JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Arbuscular Mycorrhizal Fungi Change the Growth and Metabolites of *Perilla frutescens*, with Subsequent Effects on the Development and Behavior of *Spodoptera exigua*

Yule Liu, Jingjing Jian, Lingxin Xu, Linyi Meng, Fan Yang, Shihao Li, and Junxin Yan*

Cite This: https://doi.org/10.1021/acs.jafc.5c03530 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Arbuscular mycorrhizal fungi (AMF) influence plant−insect interactions, yet how they modulate volatile organic compounds (VOCs) to affect insect behavior remains unclear. This study integrates physiological assays, GC−MS volatile metabolomics, and insect bioassays to investigate how *Funneliformis mosseae* (Fm) and *Rhizophagus intraradices* (Ri) inoculation affects *Perilla frutescens* growth and its resistance to *Spodoptera exigua*. The results showed that Fm and Ri inoculation both promoted plant growth but differed in defense strategies. Ri inoculation increased the tannin content (16.98%) and polyphenol oxidase activity (40.52%), whereas Fm inoculation showed neutral effects. VOC profiling revealed that Ri inoculation upregulated leaf monoterpenes and phenylpropanoids, while Fm inoculation reduced sesquiterpenes (e.g., β-selinene). Insect bioassays showed that Ri inoculation inhibited the insect growth, whereas Fm inoculation promoted the insect growth and enhanced its feeding preference. These results revealed that AMF triggers a plant growth−defense trade-off, with sesquiterpenoid regulation critical for insect behavioral shifts. The findings provide a basis for sustainable pest management using AMF−plant symbiosis.

KEYWORDS: *arbuscular mycorrhizal fungi, Perilla frutescens, Spodoptera exigua, insect resistance, volatile organic compounds, behavior selection*

1. INTRODUCTION

In 1885, German plant physiologist Frank first proposed the term "mycorrhiza" to describe the fungal−plant root mutualistic symbiosis.[1] Arbuscular mycorrhizal fungi (AMF), classified under the phylum *Glomeromycota*, represent the most widely distributed symbiotic fungal group in nature.[2] AMF can establish symbiotic relationships with 80−90% of terrestrial plants, forming hyphae, vesicles, and arbuscules in root cortical cells.[3] The symbiosis significantly expands the root absorption surface area, enhancing both host plant water and nutrient uptake. In return, plants transfer 20% of their photosynthetically fixed carbon to the fungi through hyphal networks to support fungal growth and development.[4] Recent studies demonstrate that AMF inoculation significantly promotes host plant growth.[5,6] Following colonization by *Funneliformis mosseae*, *Cannabis sativa* exhibits enhanced maximum PSII photochemical efficiency, increased efficiency of light energy utilization, and ultimately improved plant yield.[7] The benefits of AMF symbiosis for host plants extend beyond promoting plant growth, playing a significant role in enhancing plant stress resistance.[8,9] Numerous AMF have been commercialized and applied to improve plant adaptability to harsh environments.[10,11] Notably, AMF symbiosis has also been observed to influence host plant resistance against insects. The effects of AMF on phytophagous insects can be categorized as positive, negative, or neutral, which may depend on multiple factors including plant species, insect species, and AMF species.[12−14] Relevant studies demonstrate that AMF can enhance the defense mechanism of plants against insects. This is achieved by regulating the physiology and secondary metabolism of plants.[15,16] Inoculation with *F. mosseae* and *Rhizophagus intraradices* increased the level of accumulation of flavonoids in *Rosa rugosa* leaves. When *Lymantria dispar* larvae fed on mycorrhiza-colonized leaves, their growth was significantly inhibited.[17] Similarly, *R. intraradices* colonization significantly increased phenolic compounds and lignin content in *Vigna mungo* leaves while also reducing the feeding capacity of *Spodoptera litura*.[18] This mycorrhiza-induced insect resistance exhibits broad-spectrum efficacy, persistence, and systemic characteristics, demonstrating a stronger adaptability against phytophagous insects. Consequently, it has garnered increasing attention from researchers in recent decades.

Plant volatile organic compounds (VOCs) are key to chemical communication between plants and insects. Plants can repel insects or attract natural enemies through VOC emissions as part of direct or indirect defense strategies.[19,20] In plant−herbivore interactions, plant volatiles regulate insect behavior, and the development of attractants or repellents based on these compounds could mitigate issues associated with chemical pesticides.[21,22] Relevant studies indicate that AMF colonization alters the generation and emission of VOCs in host plants, subsequently influencing insect preferences and

Received: March 21, 2025
Revised: June 29, 2025
Accepted: July 1, 2025

performance.[16,21,23] Papantoniou et al. (2022)[24] demonstrated that *Solanum lycopersicum* inoculated by *Rhizophagus irregularis* exhibited increased attractiveness to the herbivorous insect *Macrolophus pygmaeus*, with the elevated levels of α-terpinene in mycorrhizal leaves potentially playing a key role. However, some studies have reported that AMF symbiosis suppresses VOCs in host plants or shows no significant effects, and the underlying mechanisms warrant further investigation.[25,26] These findings suggest that altered production of VOCs may represent a mechanism through which beneficial microbes mediate plant−insect interactions.

Perilla frutescens (L.) Britt., an annual herbaceous plant in the Lamiaceae family, is widely acknowledged as a species of notable medicinal and industrial importance. The primary bioactive constituents, including phenolic and volatile compounds, are extensively utilized in the food and cosmetic industries.[27] The beet armyworm (*Spodoptera exigua*) represents a polyphagous agricultural pest with strong flight capacity and global distribution, causing substantial economic losses to crops including *P. frutescens*.[28] While synthetic insecticides remain the predominant control method against *S. exigua* infestations, the extensive use of broad-spectrum chemical pesticides has led to ecological consequences such as the elimination of natural enemies, pest resurgence, insecticide resistance, and other environmental hazards.[29,30] Consequently, environmentally friendly and sustainable alternative solutions for pest management are urgently required. Although mycorrhizal symbiosis has emerged as a potential substitute for chemical pesticides and fertilizers in sustainable agriculture, divergent effects of AMF colonization on phytophagous insect performance have been observed across various mycorrhizal plants. Therefore, in order to expand the potential applications of mycorrhiza-induced resistance (MIR) in plant protection and understand the mechanisms underlying AMF-mediated enhancement of plant insect resistance, further investigation is required.[31−33]

In this study, the effect of *F. mosseae* and *R. intraradices* inoculation on *P. frutescens* growth, physiological characteristics, and resistance against *S. exigua* was investigated. Additionally, volatile metabolomic analysis was conducted on the leaves of mycorrhizal plants to determine the influence of AMF symbiosis on the feeding behavior of *S. exigua*. Using this experimental framework, the following hypotheses were examined: (1) AMF colonization enhanced *P. frutescens* growth via photosynthetic optimization; (2) AMF-induced leaf metabolites influenced *S. exigua* larval development; (3) AMF colonization altered volatile compounds' emission in leaves, shaping the *S. exigua* feeding preference.

2. MATERIALS AND METHODS

2.1. Plants, AMF, and Insects. *P. frutescens* plants used in this experiment were propagated from seeds obtained from Qingdao 100Herb Co., Ltd. (Shandong, China). Seeds were treated with 10% (v/v) hydrogen peroxide for surface sterilization for 20 min before sowing, after which they were washed twice with deionized water. Seeds were surface-dried using sterile filter paper and placed in 9 cm Petri dishes containing double-layered filter paper, followed by the addition of 5 mL of deionized water per dish. All dishes were sealed with parafilm to prevent evaporation and incubated in an artificial climate chamber (25 °C, 12/12 h light/dark photoperiod, 75% relative humidity). After 7 days, seeds exhibiting radicle emergence were transferred to autoclaved (121 °C, 120 min) river sand-filled plug trays for subsequent cultivation. Upon emergence of the second pair of true leaves, seedlings were transplanted into pots (upper diameter × base diameter × height = 20.5 cm × 14.5 cm × 17 cm) filled with the test soil, followed by AMF inoculation with one seedling per pot. The test soil, prepared as a soil: perlite: vermiculite mixture (2:1:1 v/v), was autoclaved at 121 °C for 120 min. The physicochemical properties of the test soil were as follows: pH, 5.57; organic carbon, 15.69 g kg^{-1}; hydrolyzed nitrogen, 98.87 mg kg^{-1}; and available phosphorus, 12.51 mg kg^{-1}.

F. mosseae and *R. intraradices* (AMF) were obtained from the Forestry Academy of Wuwei City, Gansu Province. The AMF strains were propagated using maize and clover as host plants, with the inoculant containing spores (13 spores g^{-1}), hyphae, root fragments, and sand.

The *S. exigua* egg masses were purchased from Keyun Biological Co., Ltd. (Henan, China). The eggs were surface-sterilized in 10% (v/v) formaldehyde solution for 40 min. The egg clusters were then rinsed with distilled water to remove the residual formaldehyde. After air-drying, the individual egg masses were placed in 500 mL plastic rearing containers and incubated at 25 °C with 60% relative humidity for hatching. After hatching, the larvae were transferred to an artificial diet (supplied by the Chinese Academy of Forestry Sciences, Beijing) and reared under controlled conditions (25 °C, 14/10 h light/dark photoperiod, 60% relative humidity) until the second instar stage.

2.2. Experimental Design. The experiment was conducted in the greenhouse of the School of Landscape Architecture at Northeast Forestry University. Uniform *P. frutescens* seedlings were transplanted into the pots. The experimental design included three treatments: inoculation with *F. mosseae* (Fm), inoculation with *R. intraradices* (Ri), and non-AMF (control). For AMF treatments, each pot received 3 kg of sterilized soil mixed with 30 g of Fm or Ri inoculum, while control pots contained 3 kg of sterilized soil without inoculum. Each treatment was comprised of 24 plants, with 8 plants per biological replicate. The greenhouse maintained natural lighting, an average temperature of 25 ± 2 °C, and a relative humidity of 70 ± 5%. During the entire experimental period, double-deionized water (ddH_2O) was applied every 2 days to ensure a normal water supply, with no additional fertilizers added. Sixty days post-inoculation, mycorrhizal colonization rates were assessed. The growth and photosynthetic parameters of seedlings across the treatments were subsequently measured, and leaves were stored at −80 °C for physiological and metabolomic analyses.

2.3. Mycorrhizal Colonization. The mycorrhizal colonization rate was measured following a modified Phillips and Hayman (1970) method.[34] Root segments were first cut into 1 cm lengths and then treated with 10% potassium hydroxide at 90 °C for 60 min in a water bath. After cooling, they were rinsed three times with distilled water, bleached in 10% hydrogen peroxide for 15 min, and acidified with 2% hydrochloric acid for 30 min after three more distilled water rinses. Roots were stained with 0.05% (w/v) trypan blue at 90 °C for 30 min. AMF structures were observed using an optical microscope (ZEISS Axio Scope A1), with ≥100 root intersections examined per sample. The colonization rate was computed based on the following equation:

$$\text{Percentage of AMF colonization (\%)} = \frac{\text{total number of infected mycorrhiza}}{\text{total number of root segments examined by microscopy}} \times 100$$

2.4. Plant Growth Index and Photosynthetic Parameters. Following plant harvest, fresh biomass was determined by using an analytical balance. The dry biomass was measured after it had been dried to a constant weight. Three randomly selected plants from each treatment were designated as one biological replicate for photosynthetic parameter measurement with three independent replicates established. Photosynthetic parameters were measured between 9:00 and 11:00 AM using an LI-6400XT system (LI-COR Biosciences, USA). The third fully expanded true leaf per plant was analyzed to determine the key photosynthetic indices per unit leaf area: net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), transpiration rate (Tr), and stomatal conductance (Gs).

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

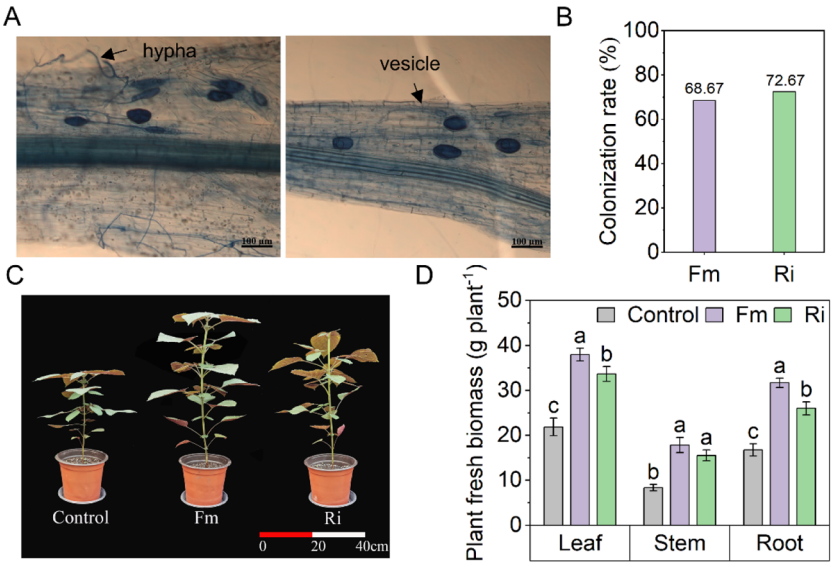

Figure 1. Mycorrhizal colonization and plant growth. (A,B) Morphological structure and colonization rate of AMF. (C,D) Effects of AMF on the growth performance and plant fresh biomass of *P. frutescens*. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. Statistical significance ($P < 0.05$) is indicated by different lowercase letters. Data represent biological replicate means ± standard deviation ($n = 3$).

2.5. Determination of the Plant's Secondary Metabolites and Enzyme Activity. In order to obtain the phenol content, 3 mL of 75% ethanol was added to 0.2 g of fresh leaves, and the mixture was then ground into a homogenate. Then, 5 mL of 75% ethanol was added for rinsing, resulting in a total of 10 mL. This was then centrifuged at 2500 rpm for 5 min, and the resulting supernatant was the total phenol extract. The total phenol content was determined via the Folin−Ciocalteu colorimetric assay.[35] The total flavonoid and tannin contents were obtained by weighing 0.5 g of fresh leaves and placing them in a 25 mL volumetric flask. 95% Methanol was added to obtain a total of 25 mL. This was allowed to soak for 12 h, ultrasonically extracted for 2 h, and then filtered to obtain the flavonoid and tannin extracts. The total flavonoid content was determined through the aluminum chloride complexation method.[36] The tannin content was determined using the vanillin−hydrochloric acid (vanillin−HCl) method.[37]

Enzymatic activities were evaluated by first placing 0.2 g of fresh leaves into a mortar; 5 mL of a phosphoric acid buffer solution with pH 7.8 was then added. The mixture was ground in an ice bath, homogenized, and poured into a centrifuge tube. The mixture was centrifuged in a refrigerated centrifuge at 4 °C for 20 min (10,000*g*). The supernatant was stored at 0−4 °C for future use. Enzymatic activities were evaluated as follows: the phenylalanine ammonia-lyase (PAL) activity was measured by monitoring L-phenylalanine deamination, polyphenol oxidase (PPO) activity via catechol oxidation kinetics,[38] and peroxidase (POD) activity employing the guaiacol−H_2O_2 system.[39]

2.6. Determination of the Leaf VOCs. Leaves for the experiments were collected, measured for mass, and rapidly frozen using liquid nitrogen prior to long-term storage at −80 °C. For analytical processing, cryogenically preserved samples were pulverized in liquid nitrogen to achieve homogeneous particulates. Precisely 500 mg (equivalent to 1 mL of volumetric displacement) of the resultant powder was aliquoted into 20 mL hermetic headspace vials (Agilent Technologies, Palo Alto) containing a sodium chloride-saturated aqueous solution to prevent enzymatic degradation. Vials were hermetically sealed by using crimp-top closures with PTFE-lined silicone septa (Agilent).

During solid-phase microextraction (SPME), samples underwent thermal equilibration at 60 °C for 5 min, followed by headspace adsorption using a 120 μm triphasic fiber (DVB/CAR/PDMS; Agilent) over 15 min at an equivalent temperature. Subsequent thermal desorption occurred in the GC inlet (7890B GC system, Agilent) maintained at 250 °C for 5 min under splitless conditions. Chromatographic separation was achieved using a DB-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) with helium carrier gas (1.2 mL/min constant flow). The temperature program was initiated at 40 °C (3.5 min hold), followed by three ramping phases: 10 °C/min to 100 °C, 7 °C/min to 180 °C, and 25 °C/min to 280 °C (5 min final hold). Detection was performed using a 7000D triple-quadrupole mass spectrometer (Agilent) operated in the electron ionization mode (70 eV) with selected ion monitoring. Critical thermal zones were maintained as follows: ion source (230 °C), quadrupole (150 °C), and transfer line (280 °C).

2.7. Insect Feeding Experiment. An insect feeding assay was conducted using second instar *S. exigua* larvae (newly molted at ≤ 12 h). After 12 h of starvation, they were placed in circular insect-rearing boxes (upper diameter × base diameter × height = 5.5 cm × 4.0 cm × 3.0 cm), with one larva in each box. Each treatment included three biological replicates, with 15 larvae per replicate. Fresh leaves from differentially treated *P. frutescens* were provided to feed the larvae (one leaf per chamber) and replaced daily to remove the frass and residual material. Larval development was monitored daily, and instar developmental durations (third, fourth, and fifth instar stages) were calculated based on the molting time of the larvae. Upon reaching the target instars, individual larval weight was measured using an analytical balance. The larvae were positioned on a millimeter grid paper and photographed. Body length measurements were obtained through image analysis using ImageJ software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA) with scale calibration based on grid references.

2.8. Behavior Selection of *S. exigua* Larvae. The selection behavior of *S. exigua* larvae was assessed using a Y-tube olfactometer.[40] The apparatus comprised of a central stem bifurcating into two symmetrical arms at a 75° angle. Each arm was connected via a Teflon tubing to separate the odor source chambers (A and B). Test plants included an AMF-inoculated treatment and a nonmycorrhizal control treatment, each placed in distinct odor chambers. Airflow purification was achieved through activated charcoal filtration prior to delivery into the system. Gas flow uniformity (250 mL/min per olfactory arm) was regulated using precision-calibrated mass flow controllers. Third instar larvae were individually positioned at the olfactometer's central stem. Behavioral trajectories were continuously tracked over a 10 min observation window, with the directional preference defined by two criteria: (1) migration exceeding one-third of the arm's total length and (2) positional persistence >60 s. Larvae

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

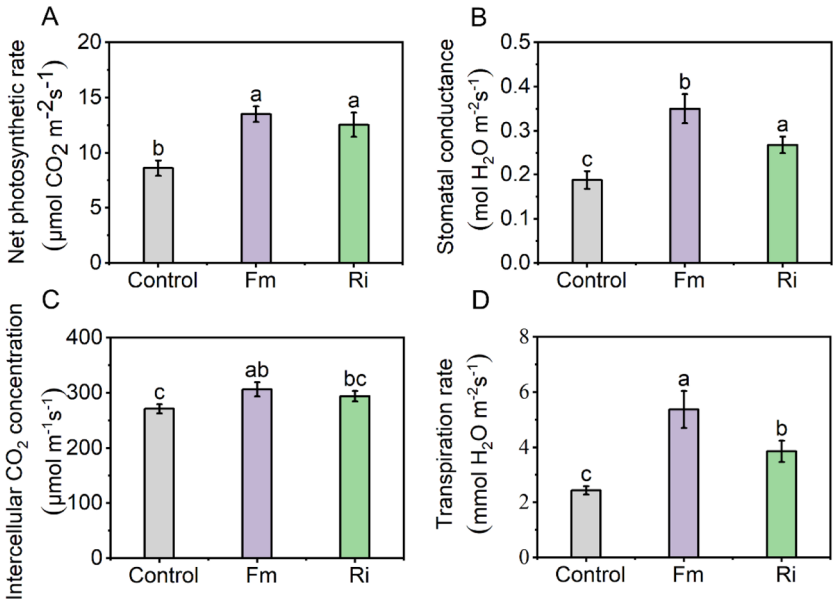

Figure 2. Effects of AMF on the net photosynthesis (A), stomatal conductance (B), intercellular CO_2 concentration (C), and transpiration rate (D) of *Perilla frutescens*. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. Statistical significance ($P < 0.05$) is indicated by different lowercase letters. Data represent biological replicate means ± standard deviation ($n = 3$).

showing no locomotory activity within the observation period were excluded from the analysis. The experimental design incorporated five replicates per treatment group, with each replicate containing a cohort of 8 larvae. Response indices were computed using the following chemotaxis equation:[20,41]

$$\text{Response rate (\%)} = (\text{total number of larvae selected for AMF group} + \text{total number of larvae selected for control group}) / (\text{total number of larvae tested}) \times 100$$

$$\text{Selection rate (\%)} = (\text{total number of larvae selected for control group or AMF group}) / (\text{total number of larvae selected for AMF group} + \text{total number of larvae selected for control group}) \times 100$$

$$\text{Selectivity coefficient} = (\text{total number of larvae selected for AMF group} - \text{total number of larvae selected for control group}) / (\text{total number of larvae selected for AMF group} + \text{total number of larvae selected for control group})$$

2.9. Statistical Analyses. Statistical analysis was processed using SPSS 26. Plant growth and physiology and larval growth data were subjected to one-factor analysis of variance with post-hoc verification through Tukey's HSD multiple comparison protocol. Larval behavior selection data were evaluated using a chi-square test. The metabolic data set underwent multivariate pattern recognition through non-supervised principal component analysis (PCA) utilizing the prcomp algorithm in the R statistical environment (version 4.3.0). Differential metabolite screening employed dual-threshold criteria: variable importance projection score >1.0 coupled with fold-change >1.5 or <0.67. Identified metabolites were annotated using the genes and genomes (KEGG) Compound database (http://www.kegg.jp/kegg/compound/); annotated metabolites were then mapped to the KEGG pathway database (http://www.kegg.jp/kegg/pathway.html).[42] Pathways with significantly regulated metabolites mapped to were then fed into MSEA (metabolite sets enrichment analysis), and their significance was determined by the hypergeometric test's *p*-values.

3. RESULTS

3.1. Effects of AMF on the Growth and Photosynthesis of *P. frutescens*. A successful symbiotic association was established between AMF and *P. frutescens*. Mycorrhizal colonization rates were determined as follows: Fm: 68.67% and Ri: 72.67% (Figure 1A,B). AMF symbiosis significantly improved the growth of *P. frutescens* compared to non-mycorrhizal plants. Fm inoculation had a greater effect on the increase in biomass, compared to Ri. Fm and Ri inoculation significantly increased the leaf fresh weight by 73.38% and 42.60%, stem fresh weight by 113.02% and 52.09%, and root fresh weight by 89.48% and 52.84%, respectively (Figure 1C,D). Compared with the control, Fm and Ri inoculation also significantly increased the dry weight of *P. frutescens* (Figure S1).

In addition, the effects of AMF colonization on the photosynthetic characteristics of *P. frutescens* leaves were also measured. It was found that AMF inoculation increased the level of photosynthesis of *P. frutescens*. Compared with the control, Fm inoculation significantly increased the net photosynthesis, stomatal conductance, intercellular CO_2 concentration, and transpiration rate by 56.86%, 86.17%, 12.92%, and 120.99%, respectively. Ri inoculation significantly increased the net photosynthesis, stomatal conductivity, and transpiration rate by 45.81%, 42.55%, and 8.41%, respectively, and showed no significant difference in intercellular CO_2 concentration compared with control (Figure 2A–D).

3.2. Effects of AMF on the Secondary Metabolic Substances and Defense Enzyme Activity of *P. frutescens*. In order to explore the mechanism of mycorrhizal colonization affecting the resistance of *P. frutescens* to *S. exigua*, defense-related secondary metabolites, and enzymes in their leaves were measured. It was found that, compared to the control, Ri inoculation significantly increased the tannin content in the *P. frutescens* leaves by 16.98%. The flavonoid and total phenol contents also showed an increasing trend, but the data were not significant. Inoculation with Fm had no significant effect on the total phenol, flavonoid, and tannin

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

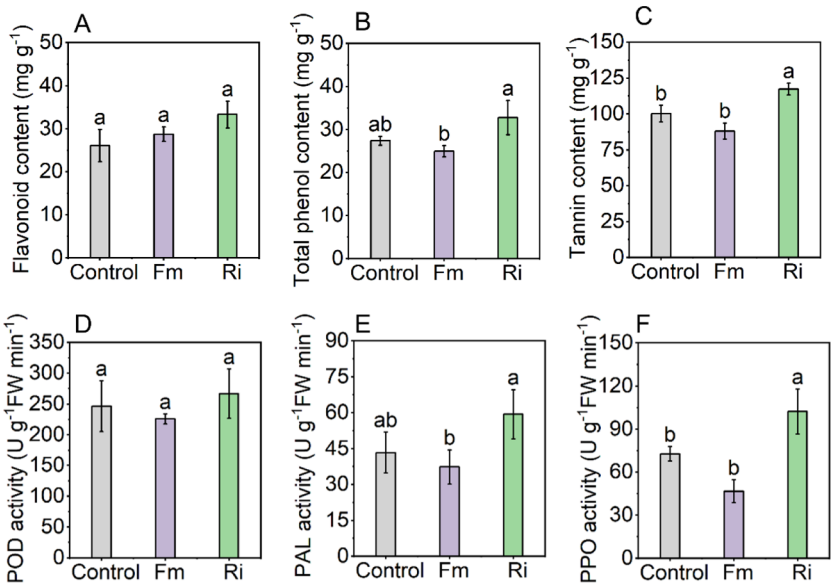

Figure 3. Effects of AMF on the secondary metabolic substances and defense enzyme activity of *Perilla frutescens*. (A, B,C) Flavonoid, total phenol, and tannin contents of *P. frutescens*, respectively. (D,E,F) POD, PAL, and PPO activities of *P. frutescens*, respectively. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. POD, peroxidase; PAL, phenylalanine ammonia-lyase; PPO, polyphenol oxidase. Statistical significance ($P < 0.05$) is indicated by different lowercase letters. Data represent biological replicate means ± standard deviation ($n = 3$).

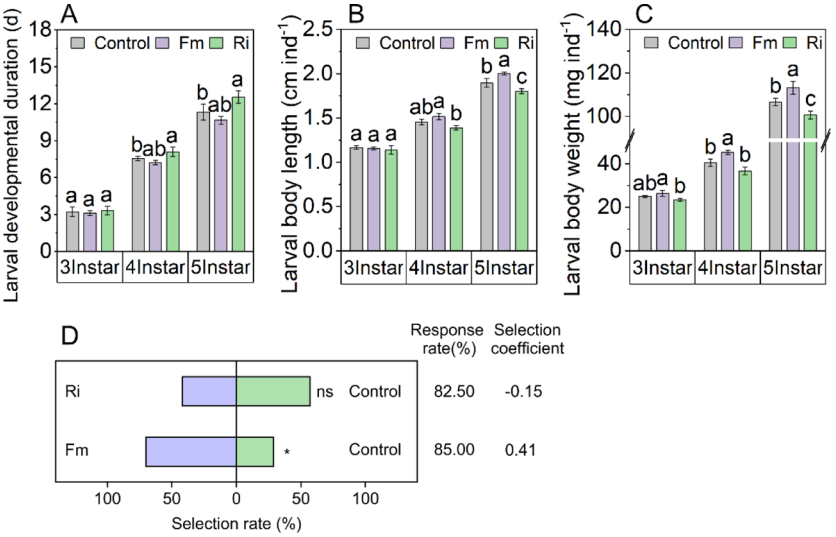

Figure 4. Larvae developmental duration (A), body length (B), body weight (C), and behavior selection (D) of *Spodoptera exigua*. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. Statistical significance ($P < 0.05$) is indicated by different lowercase letters. Data represent biological replicate means ± standard deviation ($n = 3$). Chi-square test was used to analyze the different significance of response rate of *S. exigua*. A single asterisk (*) indicates a significant difference at $P < 0.05$, and ns indicates no significant difference. Selection coefficient for positive number means that active volatile is attractive to *S. exigua*; conversely, negative number indicates a repellent effect.

contents in the *P. frutescens* leaves (Figure 3A−C). Compared with the control, Ri inoculation significantly increased the PPO activity in the *P. frutescens* leaves by 40.52%, but no significant difference was observed in POD and PAL activities between the treatments. Inoculation with Fm showed no statistically significant effect on the activity of defense-related enzymes in *P. frutescens* leaves compared to the control (Figure 3D,E).

3.3. Larval Development and Behavior Selection of *S. exigua*. The larvae were fed with different treatments of *P. frutescens* leaves, and the developmental duration, body weight, and body length of the larvae at three instars were calculated. It was found that different mycorrhizal inoculations had different effects on insect larval development. Compared to the control, the larvae of *S. exigua* fed on Ri-inoculated *P. frutescens* leaves exhibited significantly prolonged developmental durations, with the fourth and fifth instar stages extended by 7.28% and 10.86%, respectively. In contrast, larvae consuming Fm-inoculated leaves showed no statistically significant difference in developmental duration compared with the control (Figure 4A). Furthermore, larvae of *S. exigua* fed on Ri-inoculated *P. frutescens* leaves exhibited reduced body weight and shorter body length. Compared to the control, the body length and

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

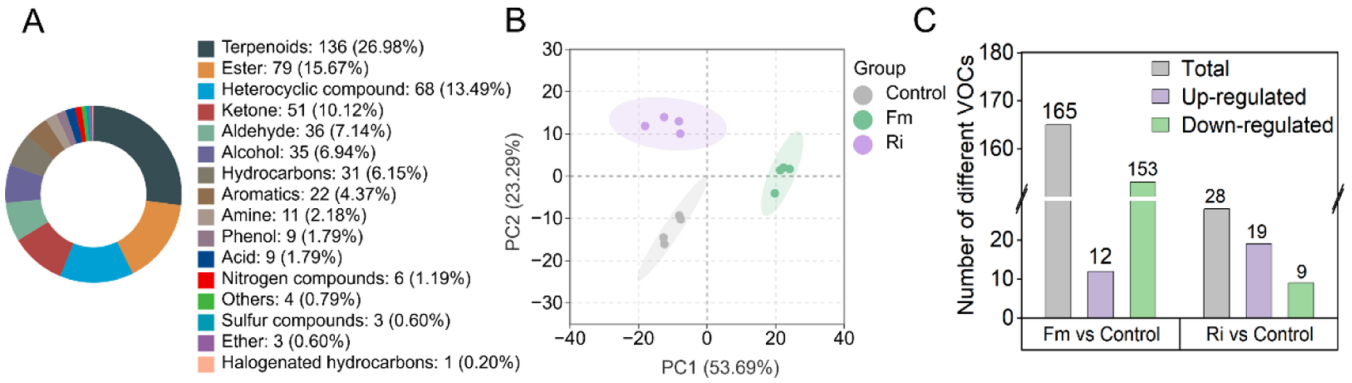

Figure 5. Effects of AMF on the VOCs of Perilla frutescens leaves (n = 4). (A) Types of VOCs. (B) PCA of leaf VOCs. (C) DEMs among different treatment groups. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation.

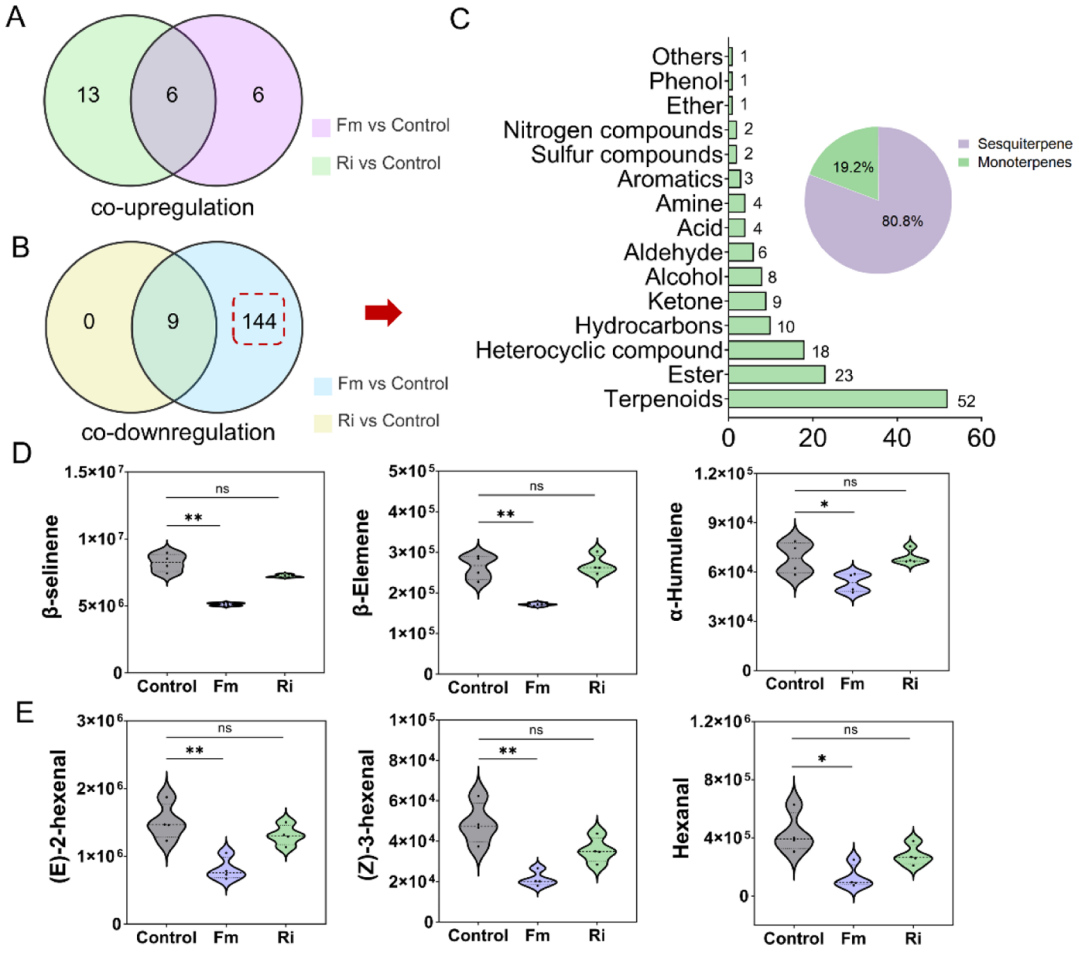

Figure 6. Venn diagram of differential metabolites with co-upregulation (A) and co-downregulation (B). Types and amounts of Fm-inoculated (C) downregulation metabolites. Sesquiterpene (D) and GLV (E) differences were observed in *P. frutescens* leaves inoculated with Fm and Ri compared to the control. Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. A single asterisk (*) indicates a significant difference at $P < 0.05$, ** indicates a significant difference at $P < 0.01$, *** indicates a significant difference at $P < 0.001$, and ns indicates no significant difference.

weight of fifth instar larvae fed on Ri-inoculated *P. frutescens* leaves significantly decreased by 5.27% and 5.65%, respectively, while fourth instar larvae showed a decreasing trend in body length and weight, though data were not significant. Nevertheless, compared to the control, the body length and weight of fifth instar larvae fed on Fm-inoculated *P. frutescens* leaves significantly increased by 5.82% and 6.13%, respectively, and the body weight of fourth instar larvae significantly increased by 11.98% (Figure 4B,C).

In the experiment of insect behavior selection, it was found that *P. frutescens* seedlings inoculated by Fm exhibited significant attraction to *S. exigua* larvae (χ^2 = 5.77, $P < 0.05$) (Figure 4D).

3.4. Effects of AMF on the VOCs of *P. frutescens* Leaves. The results indicated that a total of 504 compounds

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

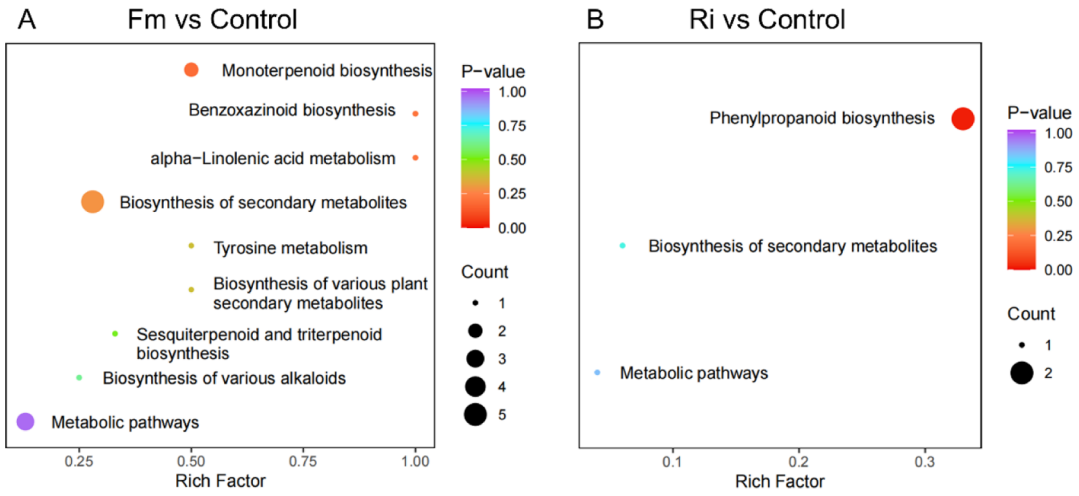

Figure 7. KEGG metabolic pathways of differential metabolites in the leaves of *Perilla frutescens* inoculated with Fm (A) and Ri (B). Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. The color chart in (A,B) represents the hypergeometric test *p*-value. The color of the points reflects the magnitude of the *p*-value. Specifically, the redder the color of the point, the more significant the enrichment.

were detected in the *P. frutescens* leaves, the complete list of which is provided in Table S1. Among them, terpenoids accounted for the largest proportion, making up 26.98% of the total compounds (Figure 5A). PCA revealed significant experimental repeatability and differences in the levels of VOCs between mycorrhizal and nonmycorrhizal plants (Figure 5B). Orthogonal partial least-squares discriminant analysis (OPLS-DA) indicated that this model was capable of effectively differentiating the samples of each comparison group (Figure S2).

Following Fm inoculation, 165 differential metabolites (DEMs) were identified, including 12 upregulated and 153 downregulated compounds. Strikingly, terpenoids dominated the downregulated DEMs (Figure S3). In the case of Ri inoculation treatment, 28 DEMs were singled out, with 19 metabolites showing upregulation and 9 metabolites displaying downregulation (Figure 5C, Tables S1 and S2). The analysis of the shared differential metabolites showed that there were 6 DEMs co-upregulated by the two types of AMF inoculation and 9 DEMs that were co-downregulated (Figure 6A-B). Fm inoculation was found to have downregulated more volatile substances, and the 144 downregulated substances mainly included terpenoids, esters, and heterocyclic compounds. Among these, 53 were terpenoids, with sesquiterpenes accounting for 80.80% of these downregulated terpenoids (Figure 6C). Some sesquiterpenes (e.g., β-selinene, β-elemene, and α-humulene) closely related to insect behavior were significantly downregulated in FM-inoculated *P. frutescens* leaves (Figure 6D). In addition, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and other green leaf volatiles (GLVs) were significantly downregulated under Ri treatment (Figure 6E).

3.5. Analysis of Metabolic Networks under AMF Inoculation. The enrichment pathways of the DEMs were analyzed by using the KEGG database. Following inoculation with Fm, the DEMs in *P. frutescens* leaves were mainly enriched in secondary metabolite biosynthesis and monoterpenoid biosynthesis. Ri inoculation resulted in preferential enrichment of phenylpropanoid biosynthesis (Figure 7A,B).

Volatile terpenoids are greatly influenced by mycorrhizal symbiosis. Consequently, the alterations in terpenoid metabolism under mycorrhizal colonization were systematically analyzed. A correlation heatmap revealed that the Fm inoculation reduced the content of most terpenoids, especially sesquiterpenoids (Figure 8A). Based on the DEMs and the metabolic pathway database, a metabolic pathway network of terpenoids in *P. frutescens* leaves was constructed. It was found that different types of mycorrhizal inoculations had distinct effects on the terpenoids of *P. frutescens* leaves. Although it was not reflected in the DEMs, several monoterpenoids, such as (+)-(*R*)-limonene, perillyl alcohol, and α-pinene-oxide, were also significantly accumulated under the Ri inoculation (Figure S4). The Fm inoculation significantly reduced the contents of myrcene and (−)-*trans*-isopiperitenol. In addition, the contents of three sesquiterpenoids, β-selinene, α-humulene, and (*S*,*E*)-nerolidol, were significantly reduced, while Ri inoculation had no significant effect on the contents of these three sesquiterpenoids (Figure 8B and Tables S1).

The results revealed that Ri inoculation significantly stimulated phenylpropanoid biosynthesis. Ri inoculation promoted the accumulation of cinnamic acid and eugenol. In contrast, Fm inoculation exhibited no significant influence on these metabolites, while at the same time, it even caused a significant decrease in chavicol content (Figures 8C and S5).

4. DISCUSSION

4.1. AMF Colonization Enhances *P. frutescens* Growth and Photosynthetic Performance. The enhancement of plant growth and biomass accumulation following AMF inoculation has been extensively documented across laboratory and field studies, a phenomenon defined as the positive mycorrhizal growth response.[43,44] This symbiotic association between AMF and plant root systems establishes extensive hypha networks that amplify the root absorption capacity. It thereby enhances water and nutrient acquisition and upregulates photosynthetic activity, playing an important role in improving plant growth.[45,46] In this study, inoculation with Fm and Ri significantly promoted the growth of *P. frutescens*, with Fm exhibiting a more pronounced effect. Generally, a high colonization rate of AMF on host plants serves as a critical guarantee for their effective application in practical environments to enhance plant growth and stress resistance.[12] The present study revealed that the colonization rates of Fm and Ri

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

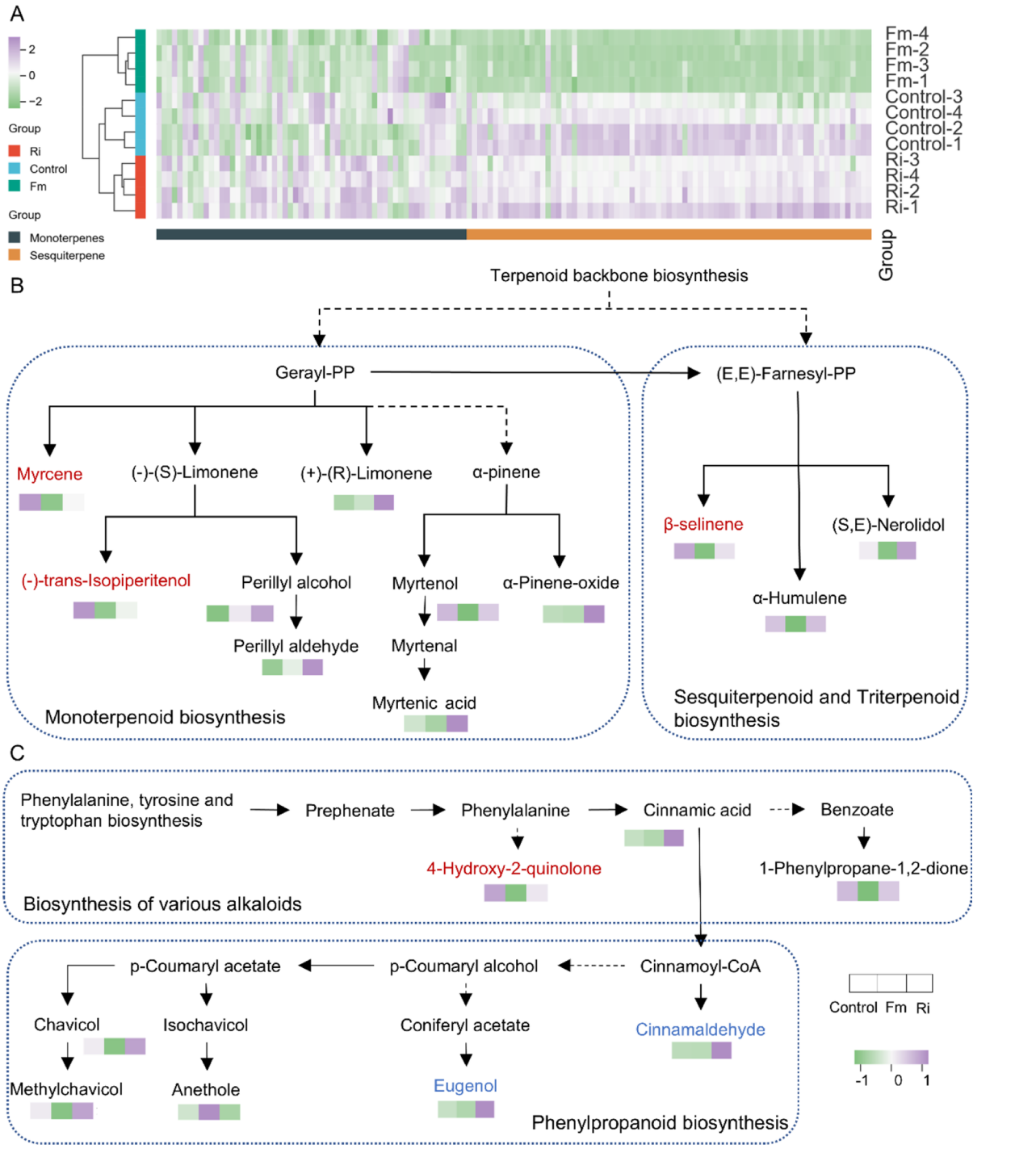

Figure 8. Cluster heatmap of terpenoids (A), terpenoid metabolic pathway network (B), and phenylpropanoid biosynthesis (C). The chromatic gradient in panels (A−C) represent relative abundance values, with the color intensity scaling proportionally from purple (elevated concentrations) to green (depressed levels). Control, uninoculated treatment; Fm, *Funneliformis mosseae* inoculation; Ri, *Rhizophagus irregularis* inoculation. The red and blue compounds in (B,C) represent the DEMs screened in Fm and Ri, respectively. The solid and dashed lines in (B,C) represent direct and indirect metabolic pathways, respectively.

on *P. frutescens* reached 68.67% and 72.67%, respectively, highlighting their substantial potential for application in medicinal and economic crops such as *P. frutescens*. Previous studies have demonstrated that during the symbiotic association between AMF and host plants, AMF competes with the host for photosynthetic products, consuming 4−20% of the plant's photo-assimilated carbon.[47] A high colonization rate may indicate increased carbohydrate allocation to fungal partners, potentially imposing a certain degree of negative impact on the host plant.[48,49] However, the findings in the present study revealed that *P. frutescens* maintained a mutualistic symbiotic association with both fungal species, even under high colonization rates. This phenomenon could be attributed to the mycorrhizal seedlings' capacity to upregulate energy metabolism, thereby compensating for the energy costs induced by AMF colonization.[12] Photosynthesis is the sole

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

pathway for plants to synthesize energy substances.[50] Therefore, the photosynthetic indices of *P. frutescens* were measured under the condition of AMF colonization. The investigation demonstrated that AMF inoculation significantly enhanced photosynthetic performance, as evidenced by marked increases in the net photosynthetic rate, stomatal conductance, and transpiration rate. It is speculated that this photosynthetic upregulation could serve as the energetic foundation supporting high AMF colonization rates and leads to a positive growth response in *P. frutescens*.

4.2. AMF Colonization Induces Alterations in Defense-Related Secondary Metabolites of *P. frutescens* to Counter *S. exigua* Herbivory. MIR, a key ecological benefit derived from mycorrhizal symbiosis, has been widely documented to enhance plant tolerance to herbivorous insects.[18] In alignment with the Slow Growth−High Mortality (SG−HM) hypothesis, insects requiring extended developmental duration exhibit reduced fitness due to incomplete metamorphosis and heightened predation pressure from natural enemies.[51] Consequently, the suppression of insect growth and developmental progression constitutes an alternative mechanism for fortifying plant resistance against herbivory. The current study found that *P. frutescens* inoculated with Fm and Ri showed different responses in resistance to *S. exigua*. Notably, larvae fed on Ri-inoculated *P. frutescens* leaves exhibited significantly reduced body weight and length in the fifth instars, coupled with prolonged developmental duration compared to nonmycorrhizal controls. In contrast, Fm colonization promoted the growth of the larvae. These findings demonstrate AMF species-specific effects on plant−insect interactions. Similar results were also found in previous studies. Zhao et al. (2024)[52] reported that Ri-inoculated *Populus pseudocathayana* × *P. deltoides* exerted neutral effects on *L. dispar* larval development, whereas Fm-inoculated counterparts unexpectedly enhanced the larval growth performance. Furthermore, studies by Lv et al. (2022)[17] and Jiang et al. (2022)[12] have corroborated the tripartite complexity of AMF−host plant−herbivore interactions. Consequently, field deployment of AMF inoculants demands rigorous risk assessment protocols to mitigate the unintended enhancement of phytophagous insect fitness, particularly in agroecosystems where pest suppression is prioritized.

The AMF-induced resistance mechanisms against herbivorous insects may involve the accumulation of insect-resistant compounds of the host plant following symbiotic colonization. The current study revealed that *P. frutescens* inoculated with Ri exhibited a significantly higher tannin content compared to nonmycorrhizal controls, with flavonoid and total phenol contents also showing an increasing trend. In contrast, Fm inoculation demonstrated no significant effects on the levels of secondary metabolites or defense enzyme activities, with even a slight decrease observed in some parameters. Correlation analysis showed that there was a correlation between larval growth, plant defense enzyme activity, and secondary metabolites (Figure S6). Tannins and flavonoids are known to reduce insect palatability due to their astringency, thereby eliciting antifeedant responses in herbivores.[14,53] Furthermore, phenolic compounds can covalently bind to proteases in the insect midgut, inhibiting digestive enzymes and impairing nutrient absorption, which constitutes a critical chemical defense mechanism.[54]

4.3. AMF Colonization Induces Alterations in VOCs of *P. frutescens* Leaves and Modulates Behavior Selection in *S. exigua*. During the investigation, it was observed with interest that Fm-inoculated seedings exhibited significantly higher attractiveness to *S. exigua* than noninoculated controls, whereas Ri-inoculated seedlings showed no effect on larval preference. Many previous studies have demonstrated that AMF can induce alterations in plant VOCs, but the effects of these alterations on the insect behavior vary depending on the host plant and insect species.[55,56] For instance, Zhang et al. (2023)[57] reported that AMF inoculation significantly increased the relative contents of benzenoid compounds and D-limonene in *Elymus nutans* leaves, which played a critical role in enhancing plant defenses and repelling herbivores. In contrast, Babikova et al. (2014)[58] found that AMF-inoculated *Vicia faba* leaves exhibited significantly reduced levels of VOCs such as (*S*)-linalool, (*E*)-caryophyllene, and (*R*)-germacrene D, while concurrently displaying increased attractiveness to *Acyrthosiphon pisum*. Based on these studies, it is hypothesized that AMF inoculation may similarly induce modifications in the VOC profiles of *P. frutescens* leaves, which could consequently influence the behavior selection of *S. exigua*. This study revealed that AMF inoculation induced reprogramming of the terpenoid backbone biosynthesis pathway in plants. However, distinct AMF inoculation exerted different effects on the terpenoid synthesis. Fm inoculation resulted in reduced concentrations of leaf terpenoids, especially sesquiterpenoids, whereas Ri inoculation moderately upregulated the accumulation of some terpenoids in *P. frutescens*. Terpenoids represent the largest and structurally most diverse classes of VOCs emitted by plants. Functionally, these compounds modulate the herbivore behavior, influence natural enemy interactions, and exert developmental effects on insects. For example, a reduction in leaf monoterpene levels in *S. lycopersicum* significantly increases the feeding attraction of *S. exigua* larvae.[59] Monoterpenes such as α-pinene and β-pinene disrupt insect nervous systems through acetylcholinesterase inhibition.[26] Furthermore, it has been found that sesquiterpenoids also play an important role in defense against predation by lepidoptera insects.[60−62] The emission of sesquiterpene α-humulene exerts repellent and toxic effects on larvae, playing a critical role in plant defense mechanisms against *S. litura* herbivory.[62] Multiple sesquiterpenoids including β-selinene and β-elemene have been validated to possess insecticidal properties as neurotoxins.[63,64] The significant downregulation of defensive terpenoids in Fm-inoculated *P. frutescens* leaves may attenuate plant resistance, thereby enhancing the *S. exigua* behavior preference. Future studies should prioritize larval behavioral bioassays of these terpenoids to clarify their roles in plant−insect chemical ecology and sustainable pest management.

The mechanisms underlying AMF-induced alterations in VOCs within plant tissues are multifaceted, with no singular explanatory model currently established to fully account for these biochemical shifts.[57,65] Generally, terpenoid biosynthesis may represent a defensive response to fungal colonization in plants.[66] It is therefore hypothesized that the elevated terpenoid content may represent a strategic response to Ri colonization, enhancing the plant's capacity to counter biotic and abiotic stress. Although Ri inoculation had no effect on the selection behavior of *S. exigua*, the production of perilla alcohol, perilla aldehyde, and other compounds could enhance the quality and economic value of *P. frutescens*. However, there are also studies that suggest the amplified defensive responses require metabolic costs, and there is a trade-off between

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

defense and growth.[67,68] Terpenoids have been regarded as one of the most energetically costly defensive metabolites produced by plants.[26] This study also found that Fm-inoculated plants exhibited the highest biomass accumulation. Based on this observation, it is speculated that Fm colonization may drive resource reallocation in the host plants, prioritizing investment in growth over defense-related metabolic expenditures. This may lead to a significant increase in growth along with a significant decrease in the number of terpenoids.

Volatile phenylpropanoids are an important category of plant secondary metabolites. They can participate in plant defenses through various mechanisms, such as repelling pests, interfering with insect behavior, and indirectly attracting natural enemies.[69,70] Cinnamic acid, as a pivotal precursor in the phenylpropanoid pathway, serves as the substrate for synthesizing antimicrobial and antifeedant compounds like eugenol and chavicol.[71] This metabolic shift likely reflects the plant's adaptive strategy to optimize chemical defenses while maintaining symbiotic benefits from Ri inoculation. Notably, the lack of Fm inoculation-induced phenylpropanoid accumulation and even the suppression of chavicol implies divergent microbial modulation of plant metabolism.[72] The reduction in chavicol, a volatile phenol with documented insect-repellent properties, may indicate a trade-off where Fm inoculation prioritizes plant growth over defense investment, potentially compromising plant resistance under pest pressure. In addition, changes in GLVs with insect-resistant properties in plants inoculated with AMF have also been observed. GLVs are plant-derived volatiles induced by biotic and abiotic stresses, functioning in direct defense (repelling herbivorous insects) and indirect defense (mediating tritrophic signaling to attract natural enemies).[73,74] The study found that substances such as (*Z*)-3-hexenal, (*E*)-2-hexene, and hexanal were significantly downregulated under the treatment of inoculation with Ri, while Fm inoculation had no significant effect on the changes in their contents, possibly leading to the strong attraction of FM-inoculated *P. frutescens* leaves to *S. exigua*.

In conclusion, AMF not only functions as biofertilizers to enhance *P. frutescens* seedling growth and photosynthetic efficiency, but *R. irregularis* inoculation further promotes the accumulation of defensive compounds in leaves, thereby improving the seedling resistance to *L. dispar*. These findings highlight the diverse outcomes of AMF−host plant−herbivorous insect interactions, which should be considered in future research, and underscore the challenges and complexities in applying AMF to agricultural production. Furthermore, it was observed that AMF inoculation induces alterations in the VOCs of *P. frutescens* leaves, potentially modulating *S. exigua* behavior selection. Future investigations should focus on elucidating the mechanistic basis of the effects of VOCs on *S. exigua*, including their binding sites on insect olfactory receptors and synergistic interactions among multiple VOCs, with the aim of developing enhanced pest management strategies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.5c03530.

Metabolic changes of leaves between AMF inoculation and control (XLSX)

Effect of AMF treatment on plant dry biomass; OPLS-DA score plot and permutation test (R2/Q2) for volatile metabolites; classification of differential metabolites; content differences of terpenoid and phenylpropanoid metabolites; and correlation analysis between larval growth and plant defensive substances (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Junxin Yan − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China;* orcid.org/0000-0002-0657-5572; Email: yanjunxin@163.com

Authors

Yule Liu − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Jingjing Jian − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Lingxin Xu − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Linyi Meng − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Fan Yang − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Shihao Li − *College of Landscape Architecture, Northeast Forestry University, Harbin 150040, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.5c03530

Funding

This work was supported by Heilongjiang Province Key Research and Development Program (2024ZXDXB60).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Y.L. designed and conducted the experiments, data collection, data analyses, and interpreted results and drafted the manuscript. J.J. conducted the experiments and conducted data analyses. L.X. conducted the experiments and data collection. L.M. conducted data analyses. F.Y. conducted data analyses. S.L. conducted data collection. J.Y. conceived the idea for this work, designed the experiments, interpreted results, and revised the manuscript.

■ REFERENCES

(1) Sportes, A.; Hériché, M.; Boussageon, R.; Noceto, P. A.; van Tuinen, D.; Wipf, D.; Courty, P. E. A historical perspective on mycorrhizal mutualism emphasizing arbuscular mycorrhizas and their emerging challenges. *Mycorrhiza* **2021**, *31* (6), 637−653.

(2) Brundrett, M. C.; Tedersoo, L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* **2018**, *220* (4), 1108−1115.

(3) Gutjahr, C. Phytohormone signaling in arbuscular mycorhiza development. *Curr. Opin. Plant Biol.* **2014**, *20*, 26−34.

(4) Parniske, M. Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nat. Rev. Microbiol.* **2008**, *6* (10), 763−775.

(5) Wu, L. M.; Zheng, Y. J.; Liu, S. L.; Jia, X. D.; Lv, H. Response of Ammodendron bifolium seedlings inoculated with amf to drought stress. *Atmosphere* **2023**, *14* (6), 989.

(6) Liu, Y. X.; Lu, J. H.; Cui, L.; Tang, Z. H.; Ci, D. W.; Zou, X. X.; Zhang, X. J.; Yu, X. A.; Wang, Y. F.; Si, T. The multifaceted roles of arbuscular mycorrhizal fungi in peanut responses to salt, drought, and cold stress. *BMC Plant Biol.* **2023**, *23* (1), 36.

(7) Yuan, H. P.; Si, H.; Ye, Y. S.; Ji, Q. Y.; Wang, H. Y.; Zhang, Y. H. Arbuscular mycorrhizal fungi-mediated modulation of physiological, biochemical, and secondary metabolite responses in hemp (Cannabis sativa L.) under salt and drought stress. *J. Fungi* **2024**, *10* (4), 283.
(8) Wang, D. Y.; Ni, Y.; Xie, K. L.; Li, Y. H.; Wu, W. X.; Shan, H. C.; Cheng, B. J.; Li, X. Y. Aquaporin ZmTIP2;3 promotes drought resistance of maize through symbiosis with arbuscular mycorrhizal fungi. *Int. J. Mol. Sci.* **2024**, *25* (8), 4205.
(9) Xie, W.; Hao, Z. P.; Zhou, J.; Fu, W.; Guo, L. P.; Zhang, X.; Chen, B. D. Integrated transcriptomics and metabolomics reveal specific phenolic and flavonoid accumulation in licorice (Glycyrrhiza uralensis Fisch.) induced by arbuscular mycorrhiza symbiosis under drought stress. *Plant Physiol. Biochem.* **2023**, *205*, 108173.
(10) Kakabouki, I.; Stavropoulos, P.; Roussis, I.; Mavroeidis, A.; Bilalis, D. Contribution of arbuscular mycorrhizal fungi (AMF) in improving the growth and yield performances of flax (Linum usitatissimum L.) to salinity stress. *Agronomy-Basel* **2023**, *13* (9), 2416.
(11) Gómez-Falcón, N.; Sáenz-Carbonell, L. A.; Andrade-Torres, A.; Lara-Pérez, L. A.; Narváez, M.; Oropeza, C. Arbuscular mycorrhizal fungi increase the survival and growth of micropropagated coconut (Cocos nucifera L.) plantlets. *In Vitro Cell. Dev. Biol.-Plant* **2023**, *59* (3), 401−412.
(12) Jiang, D.; Lin, R. X.; Tan, M. T.; Yan, J. X.; Yan, S. C. The mycorrhizal-induced growth promotion and insect resistance reduction in Populus alba × P. Berolinensis seedlings: A multi-omics study. *Tree Physiol* **2022**, *42* (5), 1059−1069.
(13) Minton, M. M.; Barber, N. A.; Gordon, L. L. Effects of arbuscular mycorrhizal fungi on herbivory defense in two Solanum (solanaceae) species. *Plant Ecol. Evol.* **2016**, *149* (2), 157−164.
(14) Jiang, D.; Tan, M. T.; Wu, S.; Zheng, L.; Wang, Q.; Wang, G. R.; Yan, S. C. Defense responses of arbuscular mycorrhizal fungus-colonized poplar seedlings against gypsy moth larvae: A multiomics study. *Hortic. Res.-England* **2021**, *8* (1), 245.
(15) Kadam, S. B.; Pable, A. A.; Barvkar, V. T. Mycorrhiza induced resistance (MIR): A defence developed through synergistic engagement of phytohormones, metabolites and rhizosphere. *Funct. Plant Biol.* **2020**, *47* (10), 880−890.
(16) Sharma, E.; Anand, G.; Kapoor, R. Terpenoids in plant and arbuscular mycorrhiza-reinforced defence against herbivorous insects. *Ann. Bot.* **2017**, *119* (5), 791−801.
(17) Lv, Y. R.; Wang, F.; Chen, H.; Zhang, T. T.; Yan, J. X.; Hu, Y. B. Unraveling arbuscular mycorrhizal fungi-induced resistance of purple branch rose (Rosa rugosa 'zizhi') to Lymantria dispar based on metabolomics. *Biol. Control* **2022**, *172*, 104971.
(18) Selvaraj, A.; Thangavel, K.; Uthandi, S. Arbuscular mycorrhizal fungi (Glomus intraradices) and diazotrophic bacterium (Rhizobium BMBS) primed defense in blackgram against herbivorous insect (Spodoptera litura) infestation. *Microbiol. Res.* **2020**, *231*, 126355.
(19) Turlings, T. C. J.; Erb, M. Tritrophic interactions mediated by herbivore-induced plant volatiles: Mechanisms, ecological relevance, and application potential. *Annu. Rev. Entomol.* **2018**, *63*, 433−452.
(20) Deng, Y. K.; Yu, X. H.; Yin, J. Q.; Chen, L.; Zhao, N. X.; Gao, Y. B.; Ren, A. Z. Epichloe endophyte enhanced insect resistance of host grass Leymus chinensis by affecting volatile organic compound emissions. *J. Chem. Ecol.* **2024**, *50* (12), 1067−1076.
(21) Meier, A. R.; Hunter, M. D. Mycorrhizae alter constitutive and herbivore-induced volatile emissions by milkweeds. *J. Chem. Ecol.* **2019**, *45* (7), 610−625.
(22) Disi, J. O.; Zebelo, S.; Kloepper, J. W.; Fadamiro, H. Seed inoculation with beneficial rhizobacteria affects european corn borer (Lepidoptera: Pyralidae) oviposition on maize plants. *Entomol. Sci.* **2018**, *21* (1), 48−58.
(23) Leitner, M.; Kaiser, R.; Hause, B.; Boland, W.; Mithöfer, A. Does mycorrhization influence herbivore-induced volatile emission in Medicago truncatula? *Mycorrhiza* **2010**, *20* (2), 89−101.
(24) Papantoniou, D.; Chang, D.; Martínez-Medina, A.; van Dam, N. M.; Weinhold, A. Root symbionts alter herbivore-induced indirect defenses of tomato plants by enhancing predator attraction. *Front. Physiol.* **2022**, *13*, 16.
(25) Zeni, V.; Grassi, A.; Santin, M.; Ricciardi, R.; Pieracci, Y.; Flamini, G.; Di Giovanni, F.; Marmugi, M.; Agnolucci, M.; Avio, L.; et al. Leaf UV-B irradiation and mycorrhizal symbionts affect lettuce voc emissions and defence mechanisms, but not aphid feeding preferences. *Insects* **2023**, *14* (1), 20.
(26) Dady, E. R.; Kleczewski, N.; Ugarte, C. M.; Ngumbi, E. Plant variety, mycorrhization, and herbivory influence induced volatile emissions and plant growth characteristics in tomato. *J. Chem. Ecol.* **2023**, *49* (11−12), 710−724.
(27) Song, T. Y.; Zhang, L. Modified green waste compost as growing substrates on the effective components of medicinal plants perilla (Perilla frutescens (L.) britt.) and mint (Mentha haplocalyx Briq.). *Ind. Crop. Prod.* **2023**, *198*, 116740.
(28) Ma, H. C.; Zhang, B.; Li, J. J.; Qiao, F. J.; Ma, Q. H.; Wan, X. W.; Jiang, Z. F.; Li, C. Y. Development of Spodoptera exigua population: Does the nutritional status matter? *Insects* **2023**, *14* (1), 13.
(29) Zhang, X. X.; Zhang, R. M.; Yu, M. Q.; Liu, R.; Liu, N. J.; Teng, H. Y.; Pei, Y. K.; Hu, Z. N.; Zuo, Y. Y. Identification and detection of the V1848i indoxacarb resistance mutation in the beet armyworm, Spodoptera exigua. *Pest. Biochem. Physiol.* **2024**, *203*, 105991.
(30) Roylawar, P.; Khandagale, K.; Nanda, S.; Soumia, P. S.; Jadhav, S.; Mahajan, V.; Gawande, S. Colonization of Serendipita indica promotes resistance against Spodoptera exigua in onion (Allium cepa L.). *Front. Microbiol.* **2023**, *14*, 11.
(31) Li, Y. D.; Nan, Z. B.; Matthew, C.; Wang, Y. J.; Duan, T. Y. Arbuscular mycorrhizal fungus changes alfalfa (Medicago sativa) metabolites in response to leaf spot (Phoma medicaginis) infection, with subsequent effects on pea aphid (Acyrthosiphon pisum) behavior. *New Phytol.* **2023**, *239* (1), 286−300.
(32) Shafiei, F.; Shahidi-Noghabi, S.; Sedaghati, E.; Smagghe, G. Arbuscular mycorrhizal fungi inducing tomato plant resistance and its role in control of Bemisia tabaci under greenhouse conditions. *Neotrop. Entomol.* **2024**, *53* (2), 424−438.
(33) Stallmann, J.; Schweiger, R. Effects of arbuscular mycorrhiza on primary metabolites in phloem exudates of Plantago major and Poa annua and on a Generalist Aphid. *Int. J. Mol. Sci.* **2021**, *22* (23), 13086.
(34) Phillips, J. M.; Hayman, D. S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc.* **1970**, *55* (1), 158.
(35) Sardar, H.; Khalid, Z.; Ahsan, M.; Naz, S.; Nawaz, A.; Ahmad, R.; Razzaq, K.; Wabaidur, S. M.; Jacquard, C.; Širić, I.; et al. Enhancement of salinity stress tolerance in lettuce (Lactuca sativa L.) via foliar application of nitric oxide. *Plants-Basel* **2023**, *12* (5), 1115.
(36) Farnad, N.; Heidari, R.; Aslanipour, B. Phenolic composition and comparison of antioxidant activity of alcoholic extracts of peppermint (Mentha piperita). *J. Food Meas. Charact.* **2014**, *8* (2), 113−121.
(37) Xia, H. Y.; Yang, Y.; Xia, L. H.; Yang, T. T.; Ruan, X. F. Variations in total phenols and condensed tannins contents in persimmon fruits from different varieties during ripening. *Food sci* **2014**, *35* (19), 66−71.
(38) Yang, S. Y.; Song, F. F.; Xie, J. C. Resistance of jasmonic acid mediated cotton seedlings against the relative growth rate of Helicoverpa armigera. *J. Northwest Sci-Tech Univ. Agric. For.(Nat.Sci. Ed.)* **2013**, *41* (5), 66−74.
(39) Ghosh, M.; Singh, S. P. A comparative study of cadmium phytoextraction by accumulator and weed species. *Environ. Pollut.* **2005**, *133* (2), 365−371.
(40) Yan, S. C.; Tan, M. T.; Zheng, L.; Wu, H. F.; Wang, K.; Chai, R. S.; Jiang, D. Defense response of Fraxinus mandshurica seedlings to Hyphantria cunea larvae under cd stress: A contradiction between attraction and resistance. *Sci. Total Environ.* **2023**, *859*, 160390.
(41) Zhou, L. M.; Jv, Q.; Qu, M. J.; Zhao, Z. Q.; Dong, S. L.; Han, Z. J.; Yu, S. L. Eag and behavioral responses of the large black chafer,

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

Holotrichia parallela (Coleoptera: Scarabaeidae)to its sex pheromone. *Acta Entomol. Sin.* **2009**, *52* (2), 121−125.
(42) Kanehisa, M.; Goto, S. Kegg: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **2000**, *28* (1), 27−30.
(43) Fan, L.; Zhang, C.; Li, J. F.; Liu, Y. The use of arbuscular mycorrhizal fungi to alleviate the growth and photosynthetic characteristics of strawberry under salt stress. *Acta Physiol. Plant.* **2024**, *46* (12), 118.
(44) Szada-Borzyszkowska, A.; Krzyzak, J.; Rusinowski, S.; Magurno, F.; Pogrzeba, M. Inoculation with arbuscular mycorrhizal fungi supports the uptake of macronutrients and promotes the growth of Festuca ovina L. and Trifolium medium L., a candidate species for green urban infrastructure. *Plants-Basel* **2024**, *13* (18), 2620.
(45) Ge, S. B.; Zhang, Z.; Hu, Q.; Wang, Q. H.; Gong, X. J.; Huang, F.; Zhang, L.; Han, W. Y.; Luo, F.; Li, X. Metabolomics analysis reveals crucial effects of arbuscular mycorrhizal fungi on the metabolism of quality compounds in shoots and roots of Camellia sinensis L. *Plant Physiol. Biochem.* **2025**, *219*, 109426.
(46) Zhang, D. J.; Tong, C. L.; Wang, Q. S.; Bie, S. Mycorrhizas affect physiological performance, antioxidant system, photosynthesis, endogenous hormones, and water content in cotton under salt stress. *Plants-Basel* **2024**, *13* (6), 805.
(47) Campo, S.; Martín-Cardoso, H.; Olivé, M.; Pla, E.; Catala-Forner, M.; Martínez-Eixarch, M.; San Segundo, B. Effect of root colonization by arbuscular mycorrhizal fungi on growth, productivity and blast resistance in rice. *Rice* **2020**, *13*, 42.
(48) Jacott, C. N.; Murray, J. D.; Ridout, C. J. Trade-offs in arbuscular mycorrhizal symbiosis: Disease resistance, growth responses and perspectives for crop breeding. *Agronomy-Basel* **2017**, *7* (4), 75.
(49) Johnson, N. C.; Wilson, G. W. T.; Wilson, J. A.; Miller, R. M.; Bowker, M. A. Mycorrhizal phenotypes and the law of the minimum. *New Phytol.* **2015**, *205* (4), 1473−1484.
(50) Li, R. Q.; He, Y.; Chen, J. Y.; Zheng, S. Y.; Zhuang, C. X. Research progress in improving photosynthetic efficiency. *Int. J. Mol. Sci.* **2023**, *24* (11), 9286.
(51) Uesugi, A. The slow-growth high-mortality hypothesis: Direct experimental support in a leafmining fly. *Ecol. Entomol.* **2015**, *40* (3), 221−228.
(52) Zhao, J. Q.; Fang, J.; Tan, M. T.; Wu, S.; Ren, Y. J.; Meng, Z. J.; Yan, S. C. Effects of arbuscular mycorrhizal fungal colonization on Populus pseudo-cathayana × P. deltoides resistance to Lymantria dispar larvae. *J. Beijing For. Univ.* **2024**, *46* (3), 53−59.
(53) Du, E. W.; Chen, Y. P.; Li, Y. H.; Zhang, F. J.; Sun, Z. X.; Hao, R. S.; Gui, F. R. Effect of arbuscular mycorrhizal fungi on the responses of Ageratina adenophora to Aphis gossypii herbivory. *Front. Plant Sci.* **2022**, *13*, 14.
(54) Rani, P. U.; Pratyusha, S. Defensive role of *Gossypium hirsutum* L. anti-oxidative enzymes and phenolic acids in response to Spodoptera litura F. Feeding. *J. Asia-Pac. Entomol.* **2013**, *16* (2), 131−136.
(55) Yu, L.; Zhang, W. T.; Geng, Y. Y.; Liu, K. S.; Shao, X. Q. Cooperation with arbuscular mycorrhizal fungi increases plant nutrient uptake and improves defenses against insects. *Front. Ecol. Evol.* **2022**, *10*, 12.
(56) Babikova, Z.; Gilbert, L.; Bruce, T.; Dewhirst, S. Y.; Pickett, J. A.; Johnson, D. Arbuscular mycorrhizal fungi and aphids interact by changing host plant quality and volatile emission. *Funct. Ecol.* **2014**, *28* (2), 375−385.
(57) Zhang, W. T.; Yu, L.; Han, B.; Liu, K. S.; Shao, X. Q. Mycorrhizal inoculation enhances nutrient absorption and induces insect-resistant defense of Elymus nutans. *Front. Plant Sci.* **2022**, *13*, 11.
(58) Babikova, Z.; Gilbert, L.; Randall, K. C.; Bruce, T. J. A.; Pickett, J. A.; Johnson, D. Increasing phosphorus supply is not the mechanism by which arbuscular mycorrhiza increase attractiveness of bean (Vicia faba) to aphids. *J. Exp. Bot.* **2014**, *65* (18), 5231−5241.
(59) Bosch, M.; Berger, S.; Schaller, A.; Stintzi, A. Jasmonate-dependent induction of polyphenol oxidase activity in tomato foliage is important for defense against Spodoptera exigua but not against Manduca sexta. *BMC Plant Biol.* **2014**, *14*, 257.
(60) Mahajan, E.; Singh, S.; Kaur, S.; Sohal, S. K.; Sohal, S. K. The genotoxic, cytotoxic and growth regulatory effects of plant secondary metabolite β-caryophyllene on polyphagous pest Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae). *Toxicon* **2022**, *219*, 106930.
(61) Egigu, M. C.; Ibrahim, M. A.; Yahya, A.; Holopainen, J. K. Yeheb (*Cordeauxia edulis*) extract deters feeding and oviposition of *Plutella xylostella* and attracts its natural enemy. *Biocontrol* **2010**, *55* (5), 613−624.
(62) Li, Z. X.; Wang, D. X.; Shi, W. X.; Weng, B. Y.; Zhang, Z.; Su, S. H.; Sun, Y. F.; Tan, J. F.; Xiao, S.; Xie, R. H. Nitrogen-mediated volatilisation of defensive metabolites in tomato confers resistance to herbivores. *Plant Cell Environ* **2024**, *47* (8), 3227−3240.
(63) Zhang, H.; Liu, E. L.; Huang, X. Z.; Kou, J. F.; Teng, D.; Lv, B. B.; Han, X. Q.; Zhang, Y. J. Characterization of a novel insect-induced sesquiterpene synthase GbTPS1 based on the transcriptome of Gossypium barbadense feeding by Cotton Bollworm. *Front. Plant Sci.* **2022**, *13*, 11.
(64) Liu, J. Y.; Hua, J.; Qu, B.; Guo, X. Y.; Wang, Y. Y.; Shao, M. N.; Luo, S. H. Insecticidal terpenes from the essential oils of Artemisia nakaii and their inhibitory effects on acetylcholinesterase. *Front. Plant Sci.* **2021**, *12*, 9.
(65) Sarkar, A. K.; Sadhukhan, S. Unearthing the alteration in plant volatiles induced by mycorrhizal fungi: A shield against plant pathogens. *Physiol. Plant.* **2023**, *175* (1), 16.
(66) Shrivastava, G.; Ownley, B. H.; Augé, R. M.; Toler, H.; Dee, M.; Vu, A.; Köllner, T. G.; Chen, F. Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. *Symbiosis* **2015**, *65* (2), 65−74.
(67) Zaret, M.; Kinkel, L.; Borer, E. T.; Seabloom, E. W. Plant growth-defense trade-offs are general across interactions with fungal, insect, and mammalian consumers. *Ecology* **2024**, *105* (5), 13.
(68) Zeng, M.; Hause, B.; van Dam, N. M.; Uthe, H.; Hoffmann, P.; Krajinski, F.; Martínez-Medina, A. The mycorrhizal symbiosis alters the plant defence strategy in a model legume plant. *Plant Cell Environ.* **2022**, *45* (12), 3412−3428.
(69) da Silva, R. R.; da Câmara, C. A. G.; Almeida, A. V.; Ramos, C. S. Biotic and abiotic stress-induced phenylpropanoids in leaves of the mango (*Mangifera indica* L., Anacardiaceae). *J. Braz. Chem. Soc.* **2012**, *23* (2), 206−211.
(70) Zeng, L.; Yang, Z. Biosyntheses of volatile phenylpropanoids/benzenoids in tea (Camellia sinensis) and their formations in response to stresses. *J. Trop. & Subtrop. Bot.* **2019**, *27* (5), 591−600.
(71) Scott, E. R.; Li, X.; Wei, J. P.; Kfoury, N.; Morimoto, J.; Guo, M. M.; Agyei, A.; Robbat, A.; Ahmed, S.; Cash, S. B.; et al. Changes in tea plant secondary metabolite profiles as a function of leafhopper density and damage. *Front. Plant Sci.* **2020**, *11*, 15.
(72) de Menezes, C. W. G.; Carvalho, G. A.; Alves, D. S.; de Carvalho, A. A.; Aazza, S.; de Oliveira Ramos, V.; Pinto, J.; Bertolucci, S. K. V. Biocontrol potential of methyl chavicol for managing Spodoptera frugiperda (Lepidoptera: Noctuidae), an important corn pest. *Environ. Sci. Pollut. Res.* **2020**, *27* (5), 5030−5041.
(73) Msisi, D.; Matojo, N. D.; Kimbokota, F. Attraction of female tomato leaf miner, Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae) to shared compounds from hosts. *Phytoparasitica* **2021**, *49* (2), 153−162.
(74) Scala, A.; Allmann, S.; Mirabella, R.; Haring, M. A.; Schuurink, R. C. Green leaf volatiles: A plant's multifunctional weapon against herbivores and pathogens. *Int. J. Mol. Sci.* **2013**, *14* (9), 17781−17811.

https://doi.org/10.1021/acs.jafc.5c03530
J. Agric. Food Chem. XXXX, XXX, XXX−XXX