyzed using a chi-square goodness of fit test, with the null hypothesis of no preference (expected proportion 50:50). To determine the discrimination in volatile blends between healthy plants and thrip-infested lemon plants, non-metric multidimensional scaling (NMDS) was performed using the metaMDS function (with autotransform = TRUE) and permutational analysis of variance (PERMANOVA) was calculated using the 'adonis2' function in the "vegan" package based on Bray–Curtis dissimilarity matrix. The multivariate homogeneity of dispersion was tested using the 'betadispr' function of the "vegan" package (v2.6.6.1). The Bray–Curtis dissimilarity matrix was visualized using NMDS implemented with the 'ggplot2' package (v3.5.2). For behavioral assays of ladybird *C. sexmaculata* in response to individual HIPVs and synthetic blends, their preference was analyzed using a chi-square goodness of fit test, as described previously.

All statistical analysis was conducted in R 4.4.1 (R Core Team, Vienna, Austria, 2024).

3. Results

3.1. Olfactory Response of C. sexmaculata to Plant Volatiles

These assays revealed significant preferences, detailed below. The predator *C. sexmaculata* showed a significant attraction to volatiles from *F. intonsa*-infested lemon plants compared to those from healthy plant (χ^2 = 4.00, df = 1, p = 0.045) and clean air control (χ^2 = 6.25, df = 1, p = 0.012). Relatively, attraction to volatiles from healthy plants was not significantly different from the control (χ^2 = 0.78, df = 1, p = 0.378) (Figure 2).

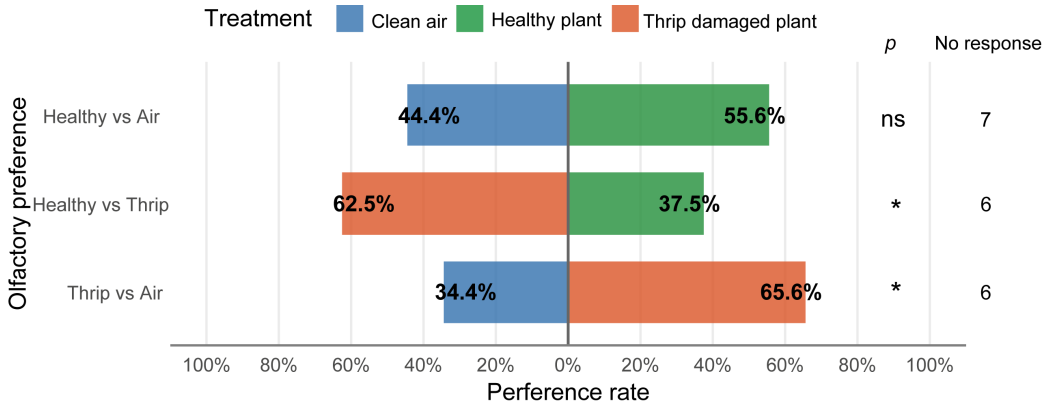


Figure 2. Responses (%) of *Cheilomenes sexmaculata* to volatiles of healthy or *Frankliniella intonsa*-infested lemon plants in a Y-tube olfactometer choice test. "ns", and "*" indicate no significant difference, and significant difference at the $p < 0.05$ level, respectively (n = 70).

3.2. Identification and Analysis of Volatiles from Lemon Plants in Different Treatments

When analyzed as whole blends, the volatile profiles of thrips-infested plants did not differ markedly from those of healthy plants (*perMANOVA*, F = 1.72, df = 1, p = 0.219). The permutational test of multivariate dispersions (*PERMDISP*) showed that dispersion was homogeneous across groups (F = 0.04, df = 1, p = 0.842). Nonetheless, the NMDS ordination showed a visible separation between the two treatments (Figure 3). Six compounds were identified in the volatile extracts of thrips-infested lemon plants based on their retention time and mass spectra: α-pinene, sabinene, β-pinene, myrcene, D-limonene, and eucalyptol (Table 1). Notably, several key compounds, including α-pinene, sabinene, and β-pinene, were not detected in the volatiles extracts from healthy plants (Table 1).

Table 1. Different volatile compounds emitted by healthy and *Frankliniella intonsa*-infested lemon plants.

Number	Retention Time/Min	Volatile Substance	CAS	Relative Content	
				Healthy Plants	*F. intonsa*-Infested Plants
1	8.70	α-Pinene	80-56-8	-	2.95 ± 0.83
2	10.12	Sabinene	3387-41-5	-	5.96 ± 2.10
3	10.26	β-Pinene	18172-67-3	28.03 ± 12.49	14.92 ± 6.77
4	10.79	Myrcene	123-35-3	-	0.93 ± 0.21
5	12.21	D-Limonene	5989-27-5	30.03 ± 8.93	39.51 ± 9.76
6	12.28	Eucalyptol	470-82-6	47.95 ± 24.91	38.07 ± 18.31

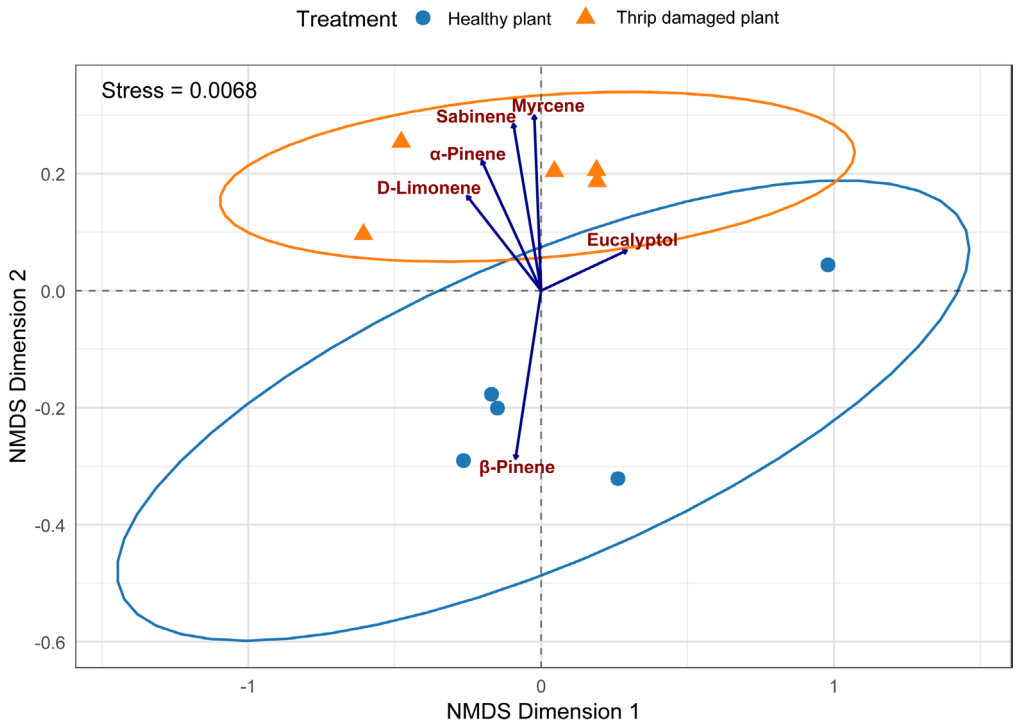


Figure 3. Visualization of non-metric multidimensional scaling (NMDS) ordination of the composition of volatiles compounds emitted from healthy and *Frankliniella intonsa*-infested lemon plants (n = 10 plants, Stress = 0.0068).

3.3. Behavioral Responses of C. sexmaculata to Detected Compounds and Synthetic Compounds

These assays revealed significant preferences; the predatory ladybird *C. sexmaculata* exhibited differential behavioral responses to individual compounds at various concentrations. Compared to the control (n-Hexane), *C. sexmaculata* was markedly attracted to α-pinene at concentrations of 1 μg/μL (χ^2 = 6.67, df = 1, p = 0.009) and 0.1 μg/μL (χ^2 = 6.67, df = 1, p = 0.009). Significant attraction to β-pinene and myrcene was observed only at the highest concentration of 10 μg/μL (χ^2 = 5.59, df = 1, p = 0.018; χ^2 = 5.79, df = 1, p = 0.016, respectively). Similarly, sabinene and eucalyptol were attractive at the lower concentrations of 1 μg/μL (χ^2 = 6.12, df = 1, p = 0.013; χ^2 = 5.67, df = 1, p = 0.017, respectively). In contrast, D-limonene elicited no significant attraction at any of the tested concentrations (χ^2 = 0.44, df = 1, p = 0.508; χ^2 = 0.15, df = 1, p = 0.696; χ^2 = 0.30, df = 1, p = 0.586, respectively) (Figure 4).

Moreover, *C. sexmaculata* was significantly attracted to the five-compound blend (α-pinene, sabinene, β-pinene, myrcene, and eucalyptol) when mixed at their optimal attractant concentration (Blend B: 0.1 μg/μL for α-pinene, 1 μg/μL for sabinene and eucalyptol, 10 μg/μL for β-pinene and myrcene) compared to the control (χ^2 = 6.67, df = 1, p = 0.009) (Figure 5). A similar attraction was observed for the high-concentration mixture (Blend A) (χ^2 = 6.67, df = 1, p = 0.009). However, the low-concentration blend (Blend C) did not elicit a significant behavioral response (χ^2 = 0.27, df= 1, p = 0.606) (Figure 5).

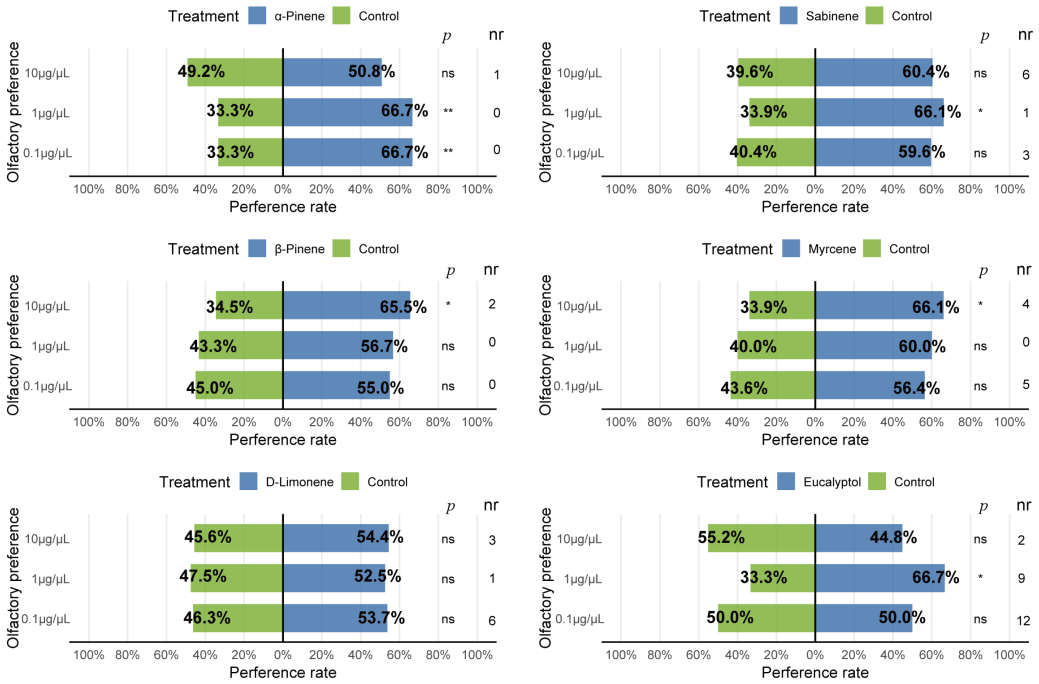


Figure 4. Behavioral responses of adult *Cheilomenes sexmaculata* to *Frankliniella intonsa*-induced volatiles at concentrations of 0.1, 1, 10 μg/μL. "ns", "nr", "*", and "**" indicate no significant difference, number of non-responsive insects, significant difference ($p < 0.05$), and significant difference ($p < 0.01$), respectively.

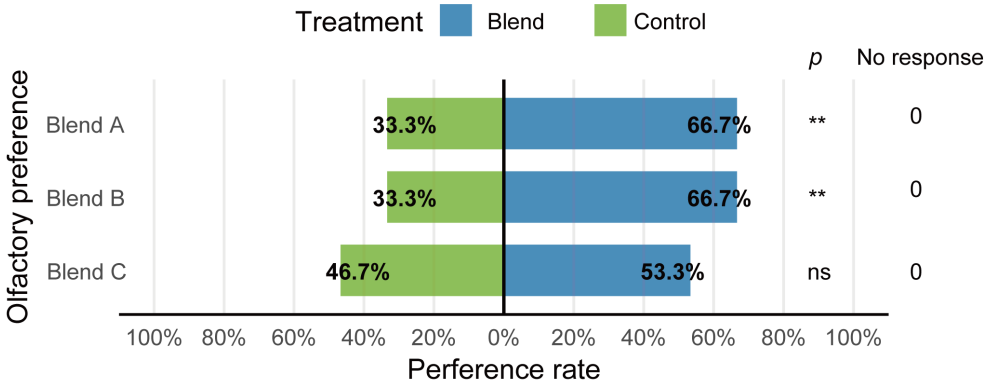


Figure 5. *Cheilomenes sexmaculata* attractiveness to different mixtures of attractant compound blends. "ns" and "**" indicate no significant difference and significant difference at the $p < 0.01$ level, respectively.

4. Discussion

HIPVs play a pivotal role in mediating the tritrophic interactions among plants, herbivores, and their natural enemies. Therefore, understanding the chemical ecology involving the local predator *C. sexmaculata* is essential for effectively incorporating HIPVs into push–pull strategies for biological control [35,36]. Our findings demonstrate that adult *C. sexmaculata* exhibited a clear preference for thrips-infested lemon plants over healthy plants. These patterns extend prior ladybird HIPV studies showing that *C. sexmaculata* is significantly attracted to *A. craccivora*-infested cowpea plants and *A. gossypii*-infested cotton plants, respectively [37,38]. Moreover, this behavioral response is not unique to *C. sexmaculata*. For instance, ladybird *Clitostethus arcuatus* (Rossi) (Coleoptera: Coccinellidae) is attracted to citrus plants (e.g., tangelo and lime) infested by whitefly *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae) [39]. Similarly, sweet pepper plants injured by *Aphis gossypii* Glover (Hemiptera: Aphididae) or *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

attract the ladybird *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Coccinellidae) [40]. The ladybirds *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and *H. axyridis* have also been consistently reported to be attracted to aphid-infested plants [41,42].

Upon thrips infestation, damaged plants can alter their volatile organic compounds (VOCs) to recruit natural enemies of the herbivores, thereby enhancing the plant's indirect defense [5]. The observed behavioral attraction of *C. sexmaculata* is likely driven by composition or concentration differences in volatile emissions between healthy and infested lemon plants, as indicated by multivariate and ordination analyses. Furthermore, we illustrated that the monoterpene HIPVs (α-pinene, β-pinene, sabinene, myrcene, and eucalyptol) emitted by *F. intonsa*-infested lemon plants individually attracted *C. sexmaculata*. The thrips-induced changes in VOC emission are likely mediated by jasmonic acid (JA), a key phytohormone regulating plant defense against herbivores [43]. This is in line with previous studies reporting increased emission of monoterpenes (e.g., α-pinene) from other infested plants, such as tomato [44–46]. Although not explicitly shown for thrips, increased emission of terpenes has been broadly associated with enhanced predator attraction. Among these compounds, α-pinene emitted by *Artemisia argyi* H.Lév. & Vaniot (Asterales: Asteraceae) damaged by *Macrosiphoniella artemisiae* (Boyer de Fonscolombe) (Homoptera: Aphididae) has been shown to effectively attract natural enemies *H. axyridis* [42]. Aphid-induced α-pinene also attracts the predator *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) [47]. Similarly, β-pinene, an isomer of α-pinene, also exhibits attractive effects on predators *H. axyridis*, *C. septempunctata*, and *Chrysoperla sinica* (Tjeder) (Neuroptera: Chrysopidae) [48,49]. Myrcene has been identified as an attractant for the parasitoid *E. Formosa* in studies on HIPVs from whitefly-infested plants [28,50]. Moreover, eucalyptol emitted by aphid *A. gossypii*-infested *Vitex negundo* L. (Lamiales: Lamiaceae) significantly attracts *H. axyridis* [51]. Our results thus confirm the role of these specific volatiles in recruiting natural enemies and provide a foundation for developing semiochemical-based tools to enhance biological control of *F. intonsa*.

Our dose–response assays revealed that the proportion of attracted *C. sexmaculata* slightly increased with higher concentration of β-pinene and myrcene. The active concentrations identified in our study are consistent with those reported previously: for instance, 10 mg/mL β-pinene triggers the highest response in electrophysiological assay for female *H. variegata* adults [47], suggesting our tested doses were within a behaviorally relevant range. Conversely, attraction to α-pinene, sabinene, and eucalyptol decreased when their concentrations were increased 10-fold, indicating that *C. sexmaculata* is sensitive to the concentration or ratio of these compounds within a blend. A similar dose-dependent response of *C. sexmaculata* to volatiles from aphid-infested cotton plants has been reported [38].

Natural enemies often rely on blends of volatiles when foraging for hosts and prey [52]. Therefore, manipulating these tritrophic interactions could improve the field efficiency of biological control [53]. Developing synthetic formulations that mimic species-specific HIPV blends may provide reliable cues in agroecosystems to recruit predatory enemies toward infested crops [54]. Our results exhibited that a five-component blend significantly enhanced predator attraction at their optimal concentrations, suggesting its promising potential as an attractant for recruiting predatory ladybirds. This aligns with a previous study showing that the wasp *Aphidius ervi* Haliday (Hymenoptera: Braconidae) responded to six-component blends across all tested concentrations [55]. These results suggest that such blends convey more ecologically relevant information to natural enemies than individual compounds [56].

However, several challenges must be addressed to translate these HIPV-based attractants into effective field applications. Firstly, to clarify their practical potential, additional parameters require further investigation, including the delivery time [57], the specific

release rate of HIPVs [58], and the effects of environmental conditions on their volatilization [57]. Secondly, given that the concentration of blends can be diluted by the wind in a dynamic olfactory landscape [55], and laboratory-reared pests may display diminished olfactory sensitivity due to insufficient exposure to natural olfactory stimuli [59], the concentration of attractants to be applied to attract natural enemies should be higher than the concentration that showed attraction when tested in laboratory assays. Thus, laboratory results warrant semi-field trials. In addition, to enhance attractant efficacy, further research should evaluate the extent to which ladybirds can distinguish composition and concentration differences between different attractive volatile blends [60].

5. Conclusions

In summary, our study demonstrates that the generalist predator *C. sexmaculata* is attracted by the volatiles emitted by *F. intonsa*-infested lemon plants. Chemical analysis and behavioral response assays identified five attractive monoterpenes (α-pinene, β-pinene, sabinene, myrcene, and eucalyptol) in the volatile profile of infested plants. Notably, a five-component blend of these monoterpenes elicited a stronger attraction in *C. sexmaculata* than individual compounds. Thus, these attractive blends can be developed into an effective lure to improve thrips management and to complement existing IPM strategies in lemon orchards. We further recommend that stakeholders reserve more natural habitats to conserve wild ladybird populations, which would promote biological control within these agroecosystems.

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