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Host plant-specific volatiles of *Beauveria bassiana*-colonized plants initiate trophic plant–aphid–predator cascades

María Cuenca-Medina,[a] Raquel Rodríguez-Solana,[b,c] José Manuel Moreno-Rojas,[d] Rafael Alcalá-Herrera,[e] Enrique Quesada-Moraga[a*] and Natalia González-Mas[a*]

Abstract

BACKGROUND: Entomopathogenic ascomycetes (EAs) have efficacy in insect pest control through direct contact and indirectly as plant endophytes. As endophytes, they lead indirectly to pest mortality, enhance plant resilience to stresses, promote plant growth and alter plant volatile emissions. These changes can influence plant interactions with beneficial fauna, such as predators, parasitoids and pollinators. However, variation in endophytic colonization across plant species and EA strains raises questions about cultivar-specific responses within the same crop species. Here we evaluated the impact of endophytic colonization by the EA *Beauveria bassiana* on the volatile organic compounds (VOCs) of three melon cultivars: 'Galia', 'Futuro' and 'Rinconete'.

RESULTS: *Beauveria bassiana* colonization rates ranged from 73% (Rinconete) to 85% (Galia) and were associated with melon crop-specific VOCs such as *cis*-3-hexenal and *N,N*-dimethyldodecanamine. The *B. bassiana* colonization also triggered cultivar-specific VOCs including allomones and synomones that play a key role in melon–insect pest interactions which are relevant to crop protection. These included *cis*-3-hexenol and β-phellandrene in Galia, cinnamaldehyde and cinnamyl alcohol in Futuro, and styrene and acetophenone in Rinconete. Differences in VOCs were evaluated in a multitrophic system involving cv. Galia, the aphid *Aphis gossypii* and the predator *Chrysoperla carnea*. Olfactometer bioassays revealed a lacewing preference for *B. bassiana* and aphid-infested plants. Significant differences in emissions of lacewing attractant VOCs were recorded between *B. bassiana*-colonized plants and controls, independent of aphid infestation.

CONCLUSION: These results reinforce the potential of endophytic *B. bassiana*-related VOCs to be managed within integrated melon protection and production strategies tailored to specific melon varieties.

Supporting information may be found in the online version of this article.

Keywords: *Aphis gossypii*; allelochemicals; chemometric analysis; *Chrysoperla carnea*; GC–MS; VOCs

1 INTRODUCTION

Integrated pest management (IPM) has emerged as one of the most robust paradigms within the field of agricultural production science. Both IPM and organic agriculture encourage the use of strategies that minimize reliance on pesticides and promote sustainable and environmentally friendly practices.[1] Biological control is a key component within these strategies and includes the use of entomophagous arthropods (predators, parasitoids) and entomopathogenic microorganisms, for sustainable pest management.[1,2] Among these, entomopathogenic ascomycete (EA) fungi stand out as effective biological control agents against arthropod pests owing to both their unique integumentary mode-of-action (MoA) and their demonstrable compatibility with other natural enemies in the agroecosystem.[3,4] Furthermore, the use of EAs as biological control agents has gained significant

* *Correspondence to: Q-M Enrique or G-M Natalia, Department of Agronomy, Maria de Maeztu Excellence Unit DAUCO ETSIAM, Universidad de Córdoba, Campus de Rabanales, Building C4 Celestino Mutis, 14071 Cordoba, Spain. E-mail: z22goman@uco.es (Enrique); E-mail: cr2qumoe@uco.es (Natalia)*

a *Department of Agronomy, Maria de Maeztu Excellence Unit DAUCO ETSIAM, Universidad de Córdoba, Cordoba, Spain*

b *Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Jerez de la Frontera, Spain*

c *MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Faro, Portugal*

d *Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Cordoba, Spain*

e *Zoology and Entomology Unit, School of Forestry Engineering and Natural Resources, Polytechnic University of Madrid, Madrid, Spain*

importance owing to their recently discovered multiple ecological roles.[5]

Some EA strains can colonize the plant as endophytes, even upon artificial application, resulting in either transient or systemic colonization of the crop with associated positive effects on the plant and negative effects on herbivorous pests.[6,7] This endophytic capacity enhances EA compatibility with other natural enemies by reducing direct contact of fungal propagules with insect predators and parasitoids.[4,8–10] Endophytic colonization (EC) by EA has been shown to influence plant responses, providing systemic protection against insect pests and phytopathogens, enhancing resilience to abiotic stresses, promoting plant growth and altering the plant volatile profile.[5] Indeed, it has been demonstrated that EC-related plant growth promotion depends on cultivar and EA fungal strain, assuming both the EA strain and cultivar strongly determine the result of the plant response.[11] Moreover, although the endophytic behaviour of EA has been reported in numerous plant species, the extent of colonization varies depending on host species and fungal strain. However, it remains unclear whether such variation also occurs at the variety or cultivar level and whether it is mediated by plant volatile profiles.[8,12–15]

In this regard, fungal EC and subsequent changes in the plant and in herbivorous insects could influence entomophagous and pollinator–plant interactions, particularly through alterations in plant volatile profiles.[16–18] When larvae of the generalist predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) were given a choice between fungal EC and control melon plants, they preferred aphids feeding on EC plants.[19] In this predatory context, the lacewing preference could be influenced by EC-mediated changes in either the prey or the plant.[8,20–24] Such changes are likely to include modifications to plant volatile profiles emitted by EA-colonized plants, which may serve as predator attractants.[17,25,26] However, it remains uncertain whether adult lacewings, which are not predators but are, remarkably, more responsiveness than immature stages to chemical cues, also show a preference for EC plants.[27,28] Adult lacewing behaviour is crucial for understanding their habitat selection and oviposition preferences, and especially critical under field cultivation conditions.[27]

Based on this framework, we evaluated whether (i) colonization by EA endophytes induces cultivar-specific changes in plant volatile profiles and (ii) these volatile modifications influence the olfactory behaviour of adult *C. carnea*.

To this end, the degree of EC of melon plants by the EA *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) was evaluated in two varieties, Reticulatus and Piel de Sapo, and a total of three different cultivars, 'Galia' F_1 in var. Reticulatus, and 'Futuro' and 'Rinconete' in var. Piel de Sapo. Additionally, the impact of EC on plant volatile organic compound (VOC) profiles was analyzed, focusing on potential variation in VOCs that were either common across all cultivars or specific to each cultivar.

In a second experiment, the ecological significance of this plant–fungus interaction was investigated by examining the habitat preference of *C. carnea*. Olfactometer assays were conducted to assay the preference of *C. carnea* adults for *B. bassiana* EC Galia melon plants, when either infested or not with the melon aphid *Aphis gossypii* Glover (Hemiptera: Aphididae), compared with control plants free of fungal inoculum. This work provides insights into the potential implications for natural lacewing populations under field conditions when integrating EA into IPM programmes across different melon varieties and cultivars grown worldwide.

2 MATERIALS AND METHODS

2.1 Plants, insects and fungal strain

Three melon cultivars from two varieties were used: *Cucumis melo* var. Reticulatus cv. Galia F_1 (Mascarell Semillas) and var. Piel de Sapo cvs Futuro and Rinconete (Semillas Fitó). Seeds were surface-sterilized in 2% sodium hypochlorite (NaOCl) for 2 min, rinsed in sterile Mili-Q water and dried under sterile conditions. For germination, seeds were placed between moist, sterilized filter papers over sterile vermiculite in 150-mm Petri dishes, sealed with Parafilm and incubated at 25 °C in darkness. A 1:1 mix of peat substrate and vermiculite was autoclaved twice (20 min at 121 °C, 24 h apart). Germinated seeds were transferred to 9-cm-diameter pots with the sterilized mix and grown at 25 ± 2 °C and 70% relative humidity (RH), under a 16 h:8 h, light:dark photoperiod. Irrigation water was sterilized and 20:20:20 NPK fertilizer (Nutrichem 60) added weekly at 3 g L^{-1}.

Beauveria bassiana strain EABb 01/33-Su was selected for the experiments owing to its demonstrated endophytic behaviour when applied by foliar spray on melon plants; its efficacy against melon aphid feeding on EC melon plants; and the attraction of lacewing larvae to aphids feeding on EC plants.[7,16,19] Moreover, EABb 01/33-Su modifies the volatile profile of EC melon plants.[16,17] This strain from the Agronomy Department of the University of Córdoba collection was originally isolated from soil of a traditional olive orchard in El Bosque (Cadiz, Spain) and is deposited under the Budapest Treaty in the Spanish Type Culture Collection located at the University of Valencia under accession number CECT 21149. Detailed molecular evaluations of EABb 01/33-Su are available in the GenBank database. Specifically, sequences EF115310 and FJ972969 correspond to the internal transcribed spacer (ITS region), FJ973025 refers to the intergenic region nad3-atp9, and FJ972914 to the intergenic region atp6-rns.

Beauveria bassiana strain EABb 01/33-Su was cultured on potato dextrose agar (PDA) in 90-mm Petri dishes for 14 days at 25 °C in darkness. Conidia were scraped into 0.01% Tween 80, filtered through sterile cheesecloth, and sonicated for 5 min. Conidial concentration was adjusted to 10^8 conidia mL^{-1} using a haemocytometer. This dose, applied at a rate of 5 mL per plant, effectively induces EC in melon and also controls aphids[7,16]. Only suspensions with >97% germination after 24 h in Czapek Dox broth with 1% yeast extract were used in experiments.

A virus-free *A. gossypii* population from the ICA-CSIC (Madrid, Spain) was reared for several generations on melon plants in insect-proof cages in an environmental chamber at 25 ± 2 °C, 70% RH, under a 16 h:8 h, light: dark regime.

Chrysoperla carnea larvae were initially supplied by Koppert S.L. (Aguilas, Spain) and reared under laboratory conditions (25 ± 2 °C, 70% RH, 16 h:8 h, light:dark). They were individually placed in 50-mm-diameter Petri dishes to avoid cannibalism and fed with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Bioline Iberia, Santa María del Águila, Spain) until reaching the final larval stage. Once new adults emerged, they were placed in a 30 × 20 × 15 cm (length × width × height) rectangular box in groups of 200. Adults were provided with sterile mineral water and a mixture of honey: pollen 1:1 (v:v). This mixture and water were replaced every 2 days. Eggs from the rectangular box were placed individually in 50-mm-diameter Petri dishes and reared with *E. kuehniella* until adult emergence.[29]

2.2 Standard methods used to inoculate melon plants and verification of *B. bassiana* EC by microbiological and molecular techniques

In all experiments, melon plants were treated by foliar spray. The aerial parts of plants at the four-leaf stage were each treated with

5 mL of the *B. bassiana* conidial suspension. An aerograph 27 085 with a piston compressor of 23 L min^{-1}, 15–50 PSI and 0.3 mm nozzle diametre (Artesania Latina S. A., Malaga, Spain) was used to achieve this. Postinoculation, each replicate plant was covered with a plastic sheet to encourage fungal activity for 24 h. For the control treatment, plants were sprayed with sterile water containing 0.01% Tween 80, using the same application procedure as that used for the fungus-treated plants.

In order to confirm that EC had occurred, two leaves were sampled from each plant at the end of experiments; one was evaluated microbiologically and the other using molecular techniques. First, both leaves were surface-sterilized in 1% NaOCl for 2 min, rinsed twice in sterile distilled water and dried on sterile filter paper. From one leaf, sections of ≈1 cm^2 were cut with a sterile scalpel and plated out independently in 90-mm-diameter Petri dishes containing selective culture medium to determine the percentage of EC. The culture medium consisted of 20 g Sabouraud Chloramphenicol dextrose agar (Biolife Italiana S. R. L., Monza, Italy), 500 mg L^{-1} Chloranphenicol (PanReac Applichem, Barcelona, Spain), 250 mg L^{-1} Cycloheximide (PanReac AppliChem) and 10 mg L^{-1} dodine 65 WP (BASF, Barcelona, Spain) Inglis *et al.*[30]. The last rinse water from each sample was plated separately to confirm the effectiveness of the surface-sterilization procedure. All plates were incubated at 25 °C in darkness until fungal growth was observed and then identified. For molecular detection and quantification of EC, the remaining surface-sterilized leaf from each plant was individually ground to a fine powder using a pestle and mortar in liquid nitrogen. DNA was extracted from 150 mg (wet weight) of each leaf using a HigherPurity Plant DNA Purification Kit (Canvax Reagents S.L., Valladolid, Spain) following the manufacturer's instructions. DNA concentration was determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All DNA samples were adjusted to a final concentration of 30 ng/μL. The quantity of fungus present was determined using quantitative (q)PCR[10] with *B. bassiana*-specific primers based on *B. bassiana* BLOC intergenic region BbF 5′-CACCATTCGCGGCCTGTTCT-3′ and BbR 5′-CGTGGCGTGCGATGCGT-3′. For the PCR reaction, iTaq Universal SYBR Green supermix (Bio-Rad, Hercules, CA, USA) was used in a final volume of 10 μL, following the manufacturer protocol. The qPCR reaction mixture contained 1 μL of the DNA sample, 5 μL iTaq Universal SYBR Green Supermix and 0.3 μL of 10 μM of each primer. Calibration curves were established by fitting the reaction wells from 15 to 1.5 10^{-5} ng μL^{-1} of *B. bassiana* DNA in melon plant DNA. Throughout the process, plant DNA was consistently maintained at 30 ng μL^{-1} as a background; PCR reactions were performed in triplicate for standard curves and samples. Quantitative PCR was performed in CFX Connect Real-Time PCR Detection System thermal cycler (Bio-Rad) under the following conditions: denaturation (95 °C for 3 min), amplification repeated 39 times (95 °C for 10 s, 65 °C for 30 s), denaturation (95 °C for 10 s), and a final melting curve stage from 65 °C to 95 °C, with increments of 0.5 °C every 5 s. The Cq values obtained from wells containing melon leaf sample DNA were interpolated into the calibration curves to quantify the relative amount of fungal DNA to total DNA.

2.3 Volatile collection from *B. bassiana* EC and control melon plants of different cultivars

Melon plants from three different cultivars (Galia F_1, Futuro and Rinconete) were grown and treated (Section 2.2). For each cultivar, three plants were sprayed with either a conidial suspension of *B. bassiana* or with the aqueous control solution without fungus. VOCs emitted by the entire treated and control melon plants were collected 48 h post-spraying. VOC collection was always conducted between 09:00 and 15:00 h to minimize potential effects of diurnal variation in VOC emissions and to ensure comparability among treatments. VOCs were collected from the six odour sources simultaneously using a volatile collection system, OLFM-6C-ADS (VCS; Volatile Collection Systems Co LLC, Gainesville, FL, USA). Plants were placed into sealed cylindrical glass chambers as odour sources. A Porapak-Q™ adsorbent filter trap (20 mg, 325 mesh; Volatile Collection Trap, https://volatilecollectiontrap.com/) was attached to each odour glass vessel. Air entered the source vessels via Teflon tubing at a rate of 1.5 L min^{-1} and was pulled through the trap at a rate of 1 L min^{-1} via Teflon-tubing, to collect VOCs over a 2-h period following a method adapted from Turlings *et al.* (1998)[31] (Fig. 1). All plants were at the same phenological stage during VOC collection, and volatile extraction from all plants across treatments was performed on the same day post-treatment to ensure uniformity in developmental stage. Once collection was complete, filter traps were each eluted with 200 μL dichloromethane (Super solvent; Merck, Dietikon, Switzerland). After collection, all olfactometer parts were cleaned by first rinsing in tap water, then deionized water, acetone and pentane, after which the parts were maintained for 12 h at 100 °C. Leaf samples were frozen at −80 °C for further analysis to verify EC of the plants; this was done after the experiment had finished to avoid damaging the plant and

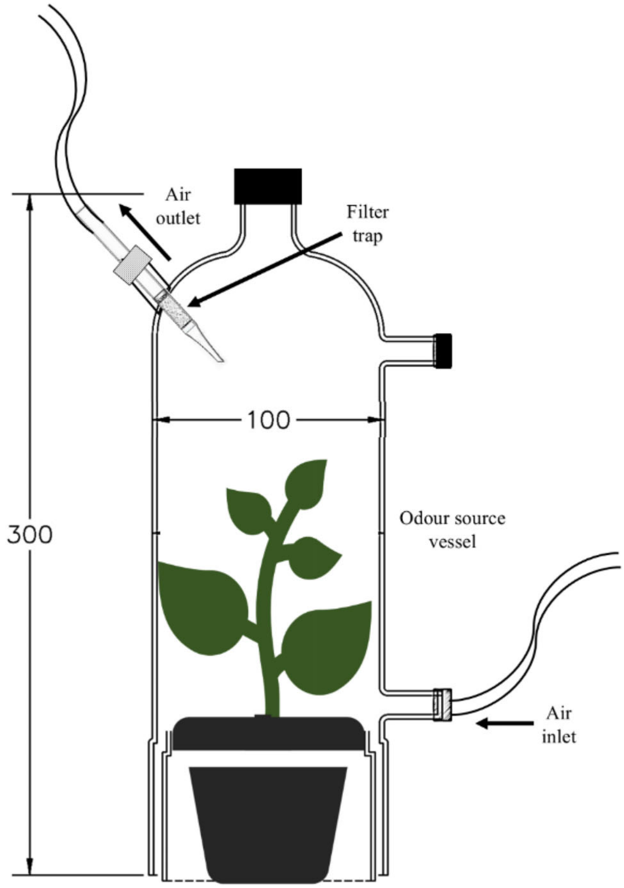

Figure 1. Schematic representation of the device used for collection of volatile organic compounds (VOCs). Measurements in mm.

triggering plant defences. The experiment was repeated once with new plants and fungal inoculum.

2.4 Effect of VOCs from aphid-infested and EC plants on *C. carnea* adult preference

The melon cultivar showing the strongest VOC response to *B. bassiana* colonization was selected for olfactometer assays with *C. carnea* adults. Lacewing responses to EC and control plants were evaluated using a four-arm olfactometer (OLFM-6C-ADS; VCS).[18] Six EC and six control plants were prepared; three from each group were each infested with 10 adult female *A. gossypii* aphids 48 h post-spraying with either the fungal or control solution. Lacewing behaviour was recorded 5 days postinfestation with aphids. The four odour sources for evaluation (one per arm) were: (i) aphid-infested EC; (ii) EC; (iii) aphid-infested control; and (iv) control. Purified air entered each odour vessel at 1.5 L min^{-1}, and VOC-laden air was drawn out at 1.0 L min^{-1} per arm. Fifty lacewings (25 males, 25 females) were tested individually by placement in the olfactometer with the odours from each plant set (four plants, one for each odour source). Each insect had 90 s to choose an arm, enter and reach the elbow; otherwise, it was recorded as 'no choice'. Three independent plant sets were tested using new insects and cleaned equipment each time. Tests ran between 09:00 and 16:00 h to minimize diurnal variation in plant volatile emissions and insect behaviour. During assays, VOCs were collected from each of the four odour sources using a Porapak-Q™ filter system (OLFM-6C-ADS; VCS). After each test, olfactometer parts were cleaned. Leaf samples were retrieved post-test to confirm EC. The same experiment was repeated on a second occasion with new plant sets, insects and inoculum.

2.5 Gas chromatography–mass spectrometry (GC–MS) analysis of VOCs collected from melon plants

Dichloromethane extracts from melon samples were analyzed by direct injection into a Trace GC Ultra (Thermo Fisher Scientific) coupled to an ISQ Single MS. Injections were in splitless mode using an HP-FFAP column (50 m × 0.32 mm, 0.50-μm film; SGE Analytical Science/Trajan Scientific and Medical, Melbourne, Australia). Helium was the carrier gas at 1.7 mL min^{-1}. The oven temperature was initially 40 °C (3 min), then increased to 100 °C by 8 °C min^{-1}, then 240 °C by 5 °C min^{-1} (held 3 min). The MS used electron ionization (70 eV) in SIM mode. Transfer line and source temperatures were 230 °C and 200 °C, respectively. VOCs were tentatively identified by matching spectra with the NIST library. LRIs were calculated using a C8–C21 *n*-alkane standard as per Eqn (1).[32]

$$\text{LRI} = 100 \times \left[\left(\left(Rt_\text{c} - Rt_\text{n}\right)\right) / \left(\left(Rt_\text{N} - Rt_\text{n}\right)\right) + n\right] \qquad (1)$$

where LRI is the linear retention index, Rt_c is the retention time of the target compound; Rt_n and Rt_N are the retention times of the standard alkanes with n and N carbon atoms, respectively, and *n* is the number of carbon atoms in the smaller alkane. LRIs were confirmed using values from HP-FFAP and similar columns (DB-Wax, ZB-Wax, HP-Wax) in the NIST library. Metabolites were assigned confidence levels based on identification criteria.[33] Semiquantitative analysis was done using Xcalibur Qual Browser (Thermo Fisher Scientific). Supporting information Table S1 lists the VOCs detected, including their retention times (*Rt*), experimental and theoretical LRIs (lRexp, lRnist), ID level, CAS number, match factor (MF) and reverse match factor (RMF).

2.6 Statistical analyses

Residuals of models evaluating the EC rates obtained from microbiological and molecular analyses, and from the dataset of volatile compound peak areas were tested for normality and heteroscedasticity using Shapiro–Wilk and Levene´s tests. Variables that failed one or more of these linear model assumptions were transformed before analysis. Specifically, EC rates expressed as percentages were submitted to arcsin transformation (arcsin $\sqrt{\frac{x}{100}}$) and qPCR data were transformed using $\sqrt{x}$, whereas for volatile peak areas the logarithmic transformation log $(x+1)$ was applied to meet normality assumptions.

Endophytic colonization results were analyzed using a two-sample Student´s *t*-test ($P \leq 0.05$ for microbiological analyses and $P \leq 0.1$ for qPCR analyses) between cultivars (cv. Galia *versus* cv. Futuro, cv. Galia *versus* cv. Rinconete, and cv. Futuro *versus* cv. Rinconete) and between treatments (*B. bassiana* EC aphid-infested *versus B. bassiana* EC plants without aphids). All statistical analyses were done using the software package Statistix 9.0 (Analytical Software, Tallahassee, FL, USA).

Data on the behavioural choices of *C. carnea* were analyzed using two complementary analyses. First, generalized linear mixed models (GLMM) with a Poisson distribution were performed to test the *C. carnea* behaviour. In each model, the response variable was the number of *C. carnea* making a treatment choice; the fixed factors were treatment (control, aphid-infested plants, EC plants and aphid-infested EC plants), *C. carnea* sex (male and female) and the interaction between treatment and *C. carnea* sex; a random factor was experimental repetition that is, the two occasions on which the experiment was done. The Walt test was used to determine the significance of each fixed factor in the model. Additionally, a *z*-test for proportions was done for each treatment option to evaluate whether any treatment was selected above random expectation (25% in a four-choice design). All analyses of behavioural choice were done using the statistical software package R (v4.2.0; R Core Team 2021). Only those insects that made a choice were included in the analysis.

Differences in volatile profiles related to the two factors studied (cultivar and fungal treatment) were assessed by two-way analysis of variance (ANOVA), followed by an LSD all-pairwise comparison test ($P < 0.05$), using the software package Statistix 9.0 (Analytical Software). To assess the impact of *B. bassiana* EC on the volatile profile emitted by the plants, a multivariate statistical approach was applied. partial least-squares discriminant analysis (PLS-DA), with a Jackknife Leave-One-Out (LOO) cross-validation method and 99% confidence intervals (CI), principal component analysis (PCA) and hierarchical clustering analysis (HCA) were done using XLSTAT Premium software (v2019.4.1; Addinsoft, Barcelona, Spain) to identify differences in volatile emissions. Comparisons were made between *B. bassiana* EC and noncolonized control plants in cultivar assays, and amongst aphid-infested EC plants, aphid-infested control plants, EC plants and control plants, in the choice experiments. Additionally, independent Venn diagrams were constructed to explore unique and shared volatile compounds associated with *B. bassiana* EC and control melon plants. Venn diagrams were generated using the online tool provided by Bioinformatics & Evolutionary Genomics (https://bioinformatics.psb.ugent.be/webtools/Venn/ accessed on 12 February 2025).

3 RESULTS

3.1 Detection and quantification of EC of melon plants by *B. bassiana*

Microbiological and molecular techniques confirmed *B. bassiana* EC in all fungal-treated melon plants used in the experiments.

Beauveria bassiana was not present in samples from plants that had been sprayed with the control solution either when they were assessed microbiologically or when they were analyzed by qPCR; no amplification products were observed. Moreover, efficacy of the surface disinfestation procedure was confirmed for all the samples; *B. bassiana* was not found in the last rinse water.

Endophytic colonization rates in plants used to evaluate differences among cultivars (collected 48 h post-treatment) were 85.0 ± 2.2% of the leaf tissue in melon plants cv. Galia; 80.00 ± 3.7% in cv. Futuro; and 73.3 ± 4.2% in cv. Rinconete. The percentage of EC of leaf tissues was significantly greater in cv. Galia than in cv. Rinconete ($t = 2.33$, $P = 0.03$). However, differences were not significant for the other comparisons: Futuro *versus* Rinconete and Galia *versus* Futuro ($t = 1.13$, $P = 0.28$ and $t = 1.08$, $P = 0.30$, respectively) [Fig. 2(A)]. Regarding the results obtained by qPCR, there were no significant differences in quantity of *B. bassiana* amongst the cultivars at $\alpha = 0.05$, although there were statistically significant differences at $\alpha = 0.1$. The quantity of *B. bassiana* DNA in melon samples from cv. Galia, cv. Futuro and cv. Rinconete were 8.7 ± 2.2 pg, 6.8 ± 1.9 pg and 4.2 ± 0.9 pg *B. bassiana* DNA per 30 ng total leaf DNA, respectively. The quantity of *B. bassiana* DNA per 30 ng leaf sample was greater in cv. Galiathan in cv. Rinconete($t = 2.46$, $P = 0.08$) [Fig. 2(A)]. By contrast, no significant differences in quantity of fungal DNA were observed for the other comparisons; Futuro *versus* Rinconete, and Galia *versus* Futuro ($t = 2.21$, $P = 0.21$ and $t = 0.55$, $P = 0.59$, respectively).

Regarding the melon plants cv. Galia F_1 used in the lacewing choice assay, no significant differences were found in the EC rates when leaf samples from *A. gossypii*-infested plants and noninfested plants were analyzed by microbiological methods (7 days after the fungal treatment) ($t = 1.17$, $P = 0.86$). Endophytic colonization was 65.0 ± 2.2% of the leaf tissue in plants that had been infested with *A. gossypii* and 70.0 ± 3.7% of the leaf tissue in noninfested plants [Fig. 2(B)]. Similar results were obtained using qPCR; no significant differences were found in the quantity (pg) of *B. bassiana* DNA in leaf samples from *A. gossypii*-infested and noninfested plants (2.5 ± 0.8 pg and 1.7 ± 1.0 pg *B. bassiana* DNA per 30 ng total leaf DNA, respectively) ($t = 0.61$, $P = 0.72$) [Fig. 2(B)].

3.2 Effect of EC by *B. bassiana* on the volatile profiles of three melon cultivars

A total of 93 volatile compounds were tentatively identified across the three melon cultivars (Galia, Futuro, Rinconete) under both *B. bassiana* EC and control conditions. The identified compounds included those naturally emitted by the plants, as well as compounds either induced in the plants by the fungal treatment or directly produced by the fungus. They were classified according to their chemical nature as: alcohols (11), aldehydes (nine), aliphatic hydrocarbons (11), aromatic hydrocarbons (15), carboxylic acids (six), esters (six), ethers (two), ketones (seven), nitrogen compounds (two), phenols and phenolic derivatives (five), terpenes (11) and miscellaneous (eight) (Supporting information Table S1).

Statistical analysis (two-way ANOVA) indicated that cultivar had a significant effect on the emission of volatile compounds under both control and EC conditions, (Table S2). Consistent with these results, agglomerative hierarchical clustering analysis (AHC) revealed that cultivar had a stronger influence on the overall volatile profile than fungal treatment. This pattern is visually supported by the dendrogram (Fig. S1), which shows control and treated samples from cvs Rinconete and Galia clustered together, reflecting cultivar-driven similarities in volatile emissions regardless of treatment (Table S2; Fig. S1).

Given the strong influence of cultivar on the overall volatile profile, a cultivar-specific analysis was done to evaluate EA treatment-related effects. When agglomerative hierarchical clustering combined with heatmap analysis (AHC-heatmap) was applied individually to each cultivar, a clear separation between EC and control samples was observed (Fig. S2). Based on these results, PLS-DA was used to identify the volatile compounds contributing most to differentiation between EC and control plants (variable importance in projection, VIP > 1) (Fig. 3).

Principal component analysis was conducted with previously selected data markers for each cultivar (data from the PLS-DA, VIPs >1, and from ANOVA, $P < 0.05$) (Table S1; Fig. 3) to identify VOCs associated with either control or EC plants, thus confirming the relevance of these compounds in differentiating treatment effects. Two principal components (PC1 and PC2) explained 85.0%, 85.8% and 92.5% of the total variance found in the data for cvs Galia, Futuro and Rinconete, respectively (Fig. 4). PC1 accounted for the majority of variance in volatile profiles for cvs Galia (74.6%), Futuro (69.8%) and Rinconete (85.5%). Amongst total volatile compounds identified per cultivar, 39.8% (33 of 83; 24 quantitative and nine qualitative) in cv. Galia, 40.5% (36 of 89; 23 quantitative and 13 qualitative) in cv. Futuro and 34.2% in cv. Rinconete (28 of 82; 16 quantitative and 12 qualitative) showed significant differentiation between control and EC plants (VIP > 1) and were considered marker compounds (VIP > 1 and $P < 0.05$). Amongst these marker compounds, a substantial proportion was emitted either exclusively or at higher concentrations in EC samples: 79.4% in Galia, 36.1% in Futuro and 42.9% in Rinconete (Table S1; Fig. 4). Conversely, 20.6%, 63.9% and 57.2% of the markers were more abundant in control plants for Galia, Futuro and Rinconete, respectively. These findings suggest that although all cultivars exhibited changes in volatile emissions in response to fungal treatment, the volatile emission profile of cv. Galia in response to fungus showed the most pronounced changes, characterized by a general increase in volatile production compared with control plants [Fig. 4(A)]. *Beauveria bassiana* EC-induced changes in the volatile profiles of Futuro and Rinconete (both from the Piel de Sapo variety) generally corresponded to a decrease in emission of specific marker compounds. This contrasts with cv. Galia, which exhibited a higher degree of EC and a general increase in volatile production in response to fungal treatment.

Marker compounds that were predominant or exclusive to either control or EC samples are represented separately in Venn diagrams (Fig. 5). Notably, 3,7-dimethyldecane, heptanoic acid and octanoic acid, were exclusively emitted in control plants across all three cultivars [Fig. 5(A)]. Some marker compounds, example for, 2-methylundecanal, octadecane, pentanoic acid and nonanoic acid, were commonly associated with control plants of the Futuro and Rinconete cultivars (both cvs of the Piel de Sapo variety) [Fig. 5(A)]. By contrast, no discriminant volatile compounds were common between cv. Galia (var. Reticulatus) and the other two cultivars [Fig. 5(A)].

Conversely, *B. bassiana* EC plants exclusively emitted the compounds *cis*-3-hexenal and *N,N*-dimethyldodecanamine, across all three cultivars [Fig. 5(B)]; these volatile emissions in response to EC occurred likewise across all cultivars. Statistical analysis revealed no significant differences in emission of *N,N*-dimethyldodecanamine amongst cultivars ($F = 0.31$, $P = 0.74$).

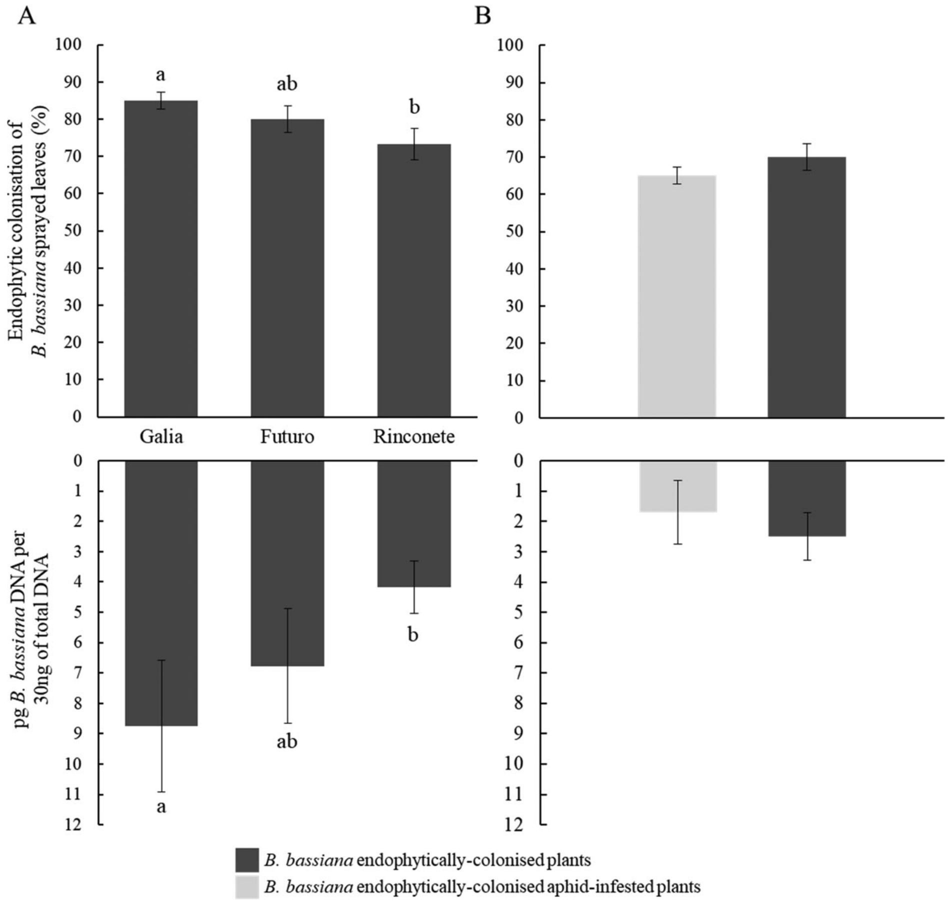

Figure 2. Detection of endophytic presence of *B. bassiana* by microbiological techniques (upper bars) and by qPCR (lower bars) in melon plants inoculated by a foliar spray with 5 mL of a conidial suspension (1.0×10^8 conidia mL^{-1}). Plant samples were collected at the end of (A) the cultivar assay, 2 days after foliar spray, and (B) the lacewing choice assay, 7 days after foliar spray and 5 days after aphid infestation initiated. Microbiologically determined endophytic colonization is expressed as the percentage of melon leaf fragments in which fungal growth was observed (% mean ± SE); molecular detection and quantification is expressed in fungal DNA pg relative to 30 ng total DNA per reaction. For qPCR quantification, bars represent the mean values of three technical replicates from each of six independent biological replicates. For each method and experiment bars with different letters are significantly different from each other as analyzed by Student's *t*-test ($P \leq 0.05$ for microbiological analyses and $P < 0.1$ for qPCR analyses).

Nonetheless, an effect of cultivar was evident for *cis*-3-hexenal emission from EC plants; emission of this volatile was significantly different amongst the three cultivars, being particularly high in cv. Futuro ($F = 3.87$; $P = 0.04$). Furthermore, ocimene, hexahydrofarnesyl acetone, benzenepropanol, hexanal and octaldehyde were emitted in higher amounts by EC plants than controls in cvs Galia and Futuro. Additionally, tetracosane and propylbenzene were associated with the EC plants of the Galia–Rinconete and Rinconete–Futuro cultivar pairs, respectively. Notably, cv. Galia emitted the largest number of cultivar-discriminant VOCs associated with *B. bassiana* colonization (19), indicating a markedly stronger metabolic response compared with Rinconete (eight) and Futuro (five).

After identifying the VOCs that distinguish between EC and control plants, those shared by two or more cultivars, illustrated in the Venn diagrams, were analyzed using PCA. The plot shows that PC1 enabled clear separation between control and EC samples, whereas PC2 revealed trends related to cultivar-specific responses. Specifically, Futuro and Rinconete exhibited similar VOC profiles under control conditions, clustering in the second quadrant. By contrast, after EC, Rinconete displayed greater similarity in the emission of VOCs to the cv. Galia (Fig. S3).

Amongst all of the identified discriminant compounds, key compounds with known ecological functions were identified and summarized in Table 1.

Taken together, these findings highlight cv. Galia as a promising candidate for future studies, given its greater susceptibility to *B. bassiana*-induced volatile changes, which may have implications for plant–fungus–insect interactions. Thus, this cultivar was used for studying predator preference.

3.3 Effect of EC by *B. bassiana* on predator preference

Only 32% of *C. carnea* adults chose one of the offered plants. Lacewing sex did not affect choice behaviour; of those that made a choice, 56.3% were females and 43.8% were males. Specifically: 37% of individuals chose EC plants infested with *A. gossypii*; 23% chose aphid-infested control plants; 17% chose EC melon plants; and 23% chose control plants (Fig. 6). However, there was no significant differences in lacewing choice amongst treatments (aphid-infested EC plants *versus* aphid-infested controls *versus* EC plants *versus* controls) (GLMMs, $\chi^2_{3\text{df}} = 4.22$; $P = 0.23$) nor when sex was considered (GLMMs, $\chi^2_{1\text{df}} = 0.31$; $P = 0.57$).

In order to further assess whether any treatment was preferred above random expectation (25%), a one-sample *z*-test for proportions was performed; aphid-infested *B. bassiana*-EC plants were chosen

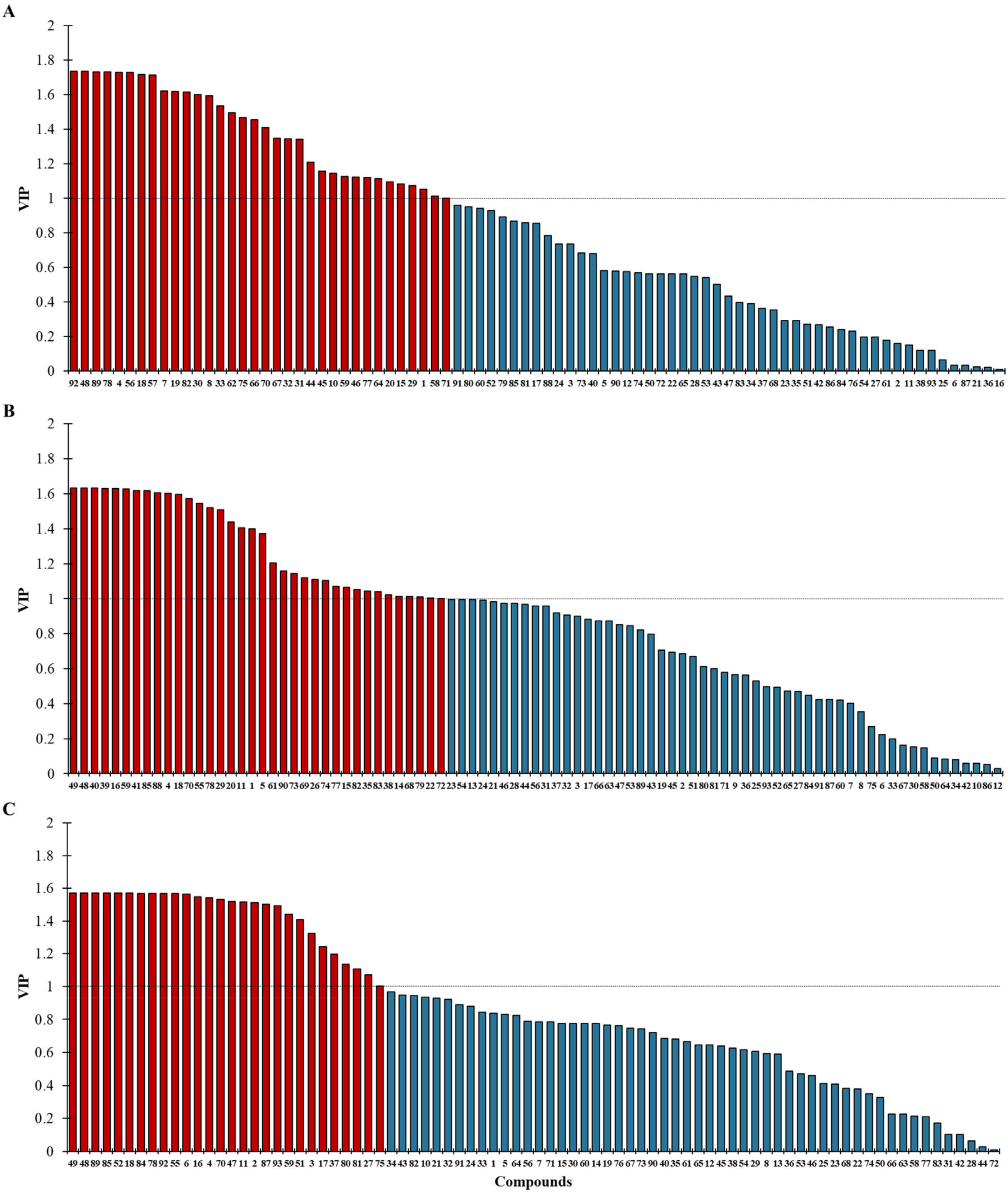

Figure 3. Variable importance in projection (VIP) plots derived from PLS-DA, showing potential of volatile organic compound (VOC) markers to differentiate between melon cultivar treatments: (A) Galia F1, (B) Futuro and (C) Rinconete. Numbers represent individual VOCs following the numbering in Table S1. Red bars indicate VOCs with VIP scores >1, that are considered as significant as contributors to treatment separation; blue bars represent non-influential compounds (VIP < 1).

significantly more often than expected by chance ($z = 2.00$, $P = 0.02$, one-tailed), indicating a significant preference and no significant deviations from random choice were observed for the other treatments.

Taken together, these results suggest that although direct comparisons amongst treatments did not reveal significant differences, *C. carnea* adults exhibited a specific preference for aphid-

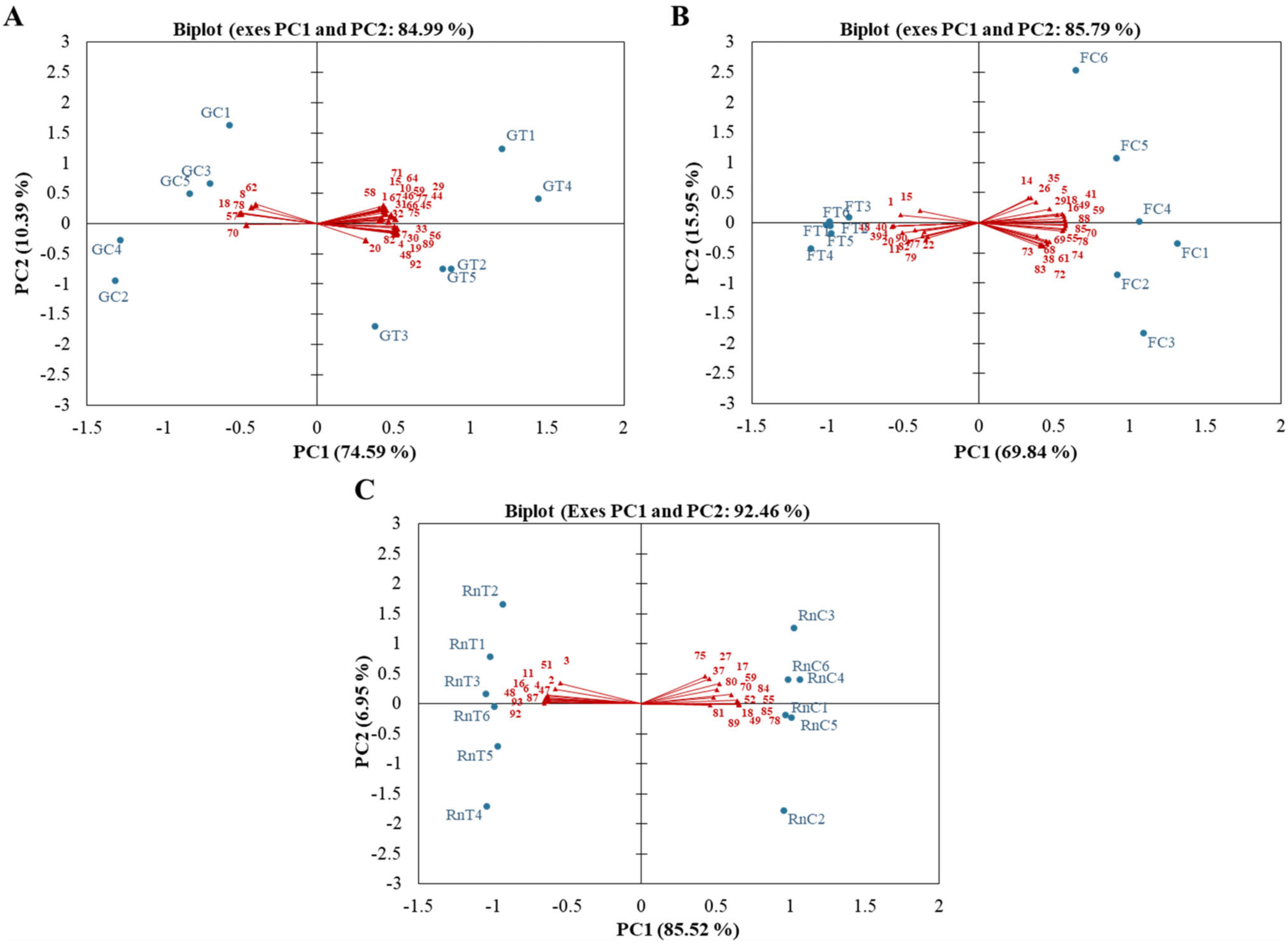

Figure 4. Principal component analysis (PCA) of VOCs identified as markers (VIP >1 and $P < 0.05$) in *B. bassiana* EC and control plants from three melon cultivars: (A) Galia, (B) Futuro and (C) Rinconete. Numbers represent individual VOCs contributing to separation of treatments as listed in Table S1. GC, Galia control plants; GT, Galia *B. bassiana* EC plants; FC, Futuro control; FT, Futuro *B. bassiana* EC plants; RnC, Rinconete control; RnT, Rinconete *B. bassiana* EC plants.

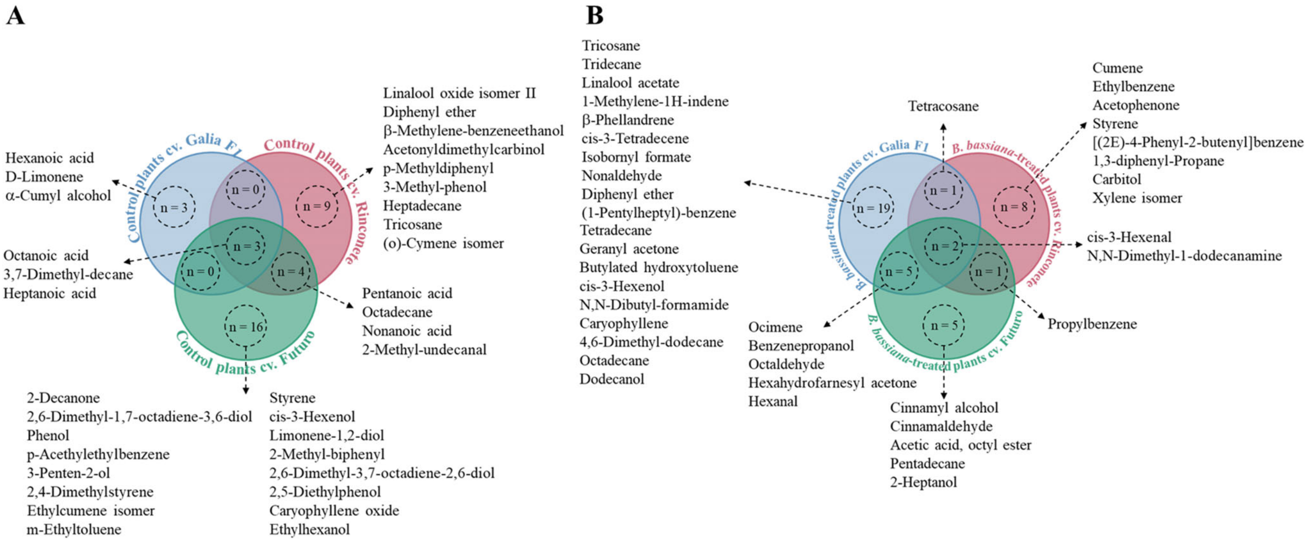

Figure 5. Venn diagrams of VOCs identified as markers (VIP > 1 and $P < 0.05$) in *B. bassiana* EC and control plants from three melon cultivars. Number of VOCs predominant or exclusive to (A) control melon plants at 48 h post-treatment with control solution, and to (B) *B. bassiana* EC melon plants, at 48 h post-treatment with fungal suspension. The number of VOCs are indicated within the circles.

Table 1. Key cultivar-specific volatile compounds identified as marker compounds in melon plants (cvs Galia, Futuro and Rinconete) with known ecological functions in plant-insect interactions

Compound	ID Compound	Galia	Futuro	Rinconete	Ecological function
Compound 2	Ethylbenzene			↑	Predator attractant
Compound 6	Cumene			↑	Predator attractant
Compound 2	cis-3-Hexenal	↑	↑	↑	Antimicrobial activity
Compound 10	*β*-Phellandrene	↑		↓	Parasitoid attractant
Compound 16	Styrene		↓	↑	Predator attractant
Compound 18	3,7-Dimethyl-decane	↓	↓	↓	Antimicrobial activity
Compound 22	2-Heptanol		↑		Repellent to phytophagous species and predator attractant
Compound 29	cis-3-Hexenol	↑	↓		Parasitoid attractant
Compound 44	Linalool acetate	↑			Aphid repellent
Compound 46	Caryophyllene	↑			Aphid repellent
Compound 48	*N,N*-Dimethyldodecanamine	↑	↑	↑	Antimicrobial activity
Compound 51	Acetophenone			↑	Predator attractant
Compound 64	Geranyl acetone	↑			Aphid repellent
Compound 70	Heptanoic acid	↓	↓	↓	Antimicrobial activity
Compound 78	Octanoic acid	↓	↓	↓	Antimicrobial activity
Compound 79	Cinnamaldehyde		↑		Toxic and repellent to phytophagous species
Compound 85	Nonanoic acid	↓	↓	↓	Antimicrobial activity
Compound 90	Cinnamyl alcohol		↑		Pollinator attractant

Note: All identified compounds are listed in Table S1. Upward arrows indicate compounds emitted in higher quantities or exclusively in EC plants by *B. bassiana* across the different cultivars. Downward arrows indicate compounds emitted in lower quantities or were absent in EC plants by *B. bassiana* across the different cultivars.

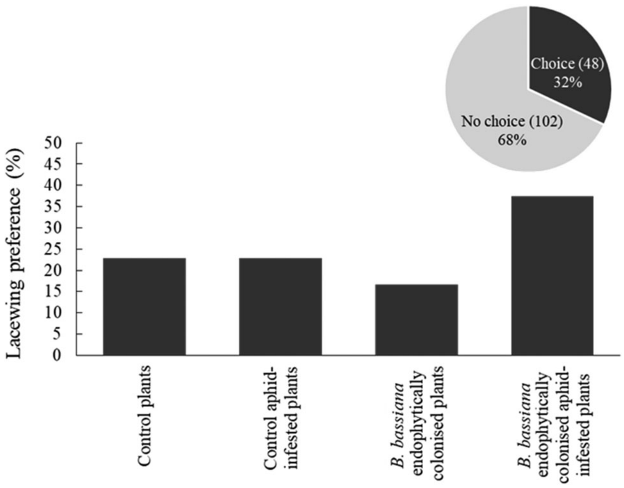

Figure 6. Olfactometer response of *C. carnea* ($N = 48$) to different VOCs emitted by (i) an aphid-infested *B. bassiana* EC plant, (ii) a *B. bassiana* EC plant, (iii) an aphid-infested control plant and (iv) a control plant that was neither EC or aphid infested. The pie chart shows the percentage of lacewings that entered an arm (dark grey) and the number of lacewings that did not make any choice and remained in the centre of the olfactometer (light grey).

infested, EC melon plants over the other treatments relative to a random choice expectation.

3.4 Effect of EC by *B. bassiana* on volatile profiles from aphid-infested melon plants of cv. Galia

A total of 83 compounds were tentatively identified, including plant-derived, aphid-induced and fungal-induced compounds belonging to the families of: alcohols (12), aldehydes (five), aliphatic hydrocarbons (nine), aromatic hydrocarbons (seven), carboxylic acids (one), esters (seven), ethers (two), ketones (five), nitrogen compounds (four), phenols and phenolic derivatives (four), terpenes (24) and miscellaneous (three) (Table S3).

The heatmap visualization enabled us to differentiate between two main treatment groups: (i) noninfested plants, including control plants (plant only) and *B. bassiana* EC plants (plant + fungus); and (ii) aphid-infested plants, including aphid-infested control plants (plant + aphid) and aphid-infested EC plants (plant + fungus + aphid) (Fig. S4). Moreover, PLS-DA identified discriminant volatile compounds (VIP >1) that enabled separation, with a higher relevance, between aphid-infested or noninfested plants, regardless of whether they were EC (Fig. 7). The PCA done with identified marker compounds (VIP >1 and $P < 0.05$) to assess variation amongst EC and control samples in aphid-infested and noninfested plants is illustrated in Fig. 8. The two principal components (PC1 × PC2) explained 74.5% of the total variance in the dataset, with PC1 accounting for 56.0% and PC2 for 18.5% of variation. The first principal component (PC1), accounting for the greatest variation, revealed the influence of several compounds on the separation of aphid-infested from noninfested plant samples. *Cis*-3-hexenol, prenylacetone, 4-tert-butylcyclohexyl acetate and [(2*E*)-4-phenyl-2-butenyl]benzene were considered important contributors to group separation and positively correlated with non-aphid-infested melon plants. These compounds were found in higher concentrations or even exclusively, as 4-tert-butylcyclohexyl acetate, in these samples (Fig. 8; Table S3). Because this group includes both control samples (plants without EC or aphid infestation) and samples that were EC only, we evaluated the influence of these compounds within each group of plants (noninfested and aphid-infested plants) (Table S4). Amongst these compounds, significant

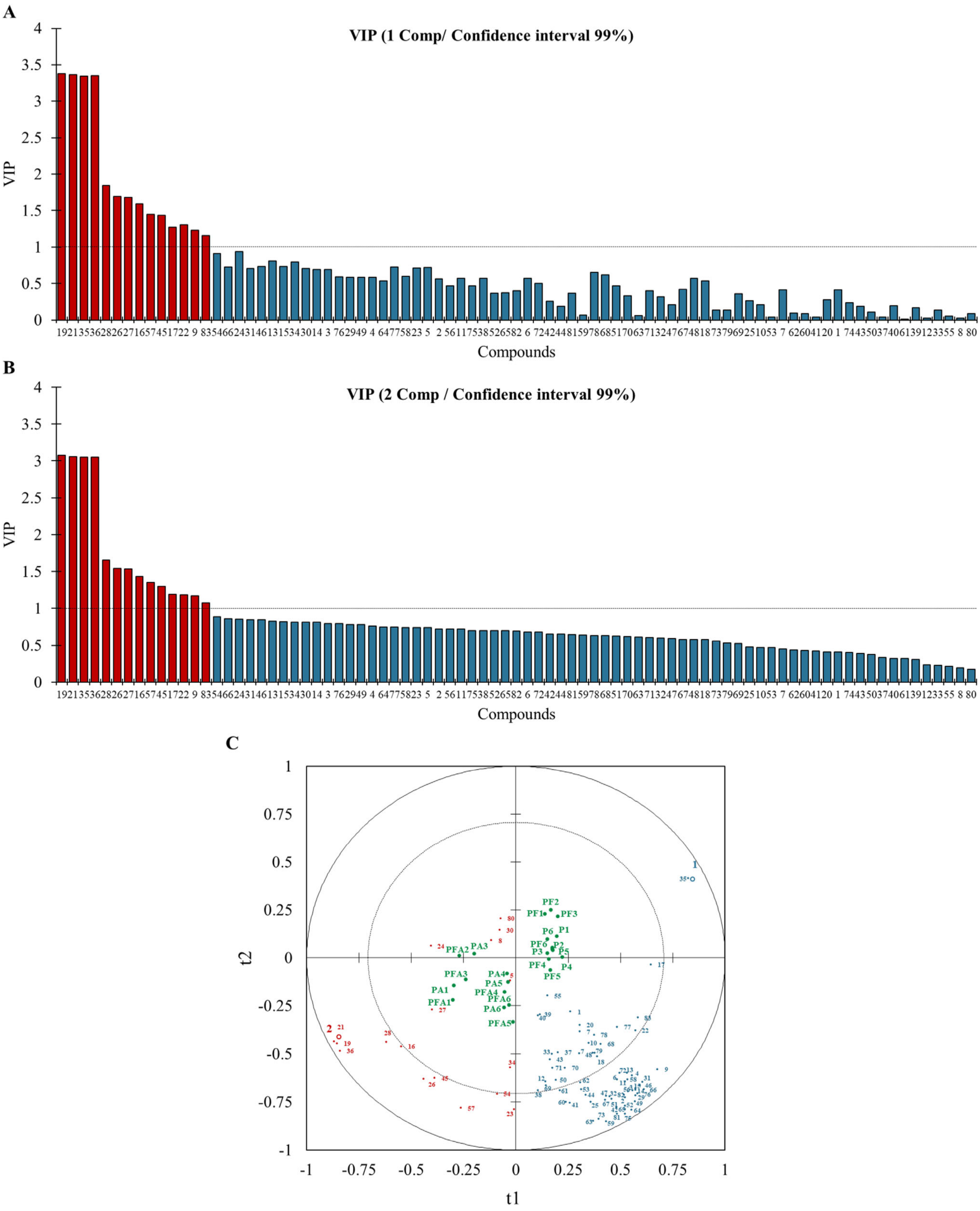

Figure 7. Multivariate statistical analyses of data on VOCs: (A, B) VIP plots of the first two components showing the most relevant VOCs (potential VOCs markers, VIP > 1) distinguishing infested and noninfested melon plants; (C) PLS-DA score plot illustrating the separation of samples (model performance parameters for two components: Q^2 cumulative $= 0.723$, R^2Y cumulative $= 0.884$, R^2X cumulative $= 0.561$), including correlations between VOCs and melon samples. Numbers represent VOCs contributing to separation amongst treatments, as listed in Table S3. Red numbers correspond to VOCs correlated with aphid-infested melon samples and blue numbers correspond to VOCs correlated with non-aphid-infested melon samples.

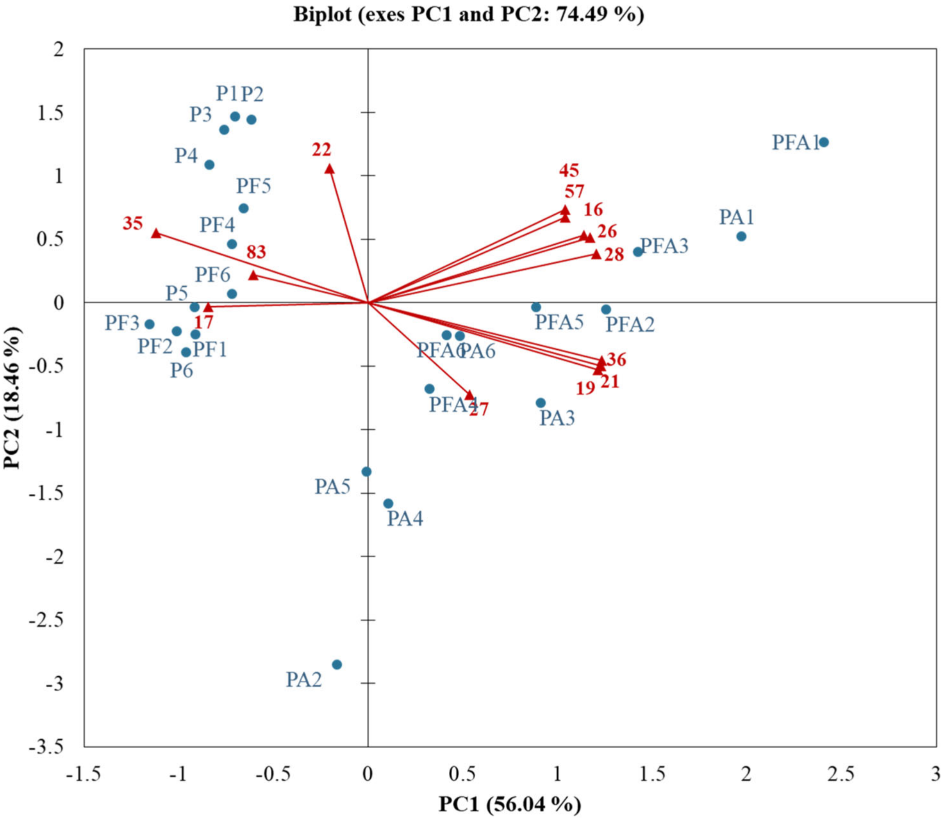

Figure 8. Principal component analysis biplot (scores and loadings) of VOCs (VIPs >1 and $P < 0.05$) markers identified in melon plants contributing to the differentiation between aphid infested (aphid-infested *B. bassiana* EC plant, PFA; aphid-infested control plant, PA) and noninfested (*B. bassiana* EC plant, PF; and control, P) melon plants ($n = 6$ per treatment). Numbers represent VOCs contributing to the separation amongst treatments, as listed in Table S3. P, Plant; PA, Plant + Aphid; PF, Plant + Fungus; PFA, Plant + Fungus + Aphid.

quantitative differences in emission were detected between EC and nontreated (noninfested) plant samples for 4-tert-butylcyclohexyl acetate ($F = 12.32$, $P = 0.0009$) and [*(2E)*-4-phenyl-2-butenyl]benzene ($F = 6.25$, $P = 0.0315$). The emission of 4-tert-butylcyclohexyl acetate was significantly increased in EC plants, whereas emission of [*(2E)*-4-phenyl-2-butenyl]benzene was significantly reduced (Table S3).

Interestingly, some nonmarker compounds (VIP < 1) showed significant differences in their emissions between EC and control melon plants in the noninfested group; emissions of 2-hexenal, *cis*-3-hexenyl acetate, 1-octen-3-ol and octylcyclohexane were significantly reduced in EC plants compared with controls when they were not infested by aphids ($F = 12.57$, $P = 0.01$, $F = 11.52$, $P = 0.01$, $F = 5.61$, $P = 0.03$, $F = 10.75$, $P = 0.01$, respectively) (Table S4).

By contrast, compounds such as farnesane, the isomer *cis* and *trans* of limonene oxide, 3-methyltridecane, 2,3,5-trimethyldecane, 1,12-tridecadiene, 5-cyclohexyleicosane, heptadecane and 7-phenyltridecane, where positively correlated with aphid-infested melon plants, regardless of whether they were EC or not (Fig. 8). Amongst these compounds, 3-methyltridecane, 2,3,5-trimethyldecane and 5-cyclohexylcosane were only found in aphid-infested melon plants, and there were no significant differences in their relative abundance on aphid-infested EC and aphid-infested nontreated plants (Table S3). Significant quantitative variations between EC and control plants, both infested with aphids, were only found for the compound 7-phenyltridecane, which was emitted in higher concentrations from EC plants than from control plants ($F = 6.71$; $P = 0.0028$) (Table S3).

Additionally, cinnamyl alcohol emissions, a nonmarker compound with VIP < 1, differed significantly between EC and control plants, both in aphid-infested and noninfested plants. For noninfested plants, EC plants emitted higher concentrations of cinnamyl alcohol than control plants ($F = 5.57$, $P = 0.03$). By contrast, aphid-infested EC plants emitted significantly less cinnamyl alcohol than untreated aphid-infested plants ($F = 6.72$, $P = 0.02$) (Table S4).

4 DISCUSSION

This study showed that *B. bassiana* can establish EC in three melon cultivars, though colonization levels varied by cultivar, as confirmed by microbiological and qPCR analyses. This suggests a genotype-dependent response amongst cultivars that was independent of variety. Endophytic colonization efficiency may depend on host plant genotype, which affects plant physiology, chemistry, structure and immunity, potentially explaining variation in colonization rates amongst melon cultivars through modulation of their interaction with *B. bassiana*.[34–36] Previous studies have linked EC variability to crop species or fungal strain[11,12,16]; this study is the first to demonstrate cultivar-specific differences, as prior work in sweet pepper did not correlate cultivar response with colonization data.[11]

In the present study, cultivar-specific VOC alterations in response to *B. bassiana* colonization were observed; in the case of cv. Rinconete, this could be associated with its lower degree of colonization and the consequently reduced modulation of

VOC emissions. Nonetheless, the relationship between entomopathogenic fungal EC and plant priming is not strictly dose-dependent. Recent evidence demonstrates that low levels of endophytic colonization or even a priming of the plants by fungal contact, without endophytic colonization, can effectively activate defence-related signalling pathways in cucurbits through jasmonic acid (JA), salicylic acid (SA) and ethylene (ETH) routes.[37] Thus, the degree of volatile profile modification reflects not only the abundance of the endophyte, but also the host–microbe interaction developed in each cultivar.[37–40] Moreover, our study revealed that, although both the genetic background (melon cultivar) and the EC rate influence changes in VOCs production, *B. bassiana* colonization also induces alterations in these VOCs linked to other unidentified factors.[41] In this regard, *B. bassiana* EC melon plants across all cultivars emitted common VOCs, *cis*-3-hexenal and *N,N*-dimethyldodecanamine, which were absent in control plants, suggesting a common *B. bassiana* EC-mediated response not associated with either the cultivar or the variety. Our results showed that these two compounds, which are known to have antimicrobial activity, were initially (48 h postinoculation) induced as part of the plant early defence response, whereas their production was subsequently suppressed (7 days postinoculation).[42–44] Previous studies have suggested that the host initially perceives endophytes as external agents, triggering systemic defences via ETH, JA, SA and auxin pathways.[37,45] Once fungal growth is controlled, defence gene expression and antimicrobial metabolite production often return to baseline levels.[37,45] These findings provide a foundation for future investigations into temporal dynamics over longer periods and in practical field conditions. Additionally, *cis*-3-hexenal plays a crucial role in attracting natural enemies such as parasitoids,[42] which highlights the need for further research on its specific ecological impact in other entomophagous species, including lacewings. Interestingly, several compounds such as 3,7-dimethyldecane, heptanoic acid, octanoic acid and nonanoic acid, with reported antimicrobial activities,[46–48] were not detected in *B. bassiana* EC plants across all cultivars, but were present in the volatile profiles of control plants; this might reflect a metabolic adjustment associated with the fungus–plant relationship.[49] Nevertheless, both qualitative and quantitative differences in VOCs emitted by each cultivar between EC and control plants suggest that part of the plant response to EC is cultivar-specific.[14,50]

A key finding of our work is that *B. bassiana* colonization modulates plant chemical defences influencing key plant–insect interactions related to crop protection. For example, VOCs emitted at significant levels across one of the three cultivars include allomones, which act as repellents for phytophagous insects, and synomones, which attract natural enemies. In cv. Galia, *cis*-3-hexenol, β-phellandrene (parasitoid attractants), linalool acetate, caryophyllene and geranyl acetone (aphid repellents) were emitted in higher quantities in *B. bassiana* EC plants.[51–53] Notable emissions in cv. Futuro were 2-heptanol, which is a known repellent of insect pests and attractant of predator species,[54,55] and cinnamaldehyde, which is toxic and repellent to phytophagous species.[56,57] It is worth noting that cinnamyl alcohol has been identified as a bee attractant, supporting our previous finding that *B. bassiana* colonization was associated with enhanced plant productivity via pollinator attraction.[18,58] Lastly, in EC plants of cv. Rinconete, we found higher production of the aromatic compounds ethylbenzene, cumene, styrene and acetophenone than in control plants; all of these are attractants of predator species.[59,60] The defensive potential of *B. bassiana*-colonized plants for biological control beyond lethal and sublethal effects on crop pests is underscored by the fact that each cultivar has unique genetic mechanisms for responding to fungal colonization which result in production of distinct VOCs, but all are aimed at protecting the plant. The distinct endophytic fungal response in the different melon cultivars raises new questions about how these changes in the VOCs profile might influence attraction of damaging pests but also beneficial insects, especially under field conditions where insects could have the potential to make choices between EC and non-EC plants. Recent studies have shown that *B. bassiana* EC can significantly influence interactions amongst plants, insect pests and their natural enemies.[4] In particular, it has been suggested that *B. bassiana*-colonized plants emit VOCs that attract larvae of the generalist predator *C. carnea*.[19] The present study advances this line of research by providing the first report exploring the multitrophic interactions involving EC plants, aphids and an adult generalist predator. From an applied IPM perspective, knowledge of cultivar-specific VOCs induced by *B. bassiana* EC could be leveraged to select cultivars that enhance indirect plant defences by attracting beneficial insects, or to further promote this attraction in cultivars where an increase in these volatile attractants was not observed using additional techniques.[61] Nonetheless, this effect should be confirmed under field conditions.

Cultivar Galia was selected owing to its higher EC rate compared with the other melon cultivars assessed, and the presence of more discriminant VOCs indicative of such volatile profile alteration by the EC.

It should be noted that it was observed a relatively low response in the lacewings, which is consistent with observations frequently reported in insect olfactometer choice experiments.[18,62,63] Such variability may result from physiological or behavioural differences among individuals, handling stress or suboptimal experimental conditions that are difficult to control.

Moreover, when the presence of *B. bassiana* in melon plants used in choice assays was verified and quantified using both microbiological and molecular techniques, no significant differences in the degree of EC were observed between aphid-infested and noninfested plants. This study is the first to assess the effect of aphid feeding on endophytic colonization, considering only the absence of aphids and a single density of 10 adult aphids per plant. These results establish a foundation for future studies, in which a broader range of aphid densities could be evaluated to unravel whether a threshold exists beyond which endophytic colonization intensity may decline and/or volatile emissions may change, altering natural enemy attraction.[64,65]

Regarding lacewing behaviour results, they revealed no significant differences in adult lacewing preference among any of the offered options (EC and control plants; aphid-infested and noninfested plants). Nonetheless, targeted analysis revealed that lacewings selected EC plants infested with aphids significantly more frequency than predicted under a random distribution. This suggests potential for specific attraction to plants that were simultaneously colonized by the fungus and infested by aphids. Plants that were both EC and aphid-infested elicited a measurable, interaction-driven response, in which the combination of fungus and aphids altered the plant odour and produced a small but detectable bias in lacewing orientation, which arises from their combined effect rather than from either factor alone. Thus, we can hypothesize that there would be a trend toward increased attraction of adult lacewings to EC- and aphid-infested plants under field conditions compared with the other choices which we offered in the olfactometer assay. This implies that the benefits conferred by the fungus may become evident upon pest attack, functioning as a bodyguard.[66]

For example, VOC analysis showed that aphid infestation reduced *cis*-3-hexenol and *cis*-3-hexenyl acetate emissions in control plants, but not in those colonized by *B. bassiana*, where levels remained stable. As these compounds attract lacewings, EC plants may attract more lacewings under field conditions.[25,67] This also was the case for the VOCs 7-phenyltridecane, 1,12-tridecadiene, heptadecane, farnesane and cis-limonene oxide that were emitted at higher quantities only in *B. bassiana* EC plants infested with aphids. However, these compounds were detected in similar amounts in non-aphid-infested plants, regardless of whether they were EC or controls, indicating that their higher emission in aphid-infested EC plants emerges from the combined effect of aphid damage and endophytic colonization at the level of overall volatile profile, rather than from pairwise differences between treatments. This may partially explain the limited effect observed on *C. carnea* adult behaviour in the olfactometer assays. The response also could be to the result of EC reducing emission of other compounds that attract lacewings, such as *D*-limonene.[25]

Heptadecane is not only an attractant and oviposition stimulant for phytophagous species, but also an attractant of aphid parasitoids.[68–71] Our findings suggest that the increased emission of heptadecane by aphid-infested *B. bassiana* EC plants not only affects herbivore behavior, but also could enhance host location by aphid parasitoids, thereby contributing to modulation of tritrophic interactions. Indeed, aphid parasitoid attraction by EC plants has been recently reported.[9,71] Interestingly, farnesane is a plant-derived compound produced in response to herbivore attack.[72] It also has been identified as a hydrogenation product of β-farnesene, which was first discovered as an aphid alarm pheromone and plays an important role in aphid communication, particularly in predator avoidance.[24,72,73] Thus, the higher emission of β-farnesene by *B. bassiana*-colonized plants infested with aphids suggests a bodyguard-like synergistic effect of combined aphid infestation and fungal colonization, in which EC primes the plant for a stronger defence response. *Cis*-limonene oxide has been reported as an essential oil component with known antimicrobial and insect-repellent properties. The increased emission of *cis*-limonene oxide in aphid-infested EC plants suggests a bodyguard-like role in the plant defence mechanism against phytophagous insects.

In summary, several of the VOCs identified across experiments are known to mediate plant–insect interactions, reinforcing the ecological relevance of the observed chemical changes. In the cultivar experiments, *B. bassiana* EC plants increased the emission of cis-3-hexenol, cis-3-hexenal, β-phellandrene, linalool acetate, caryophyllene and geranyl acetone, compounds broadly recognized as repellents of herbivores and attractants of parasitoids.[42,51–53] In the choice experiments, aphid-infested EC plants released higher levels of heptadecane, farnesane and cis-limonene oxide, which have been reported as olfactory cues involved in repellence of herbivores, aphid alarm signalling and parasitoid host location.[24,68,70,72–74] Together, these volatiles represent potential chemical markers of plant defence activation, serving as indirect plant defences as olfactory cues for the recruitment of natural enemies under field conditions. Our findings may have practical implications for melon breeding and crop management, as cultivar-dependent VOC responses to *B. bassiana* endophytic colonization could be considered when selecting varieties with improved capacity to recruit natural enemies or enhance indirect defences under field conditions. Although further targeted analyses are required to evaluate volatile responses evolution over time, lacewing responses to individual compounds and their blends, as well as their behaviour under field conditions, this study provides a foundation for exploiting endophytic fungi within IPM strategies that combine biological control and plant-mediated indirect defences.

5 CONCLUSIONS

The present research approach provides insight into the trophic melon-aphid-lacewing predator cascade. Endophytic colonization by *B. bassiana* drives emission of plant- and cultivar-specific VOCs that affect the success of biological control strategies by triggering indirect bodyguard-like defences involving natural enemies. Nevertheless, further studies should validate these results using other fungal strains and aphid densities, as well as test the lacewing exposure to individual compounds and their blends. These results represent the basis for future research under field conditions.

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AUTHOR CONTRIBUTION

EQM and NGM conceived and designed the experiments; MCM and RAH did the experiments; MCM, RRS, and RAH analyzed the data; MCM wrote the manuscript; EQM, NGM and JMMR revised the manuscript. All authors read and approved the manuscript before submission.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Baker BP, Green TA and Loker AJ, Biological control and integrated pest management in organic and conventional systems. *Biol Control* **140**: 104095 (2020).

2 Stenberg JA, Sundh I, Becher PG, Björkman C, Dubey M, Egan PA *et al.*, When is it biological control? A framework of definitions, mechanisms, and classifications. *J Pest Sci* **94**:665–676 (2021).

3 Smagghe F, Spooner-Hart R, Chen ZH and Donovan-Mak M, Biological control of arthropod pests in protected cropping by employing entomopathogens: efficiency, production and safety. *Biol Control* **186**:105337 (2023).

4 Quesada-Moraga E, Garrido-Jurado I, Yousef-Yousef M and González-Mas N, Multitrophic interactions of entomopathogenic fungi in BioControl. *BioControl* **67**:457–472 (2022).

5 Quesada-Moraga E, Garrido-Jurado I, González-Mas N and Yousef-Yousef M, Ecosystem services of entomopathogenic ascomycetes. *J Invertebr Pathol* **201**:108015 (2023).

6 Quesada-Moraga E, Lopez-Diaz C and Landa B, The hidden habit of the entomopathogenic fungus *Beauveria bassiana*: first demonstration of vertical plant transmission. *PlosOne* **9**:e89278 (2014).

7 Garrido-Jurado I, Resquín-Romero G, Amarilla S, Ríos-Moreno A, Carrasco L and Quesada-Moraga E, Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *J Pest Sci* **90**:319–330 (2016).

8 García-Espinoza F, Yousef-Yousef M, García del Rosal MJ, Cuenca-Medina M and Quesada-Moraga E, Greenhouse melon crop protection and production through the compatible use of a parasitoid with endophytic entomopathogenic ascomycetes. *J Pest Sci* **97**:1–14 (2024).

9 Wilberts L, Vuts J, Caulfield JC, Thomas G, Withall DM, Wäckers F *et al.*, Effects of root inoculation of entomopathogenic fungi on olfactory-mediated behavior and life-history traits of the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). *Pest Manag Sci* **80**:307–316 (2024).

10 Cuenca-Medina M, González-Mas N, Martínez-Anguita Ó, Sandoval-Lozano A and Quesada-Moraga E, A new aphid IPM strategy based on the use of endophytic entomopathogenic ascomycetes that reduces treatment risks to the generalist predator *Chrysoperla carnea* (Stephens). *J Invertebr Pathol* **211**:108357 (2025).

11 Wilberts L, Rojas-Preciado N, Jacquemyn H and Lievens B, Fungal strain and crop cultivar affect growth of sweet pepper plants after root inoculation with entomopathogenic fungi. *Front Plant Sci* **14**: 1196765 (2023).

12 Resquín-Romero G, Garrido-Jurado I, Delso C, Ríos-Moreno A and Quesada-Moraga E, Transient endophytic colonizations of plants improve the outcome of foliar applications of mycoinsecticides against chewing insects. *J Invertebr Pathol* **136**:23–31 (2016).

13 Vega FE, The use of fungal entomopathogens as endophytes in biological control: a review. *Mycologia* **110**:4–30 (2018).

14 Bazghaleh N, Prashar P, Woo S and Vandenberg A, Effects of lentil genotype on the colonization of beneficial *Trichoderma* species and biocontrol of *Aphanomyces* root rot. *Microorganisms* **8**:1290 (2020).

15 Ravnskov S and Larsen J, Functional compatibility in cucumber mycorrhizas in terms of plant growth performance and foliar nutrient composition. *Plant Biol* **18**:816–823 (2016).

16 González-Mas N, Sánchez-Ortiz A, Valverde-Garcia P and Quesada-Moraga E, Effects of endophytic entomopathogenic ascomycetes on the life-history traits of *Aphis gossypii* glover and its interactions with melon plants. *Insects* **10**:65 (2019).

17 González-Mas N, Gutiérrez-Sánchez F, Sánchez-Ortiz A, Grandi L, Turlings T, Manuel Muñoz-Redondo J *et al.*, Endophytic colonization by the entomopathogenic fungus *Beauveria Bassiana* affects plant volatile emissions in the presence or absence of chewing and sap-sucking insects. *Front Plant Sci* **12**:660460 (2021).

18 González-Mas N, Cuenca-Medina M, García-Mozo H, Muñoz-Redondo JM, Moreno-Rojas JM, Padilla-Álvarez F *et al.*, Endophytic *Beauveria bassiana* modifies flowering phenology, floral volatile profile and pollinator behaviour in melon. *Entomol Gen* **43**:961–969 (2023).

19 González-Mas N, Cuenca-Medina M, Gutiérrez-Sánchez F and Quesada-Moraga E, Bottom-up effects of endophytic *Beauveria bassiana* on multitrophic interactions between the cotton aphid, *Aphis gossypii*, and its natural enemies in melon. *J Pest Sci* **92**:1271–1281 (2019).

20 Netwing W, Sorg M and Eichenberger J, Chemical extracts of plants as possible attractants for the green lacewing *Chrysoperla carnea* (Planipennia, Chrysopidae). *J Plant Dis Prot* **109**:318–326 (2002).

21 Lawo NC, Wäckers FL and Romeis J, Characterizing indirect prey-quality mediated effects of a *Bt* crop on predatory larvae of the green lacewing, *Chrysoperla carnea*. *J Insect Physiol* **56**:1702–1710 (2010).

22 Nesbit CM, Wilby A, Roberts MR and Menendez R, Selection of aphid prey by a generalist predator: do prey chemical defences matter? *Ecol Entomol* **40**:767–775 (2015).

23 Koczor S, Szentkirályi F, Fekete Z and Tóth M, Smells good, feels good: oviposition of *Chrysoperla carnea*-complex lacewings can be concentrated locally in the field with a combination of appropriate olfactory and tactile stimuli. *J Pest Sci* :311–317 (2017).

24 Cuenca-Medina M, Die JV, Pérez-Rial A, Quesada-Moraga E and González-Mas N, Transcriptome analyses reveal *Beauveria bassiana* endophyte induced disruption of aphid physiology. *J Invertebr Pathol* **212**:108377 (2025).

25 Huang S, Zhang W, Zhang Y, Jia H, Zhang X, Li H *et al.*, Volatile chemical cues emitted by an agricultural companion plant (*Cnidium monnieri*) attract predatory lacewings (*Chrysoperla sinica*). *Biol Control* **192**: 105516 (2024).

26 Yu H, Khashaveh A, Li Y, Li X and Zhang Y, Field trapping of predaceous insects with synthetic herbivore-induced plant volatiles in cotton fields. *Environ Entomol* **47**:114–120 (2018).

27 Kunkel BA and Cottrell TE, Oviposition response of green lacewings (Neuroptera: Chrysopidae) to aphids (Hemiptera: Aphididae) and potential attractants on pecan. *Environ Entomol* **36**:577–583 (2007).

28 Borges DJV, Souza RAC, de Oliveira A, de Sousa RMF, Venâncio H, Demetrio GR *et al.*, Green lacewing *Chrysoperla externa* is attracted to volatile organic compounds and essential oils extracted from *eucalyptus urograndis* leaves. *Plants* **13**:2192 (2024).

29 Hassanpour M, Nouri Ganbalani G, Mohaghegh J and Iranipour S, *Life Table and Age-Depenent Reproduction of Chrysoperla Carnea on Eggs of Ephestia Kuehniella and Helicoverpa Armigera under Laboratory Conditions*. *XXIII International Congress of Entomology*, Durban, South Africa (2008).

30 Inglis GD, Enkerli J and Goettel MS, Laboratory techniques used for entomopathogenic fungi: Hypocreales, in *Manual of Techniques in Invertebrate Pathology*, ed. by Lacey LA. Elsevier, Yakima, WA, pp. 189–251 (2012).

31 Turlings TCJ, Lengwiler UB, Bernasconi ML and Wechsler D, Timing of induced volatile emissions in maize seedlings. *Planta* **207**:146–152 (1998).

32 Rodríguez-Solana R, Salgado JM, Domínguez JM and Cortés-Diéguez S, Characterization of fennel extracts and quantification of estragole: optimization and comparison of accelerated solvent extraction and Soxhlet techniques. *Ind Crop Prod* **52**:528–536 (2014).

33 Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA *et al.*, Proposed minimum reporting standards for chemical analysis. *Metabolomics* **3**:211–221 (2007).

34 Mattoo AJ and Nonzom S, Endophytic fungi: understanding complex cross-talks. *Symbiosis* **83**:237–264 (2021).

35 Redkar A, Sabale M, Zuccaro A and Di Pietro A, Determinants of endophytic and pathogenic lifestyle in root colonizing fungi. *Curr Opin Plant Biol* **67**:102226 (2022).

36 Christian N, Sedio BE, Florez-Buitrago X, Ramírez-Camejo LA, Rojas EI, Mejía LC *et al.*, Host affinity of endophytic fungi and the potential for reciprocal interactions involving host secondary chemistry. *Am J Bot* **107**:219–228 (2020).

37 García-Espinoza F, García del Rosal MJ, Quesada-Moraga E and Yousef-Yousef M, Entomopathogenic fungus-related priming defense mechanisms in cucurbits impact *Spodoptera littoralis* (Boisduval) fitness, ed. by Druzhinina IS. *Appl Environ Microbiol* **89**:e0094023 (2023).

38 Byregowda R, Prasad SR, Oelmüller R, Nataraja KN and Prasanna Kumar MK, Is endophytic colonization of host plants a method of alleviating drought stress? Conceptualizing the hidden world of endophytes. *Int J Mol Sci* **23**:9194 (2022).

39 Hammerbacher A, Coutinho TA and Gershenzon J, Roles of plant volatiles in defence against microbial pathogens and microbial exploitation of volatiles. *Plant Cell Environ* **42**:2827–2843 (2019).

40 Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A *et al.*, The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* **79**:293–320 (2015).

41 Shi J, Wu H, Xiong M, Chen Y, Chen J, Zhou B *et al.*, Comparative analysis of volatile compounds in thirty nine melon cultivars by

headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Food Chem* **316**:126342 (2020).
42 Mao K, Li C, Zhai H, Wang Y, Lou Y, Xue W *et al*., OsRCI-1-mediated GLVs enhance rice resistance to brown planthoppers. *Plants* **13**:1494 (2024).
43 Massawe VC, Hanif A, Farzand A, Mburu DK, Ochola SO, Wu L *et al*., Volatile compounds of endophytic *bacillus* spp. have biocontrol activity against *Sclerotinia sclerotiorum*. *Phytopathology* **108**:1373–1385 (2018).
44 Tajul MI, Motoyama T, Hatanaka A, Sariah M and Osada H, Green-odour compounds have antifungal activity against the rice blast fungus *Magnaporthe oryzae*. *Eur J Plant Pathol* **132**:91–100 (2012).
45 Shoresh M, Yedidia I and Chet I, Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology* **95**:76–84 (2005).
46 Upadhyay A, Upadhyaya I, Mooyottu S, Kollanoor-Johny A and Venkitanarayanan K, Efficacy of plant-derived compounds combined with hydrogen peroxide as antimicrobial wash and coating treatment for reducing *Listeria monocytogenes* on cantaloupes. *Food Microbiol* **44**:47–53 (2014).
47 Majithia D, Metrani R, Dhowlaghar N, Crosby KM and Patil BS, Assessment and classification of volatile profiles in melon breeding lines using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Plants* **10**:2166 (2021).
48 do Bastos M S, de Ferreira-Soares N F, de Nélio J A, Aline CA and Elesbão-Alves R, The effect of the association of sanitizers and surfactant in the microbiota of the cantaloupe (*Cucumis melo* L.) melon surface. *Food Control* **16**:369–373 (2005).
49 Vylkova S, Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. *PLoS Pathog* **13**:e1006149 (2017).
50 Schröder ML, Glinwood R, Webster B, Ignell R and Krüger K, Olfactory responses of *Rhopalosiphum padi* to three maize, potato, and wheat cultivars and the selection of prospective crop border plants. *Entomol Exp Appl* **157**:241–253 (2015).
51 Cantó-Tejero M, Casas JL, Marcos-García MÁ, Pascual-Villalobos MJ, Florencio-Ortiz V and Guirao P, Essential oils-based repellents for the management of *Myzus persicae* and *Macrosiphum euphorbiae*. *J Pest Sci* **2004**:365–379 (2022).
52 Dardouri T, Gautier H, Ben Issa R, Costagliola G and Gomez L, Repellence of *Myzus persicae* (Sulzer): evidence of two modes of action of volatiles from selected living aromatic plants. *Pest Manag Sci* **75**: 1571–1584 (2019).
53 Müller M and Buchbauer G, Essential oil components as pheromones. A review. *Flavour Fragr J* **26**:357–377 (2011).
54 Chang X, Wang F, Fang Q, Chen F, Yao H, Gatehouse AMR *et al*., Virus-induced plant volatiles mediate the olfactory behaviour of its insect vectors. *Plant Cell Environ* **44**:2700–2715 (2021).
55 Chang X, Guo Y, Xie Y, Ren Y, Bi Y, Wang F *et al*., Rice volatile compound (*E*)-*β*-caryophyllene induced by rice dwarf virus (*RDV*) attracts the natural enemy *Cyrtorhinus lividipennis* to prey on *RDV* insect vectors. *Pest Manag Sci* **80**:874–884 (2024).
56 Kumar Chaubey M and Kumar N, Evaluation of insecticidal properties of cinnamaldehyde and cuminaldehyde against *Sitophilus zeamais*. *Int J Entomol Res* **9**:42–48 (2024).
57 Zaio YP, Gatti G, Ponce AA, Saavedra Larralde NA, Martinez MJ, Zunino MP *et al*., Cinnamaldehyde and related phenylpropanoids, natural repellents, and insecticides against *Sitophilus zeamais* (Motsch.). A chemical structure-bioactivity relationship. *J Sci Food Agric* **98**:5822–5831 (2018).
58 McArt SH, Miles TD, Rodriguez-Saona C, Schilder A, Adler LS and Grieshop MJ, Floral scent mimicry and vector-pathogen associations in a pseudoflower-inducing plant pathogen system. *PLoS One* **11**: e0165761 (2016).
59 Zhu J and Park K-C, Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. *J Chem Ecol* **31**:1733–1746 (2005).
60 Zhao J, Wang Z, Li Z, Shi J, Meng L, Wang G *et al*., Development of lady beetle attractants from floral volatiles and other semiochemicals for the biological control of aphids. *J Asia Pac Entomol* **23**:1023–1029 (2020).
61 Niu D, Xu L and Lin K, Multitrophic and multilevel interactions mediated by volatile organic compounds. *Insects* **15**:572 (2024).
62 Natale D, Mattiacci L, Hern A, Pasqualini E and Dorn S, Bioassay approaches to observing behavioural responses of adult female *Cydia molesta* to host plant odour. *J Appl Entomol* **128**:182–187 (2004).
63 Turlings TCJ, Davison AC and Tamò C, A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol Entomol* **29**:45–55 (2004).
64 Niinemets Ü, Kännaste A and Copolovici L, Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Front Plant Sci* **4**:262 (2013).
65 Tan X and Liu T, Aphid-induced plant volatiles affect the attractiveness of tomato plants to *Bemisia tabaci* and associated natural enemies. *Entomol Exp Appl* **151**:259–269 (2014).
66 Yousef-Yousef M, García-Espinoza F, García del Rosal MJ and Quesada-Moraga E, Guardians within: entomopathogenic fungi-driven antibiosis and compensatory growth combines to reduce the damage of herbivores to melon plants. *J Pest Sci* **98**:291–308 (2024).
67 Xu X, Cai X, Bian L, Luo Z, Xin Z and Chen Z, Electrophysiological and behavioral responses of *Chrysopa phyllochroma* (Neuroptera: Chrysopidae) to plant volatiles. *Environ Entomol* **44**:1425–1433 (2015).
68 Debnath R, Mitra P, Das S and Barik A, Leaf surface wax chemicals in *Trichosanthes anguina* (Cucurbitaceae) cultivars mediating short-range attraction and oviposition in *Diaphania indica*. *J Chem Ecol* **47**:664–679 (2021).
69 Feng H, Gou C, Aimaiti D, Sun P, Wang L and Hao H, Plant volatile organic compounds attractive to *Lygus pratensis*. *Open Life Sci* **17**: 362–371 (2022).
70 Milonas PG, Anastasaki E, Psoma A, Partsinevelos G, Fragkopoulos GN, Kektsidou O *et al*., Plant viruses induce plant volatiles that are detected by aphid parasitoids. *Sci Rep* **13**:8721 (2023).
71 Van Hee S, Alınç T, Weldegergis BT, Dicke M, Colazza S, Peri E *et al*., Differential effects of plant-beneficial fungi on the attraction of the egg parasitoid *Trissolcus basalis* in response to *Nezara viridula* egg deposition. *PLoS One* **19**:e0304220 (2024).
72 Borges E de O, Martins CBC, da Silva R and Zarbin PHG, Terpenoids dominate the bouquet of volatile organic compounds produced by *Passiflora edulis* in response to herbivory by *Heliconius erato phyllis* (Lepidoptera: Nymphalidae). *Arthropod Plant Interact* **12**:123–131 (2018).
73 Wang B, Jacquin-Joly E and Wang G, The role of (*E*)-*β*-farnesene in tritrophic interactions: biosynthesis, chemoreception, and evolution. *Annu Rev Entomol* **70**:313–335 (2025).
74 Fernando SSST, Jayasooriya RGPT, Samarakoon KW, Wijegunawardana NDAD and Alahakoon SB, Citrus-based bio-insect repellents—a review on historical and emerging trends in utilizing phytochemicals of citrus plants. *J Toxicol*:179 (2024).