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RESEARCH ARTICLE

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Feeding specialization shapes the bottom-up effect of arbuscular mycorrhizal fungi across a plant–aphid–parasitoid system

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Societal Impact Statement

Arbuscular mycorrhizal fungi (AMF) impact the relationships between plants, aphids (insects that feed on plant phloem), and their natural enemies (insects that prey on or parasitize aphids). The presence of AMF influences the growth and population of different aphid species and affects the development of aphid-killing wasps and their attraction to plants. This research has been conducted also considering the insects' feeding strategy and their feeding specialization. This study provides novel perspectives on how these fungi shape interactions in the natural world, offering potential insights for the development of sustainable pest management strategies in agriculture.

Summary

- Arbuscular mycorrhizal fungi (AMF) are major root symbionts regulating plant physiology. Their presence affects the performance of aboveground insect herbivores in relation to their feeding strategy and their feeding specialization. For example, the effect of the arbuscular mycorrhizal (AM) symbiosis on chewing insects, positive for specialists and negative for generalists, has been previously demonstrated. Conversely, the impact of AMF on phloem-suckers with relatively different levels of specialization remains unexplored.
- We tested the influence of the AM *Funneliformis mosseae* on the fitness of the specialist aphid *Acyrthosiphon pisum* and the generalist aphid *Myzus persicae* on *Vicia faba* plants. Further, we investigated the effects of AMF on the higher trophic level, the aphid parasitoids *Aphidius ervi* (specialist) and *Aphidius colemani* (generalist), by evaluating plant attractiveness and parasitoid fitness. To support the results of behavioral and biological bioassays we characterized the photosynthetic parameters, the volatilome and the transcriptome of tested plants.
- Mycorrhizal plants proved unsuitable for the generalist *M. persicae* but enhanced the fitness of the specialist *A. pisum*. The AM symbiosis had no effects on the

behavioral response of *A. colemani* and enhanced the attraction and fitness of *A. ervi*.
- Volatilome and transcriptome profiling corroborated the results of bioassay highlighting a bottom-up effect of the AMF across a plant–aphid–parasitoid system.

KEYWORDS
arbuscular mycorrhizal fungi, integrated pest management, multi-trophic interactions, specialist and generalist aphids, *Vicia faba*

1 | INTRODUCTION

In recent years the impact of soil symbionts on aboveground insect communities mediated by the plant has been shown in different multi-trophic systems (Coppola, Cascone, et al., 2019; Yu et al., 2022). The outcome of these interactions is an important source of information that could be exploited for sustainable plant protection. One of the most significant interactions that occurs in the below-ground part is attributed to arbuscular mycorrhizal fungi (AMF) which, in spite of some degree of preference under certain ecological conditions (Cesaro et al., 2008), are considered unspecific symbionts, because a small number of fungal species (a few hundred) are able to establish a symbiosis with several thousands of species belonging to very distant clades of Embryophytes (Smith & Read, 2008). In fact, while the AMF receive the needed carbon supply from the plant, the plant exploits the fungi's ability to enhance the availability and the uptake of nutritional elements from the soil, particularly phosphorous (Smith & Read, 2008). So far, a large body of evidence indicates that AMF are able to enhance plant defenses (Frew et al., 2022; García-Garrido & Ocampo, 2002; Gianinazzi-Pearson et al., 1996; Jung et al., 2012; Li et al., 2019; Wang et al., 2023), but the effect on herbivore insects is variable. For instance, in some cases, a positive effect of AMF on aphid weight and fecundity was reported (Gange et al., 1999; Maurya et al., 2018), while in other cases the aphids were negatively affected (Balog et al., 2017; Guerrieri et al., 2004). The impact of belowground symbionts on aboveground insects is also possibly related to the feeding habit of the herbivore, whether it is a chewer (e.g., caterpillar) or a sucker (e.g., aphid) (Koricheva et al., 2009). Nonetheless, the common aspect that emerges from all these studies is the species-specificity of these belowground-aboveground interactions: different symbiont strains (e.g., *Trichoderma*) or species (e.g., AMF spp.) differentially affect phytophagous insects (Charters et al., 2022; Gange, 2001; Simon et al., 2017; Stallmann & Schweiger, 2021; Tomczak & Müller, 2017) and their natural enemies (insect predators and parasitoids) by altering the volatile organic profile released by colonized plants (Babikova et al., 2014).

In general, the performance of an herbivorous insect depends on the nutritional quality of the host plant and on the effectiveness of its constitutive/induced defenses. In the never-ending battle between plants and herbivorous insects, specialist species have evolved to overcome plant defenses, for example by using plant-derived powerful toxic compounds to their own advantage. The glucosinolates, a group of compounds characterizing the plant species of the family *Brassicaceae* which are extremely toxic for the vast majority of herbivore insects, are in fact exploited by the specialist aphid *Brevicoryne brassicae* to speed up its development and eventually to poison its antagonists (Sun et al., 2021). Conversely, the success of a generalist species usually links to its ability to either excrete plant-derived toxic compounds, hampering their detrimental effect, or to disperse effectively in search of more suitable host plants (Ali & Agrawal, 2012). These complex scenarios are altered by the presence of root symbionts (Koricheva et al., 2009). Indeed, the concomitant presence of a root symbiont, inducing a plant response, and of an herbivore insect, activating direct and indirect defensive responses, could either combine in a synergic way or act in opposite ways, counterbalancing each other. Because the root symbioses are widely widespread in nature, it is important to understand if and how they affect the communities of generalist and specialist insects and in turn ecosystem dynamics. With respect to host plant colonization, AMF can be considered generalist species: single fungal isolates, like *Rhizophagus irregularis*, have been shown to colonize hundreds of plant species across several plant families (Zeng et al., 2018, and references therein). The effect of the arbuscular mycorrhizal (AM) symbiosis on aboveground insects has been long studied on a number of experimental and natural systems. It has been reported that these effects change with the insect species, and this applies to both herbivores and antagonist species (Gange, 2001; Guerrieri et al., 2004). Far scarcer are the data on the impact of root symbionts on aboveground insects characterized by the same feeding habit but by different levels of host plant specialization. Some information is available on the effect of AM symbiosis on specialist and generalist chewers (Koricheva et al., 2009, and references therein): to summarize, mycorrhizal symbiosis favours the specialists and hampers the generalists (Koricheva et al., 2009). Conversely, there is a lack of data referring to sap-sucking insects and relative antagonists, despite the importance of this category which includes aphids and whiteflies, also known for their ability to act as vectors of economically important phytopathogens (e.g., viruses). Aphids stand as one of the most destructive groups of insect pests globally, leading to annual yield losses ranging from tens

of millions to billions of US dollars across various food and commodity crops (van Emden & Harrington, 2007). Hence, besides the scientific value, the thorough characterization of these multitrophic and multilevel interactions could have important practical relevance for the sustainable protection of agricultural crops. The use of AMF represents a significant potential for reducing pesticide usage if their colonization has a negative impact on aphids and/or attracts natural enemies. Studies involving aphids, AMF and their parasitoids are relatively scarce (Bennett et al., 2016; Guerrieri et al., 2004; Hempel et al., 2009; Maurya et al., 2018; Volpe et al., 2018). As far as we know, the impact of this kind of study has not been extended to broad bean, which is an economically important crop. In this paper, we designed several multidisciplinary experiments aiming at assessing the impact of the AMF *Funneliformis mosseae* on (i) the development and the reproduction of a specialist and a generalist aphid species (as a measure of plant direct defenses), (ii) the attraction of natural antagonists of the specialist and generalist aphid species (as a measure of plant indirect defenses), and (iii) a selection of key morphological parameters of the natural antagonists of the specialist and generalist aphid species (as an indirect measure of their fitness). The results of these tests were corroborated by the characterization of (i) RNAseq of plants used in all treatments including mycorrhizal symbiosis and aphid attack and (ii) plant volatilome collected from plants used in all treatments including mycorrhizal symbiosis and aphid attack.

2 | MATERIALS AND METHODS

2.1 | Plants, insect rearing, and mycorrhizal inoculant

The broad bean plants (*Vicia faba* cultivar "Aguadulce Supersimonia") were grown in a glasshouse at the Institute for Sustainable Plant Protection under the following controlled conditions: relative humidity (RH) of 65 ± 5%, photoperiod of 16L:8D h, light irradiance of 700 µmol m^{-2} s^{-1}, and light/dark thermoperiod of 24/22°C. The aphids *Acyrthosiphon pisum* Harris and *Myzus persicae* Sulzer (both, Hemiptera: Aphididae) were permanently reared in separated mesh cages (Vermandel, The Netherlands) on broad bean plants under the same conditions as described above. Similarly, the parasitoid wasps *Aphidius ervi* Haliday and *Aphidius colemani* Viereck (both, Hymenoptera: Braconidae) were continuously reared on their relative natural aphid hosts, *A. pisum* and *M. persicae*, maintained on potted broad bean plants in mesh cages as described above. Aphids and parasitoids rearings were located in separate glasshouse compartments. *F. mosseae* BEG12 (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler was provided by the European Bank of Glomales (Dijon) and propagated in *Sorghum bicolor* plants grown on sterile quartz sand for about 3 months as described in detail by Cesaro et al., 2020. The inoculum consisted of fragmented hyphae, spores, and small pieces of colonized roots in quartz sand (62 propagules g^{-1}).

2.2 | Experimental design

All specialist and generalist organisms, experimental treatments, and experiments performed are summarized in Figure 1.

In all experiments, broad bean seeds were placed in 0.5 L-plastic pots (one seed/pot) filled with sterile quartz sand. For mycorrhizal treatments (Fm--), each seed was inoculated at the time of sowing with 30 mL of the AM fungus *F. mosseae* BEG12, placing the inoculum under the seed. For relative control treatments (Nm--) seeds were placed on the same quantity of sterile substrate. The seeds were then covered with sand and watered with modified Long Ashton solution P 64 µM (Trotta et al., 1996). The same solution was used for watering the plants three times a week. After seed germination, broad bean plants were maintained in a growth chamber for 4 weeks under the following controlled conditions: RH of 65 ± 5%, photoperiod of 16L:8D h, light irradiance of 150 µmol m^{-2} s^{-1}, and light/dark thermoperiod of 24/22°C. Both mycorrhizal and control plants were transferred to a glasshouse under the conditions described above and reared before running the experimental tests (Figure 1c). At the end of all three experiments, below described, mycorrhizal colonization was checked for all Fm-- treatments and its absence was verified in all Nm-- treatments. Fifty 1-cm-long root pieces were cut from each root system, fixed in 70% ethanol and stored at 4°C until analysis. Then, they were cleared in 10% potassium hydroxide (KOH) solution for about 30 min at 60°C, stained with 1% methyl blue in lactic acid, and mounted on a slide. Mycorrhizal colonization was microscopically estimated according to Giovannetti and Mosse (1980). The microscopic analyses also allowed us to assess the complete absence of root nodules induced by nitrogen-fixing bacteria.

2.3 | Experiment 1: aphid survival and reproduction assays

Two bioassays were conducted using the aphids *A. pisum* and *M. persicae*. For each bioassay and species, 40 four-week-old broad bean mycorrhizal plants (Fm) and 40 control plants (Nm) were infested with one newly born first-instar nymph (<24 h old) transferred to them by a soft brush. All plants were checked daily to assess the presence of aphids, the presence of exuviae (evidence of moulting), and the presence and number of newly laid nymphs and dead aphids. When reproduction started, the offspring were removed daily leaving only the initial individual on the plant. The mortality of the aphids and the number of newly laid nymphs were recorded daily until death. The following population parameters were calculated as described in Cascone et al., 2018: the intrinsic rate of increase (r_m), the finite rate of increase (λ), the net reproductive rate (R_0), the mean generation time (T), the doubling time (Dt), the age-specific survival rate (lx), and the age-specific fecundity (mx). Survival and fertility curves were analyzed using the Kaplan–Meier method using the log-rank test, and the indexes R_0, r_m, T, and Dt and their variance were estimated using the jack-knife method (Cascone et al., 2018).

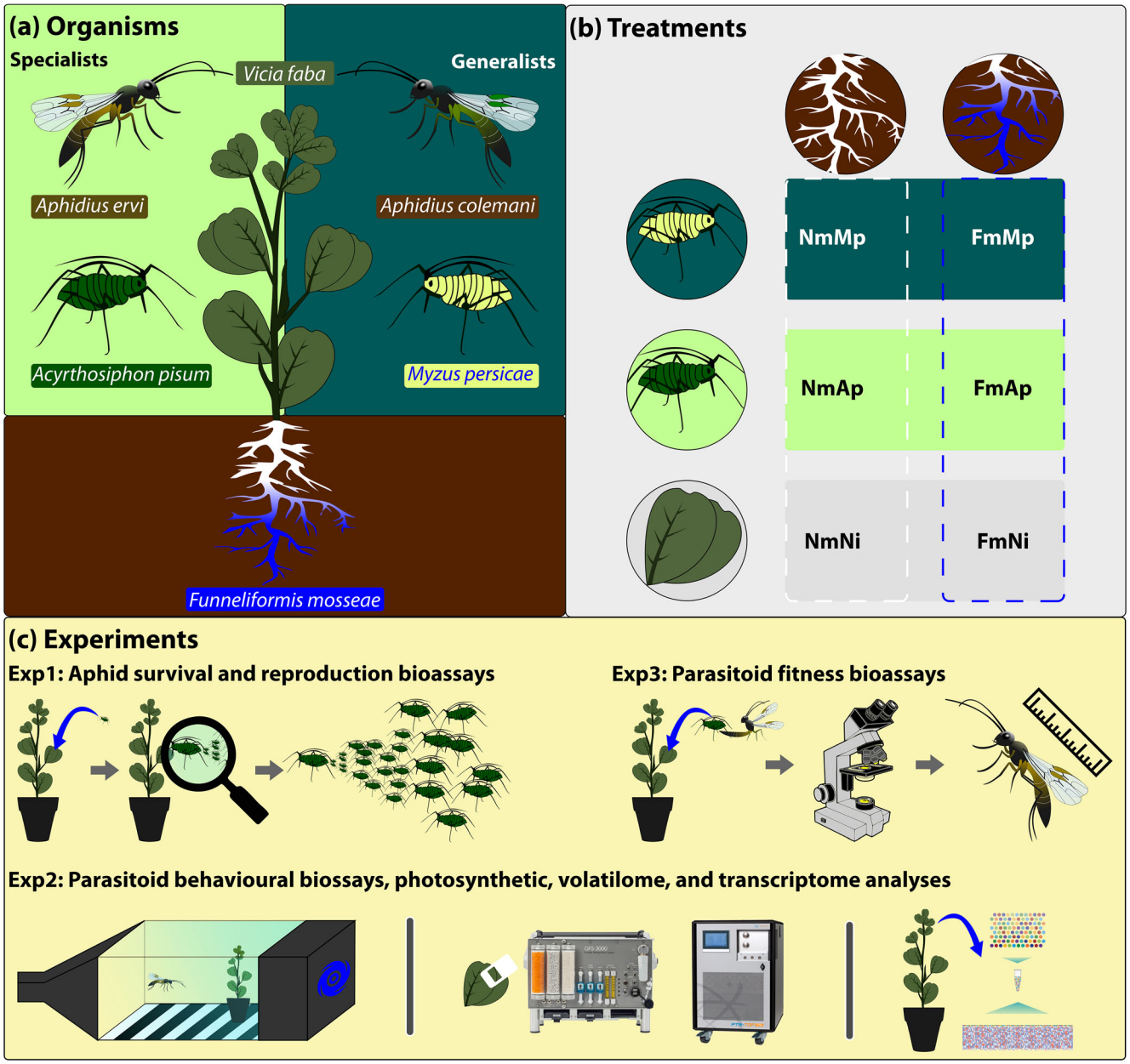

FIGURE 1 Experimental design for determining the effect of the arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* on the specialist aphid *Acyrthosiphon pisum* and the generalist aphid *Myzus persicae*, and relative parasitoid *Aphidius ervi* and *Aphidius colemani*. Graphical representation of all specialist (lime green background) and generalist (sky blue background) organisms (a), treatments (b) and experiments (c) under study in this research. NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant.

2.4 | Experiment 2: parasitoid behavioral bioassay

To assess for possible effects of AM colonization on parasitoid behavior, the attractiveness of the broad bean plants subjected to each treatment combination (Figure 1b) was estimated in a wind tunnel by testing a total of 60 female parasitoids over six different days on six different plants. For treatments involving *A. pisum* (--Ap) and *M. persicae* (--Mp), 4-week-old plants, mycorrhizal (Fm--) or not (Nm--), were infested for 7 days by 100 aphids of mixed ages ranging from third instar to adult for each aphid species. Relative control un-infested plants (--Ni) were grown under the same conditions for 7 days. Experimental tests were run combining all possible treatments as follows: NmMp, NmAp, NmNi, FmMp, FmAp, and FmNi. The percentage of responses (oriented flights, landings) to each target was calculated. The number of parasitoids responding, as oriented and unoriented flight, to each target was compared by a G-test for independence, as described in Coppola et al., 2018. To support the results of behavioral bioassays we have performed photosynthetic, volatilome, and transcriptome analyses for each treatment.

2.4.1 | Photosynthetic and VOC measurements from broad bean plants

Measurements (three plants for each of the six treatments) were carried out with a portable system of simultaneous gas exchanges and chlorophyll fluorescence analysis (Heinz Walz GmbH GFS-3000, Effeltrich, Germany). All measurements were conducted between 8:00 a.m. and 3:00 p.m. After leaves were dark-adapted for 30 min in an 8 cm^3 leaf cuvette, a saturating pulse was applied to obtain the maximum quantum yield of photosystem II (PSII) (Fv/Fm). Photosynthetic measurements were carried out with light intensity 1000 µmol m^{-2} s^{-1}, leaf temperature 30°C, relative air humidity 50%, and CO_2 set to 400 ppm, matching ambient CO_2 levels. The leaf was stabilized under the standard conditions until the stomata opened and a steady state of CO_2 and water vapor exchange rates were reached. The parameters *A*, *gH2O*, *ETR*, *PhiPSII*, and *ci* were obtained from the data.

Volatile organic compound (VOC) analyses were performed with proton transfer reaction–time of flight–mass spectrometer (PTR-Qi-TOF-MS) (Ionicon Analytik GmbH, Innsbruck, Austria) as described in Coppola, Di Lelio, et al. (2019). Tentative peak identification was performed using an in-house library developed by the authors and available literature reported as in List S1. Peaks below the 5 cps threshold (count per second signal from the ion detector, raw signal) were discarded as background noise. ANOVA assumptions of normality and homogeneity of variances of the area peaks were tested using the "gvlma" package (Peña & Slate, 2006). When ANOVA assumptions were satisfied, a one-way ANOVA analysis was run to assess the effect of *F. mossae* and aphids (*M. persicae* vs. *A. pisum*) on the VOC emitted and photosynthetic measurements. An alternative analysis was made using the non-parametric Kruskal–Wallis test. The volatile emission patterns, measured as peak areas, were analyzed through multivariate data analysis using projection to latent structures discriminant analysis (PSL-DA) using the R package "ropls" (Thévenot et al., 2015).

2.4.2 | Transcriptome analysis: RNA extraction and library construction from broad bean plants

Total RNA was extracted from at least 1 g of leaves (four replicates for each of the six treatments) using the RNeasy Plant RNA isolation kit (Qiagen, Italy) and it was digested after purification with RNase-free DNase I (Qiagen, Italy), in accordance with the manufacturer's instructions. In the first step, RNA quantity and quality were assessed by spectrophotometry (Beckman Coulter DU800, Milan, Italy), and by denaturing agarose gel electrophoresis. RNA samples were then sent to Iga technology service (Udine, Italy) for library preparation and sequencing. Table S1 shows the number of reads produced at this step for each sample. A quality check was performed on the raw Illumina sequencing data, low-quality portions were removed while the longest high-quality parts of next generation sequencing (NGS) reads were preserved. The software BBDuk (Bushnell et al., 2017) was used for the trimming step setting up the following parameters: the minimum length equal to 35 bp and the quality score was 25. The quality of the reads was checked before and after the trimming step with FastQC (Andrews, 2010).

2.4.3 | Data analysis for transcriptome sequencing: de novo assembly and functional annotation

The software Trinity v2.13.0 (Grabherr et al., 2011) was used on the trimmed Illumina reads to obtain a bulk of normalized reads representing the entire experimental groups. As a second step, the software TransAbyss (Robertson et al., 2010) was used to generate a de novo transcriptome assembly setting a k-mer length of 31 and a minimum length of 300 bp. Then, transcriptome redundancy was removed with the software BLAST-like alignment tool (BLAT) setting a minimum identity of 95%. Finally, the expression of all the transcripts was calculated in all the samples using Kallisto (Bray et al., 2016) with the options "--bias --fr-stranded" and the transcripts with no expression in any samples were considered artefacts and removed. The completeness of the transcriptome assembly was evaluated using the software Benchmarking Universal Single-Copy Orthologs (BUSCOv4) (Manni et al., 2021) against the databases of Eukaryote and Viridiplantae conserved genes (Figure S1). The software Kallisto was employed with the RNA-seq reads to quantify the expression of the predicted transcripts with the options "--bias --fr-stranded." Both the effective counts and the normalized expression values in transcripts per kilobase million (TPM) were obtained. The obtained expression matrix was then analyzed with R with the package NOISeq (Tarazona et al., 2015), together with the ASCA removal of systematic noise (ARSyN) approach to remove background and unwanted noise from the expression data, in order to identify differentially expressed transcripts (DETs). Only the DET with a probability of differential expression higher or equal to .95 was considered significant. Based on the experimental groups, the following comparisons were performed: NmAp versus NmNi, NmMp versus NmNi, FmAp versus FmNi, and FmMp versus FmNi. *V. faba* is not supported by Kyoto Encyclopedia of Genes and Genomes (KEGG). The closest to *V. faba* model species in KEGG databases, *Medicago truncatula* L., was used as a proxy. A local blast with the following parameters: task dc-megablast, identity >30%, e-value 0.00001, and query coverage per hsp >20 was performed to search significant transcripts against the cds of *M. truncatula* retrieved from the National Center for Biotechology Information (NCBI, accession GCF_003473485.1). A mean ± SE of 77.86 ± 2.34% of *V. faba* transcripts for the four treatment comparisons have been converted in *M. truncatula* transcripts. The KEGG pathway enrichment analysis was conducted by the ClusterProfiler packages in R (Wu et al., 2021). All transcripts were used as background for enrichment ($n = 15{,}405$). A *p*-value of <.01 cutoff was used on gene ontological terms with the addition of a fold-enrichment cutoff of >1.5 relative to the background. In order to visualize the KEGG enrichment result, the compareCluster function was utilized.

2.5 | Experiment 3: parasitoid fitness bioassay

To assess for possible effects of AMF treatments on the fitness of the parasitoid *A. ervi* the following morphological traits and ratios were measured on 6 mothers and 45 relative progenies grown on aphids fed by mycorrhizal and non-AM control plants. Morphological traits (head width, frontovertex width, mid tibia, and hind tibia length) and relative proportions (head width:frontovertex width) have been calculated at a dissection binocular provided with a micrometric lens. ANOVA assumptions of normality and homogeneity of variances of the proportions were tested using the "gvlma" package (Peña & Slate, 2006). When ANOVA assumptions were satisfied a Welch Two Sample *t*-test was run to assess the effect of *F. mosseae*, and alternative analyses were made using the non-parametric Kruskal–Wallis test.

3 | RESULTS

3.1 | Mycorrhizal impacts on aphid fitness

The age-specific survival rate (LX) and fecundity (MX) of *A. pisum* (the specialist) and *M. persicae* (the generalist) reared on *V. faba* plants were calculated from the data obtained by the first experiment. Figure 2 indicates a significant positive effect for both parameters (survival: log-rank test, $\chi^2 = 6.401$ $p = .011$; fertility: log-rank test, $\chi^2 = 45.135$ $p < .001$) of the AMF *F. mosseae* on the specialist aphid (Figure 2a,c), and a significant opposite trend (survival: log-rank test, $\chi^2 = 8.722$ $p = .003$; fertility: log-rank test, $\chi^2 = 92.482$ $p = <.001$) for the generalist (Figure 2b,d).

These data were used to calculate the life table parameters reported in Figure 3. AMF improved *A. pisum* (specialist) fitness that showed an intrinsic rate of increase (r_m) enhanced by 27%, a finite rate of increase λ enhanced by 8%, and a net reproductive rate R_0 increased by 92% on mycorrhizal plants compared with non-AM controls (Figure 2a). Hence, the population of specialist aphids on mycorrhized plants needed approximately half a day less to double and to complete one generation with respect to non-AM control (Figure 2a). Conversely, AMF impaired the fitness of *M. persicae* (generalist), resulting in an r_m, λ, and R_0 decreased by 32%, 12%, and 69%, respectively, compared with non-AM controls (Figure 2b). Consequently, the population of the generalist fed on mycorrhizal plants needed 0.87 and 1.03 more days to double and to complete one generation, respectively, compared with non-AM control plants. All demographic indexes were significantly different between the aphid population reared on AM colonized plants and relative non-AM control plants (jack-knife test, $p < .001$).

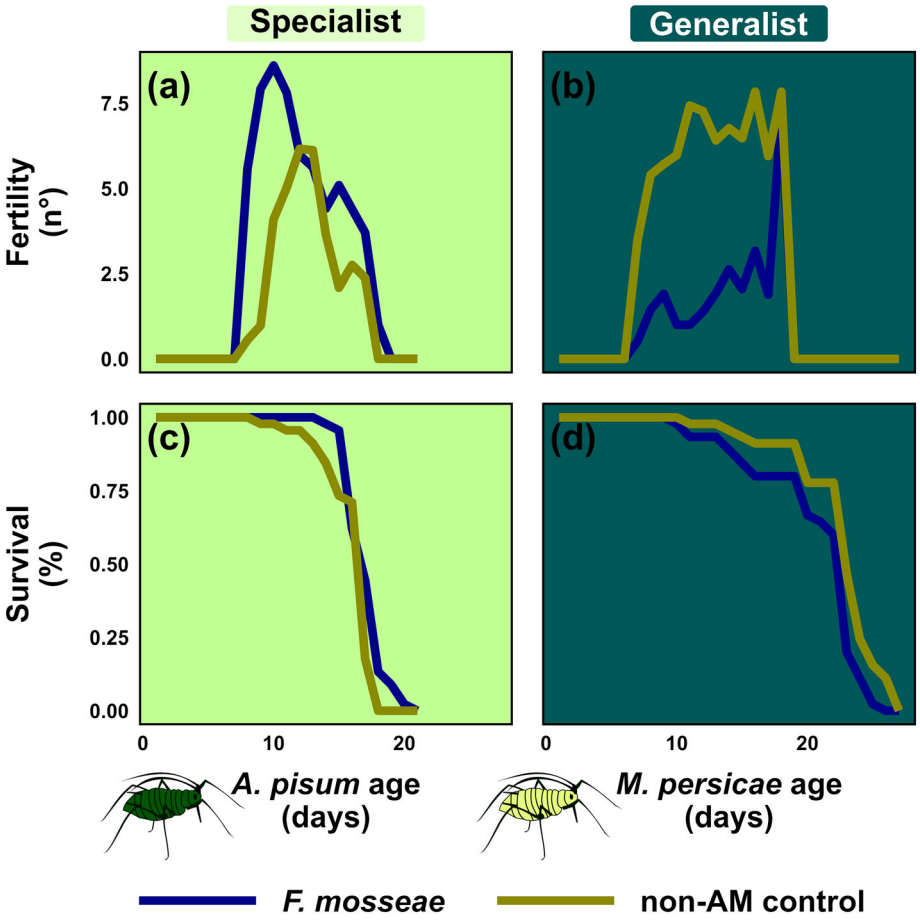

FIGURE 2 The effect of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* colonization on aphids *Acyrthosiphon pisum* and *Myzus persicae* fitness. Age-specific fecundity (MX panels a and b) and age-specific survival rate (LX, panels c and d) of the specialist aphid *A. pisum* (panels a and c, background lime green) and the generalist aphid *M. persicae* (panels b and d, background sky blue) reared on *Vicia faba* plants.

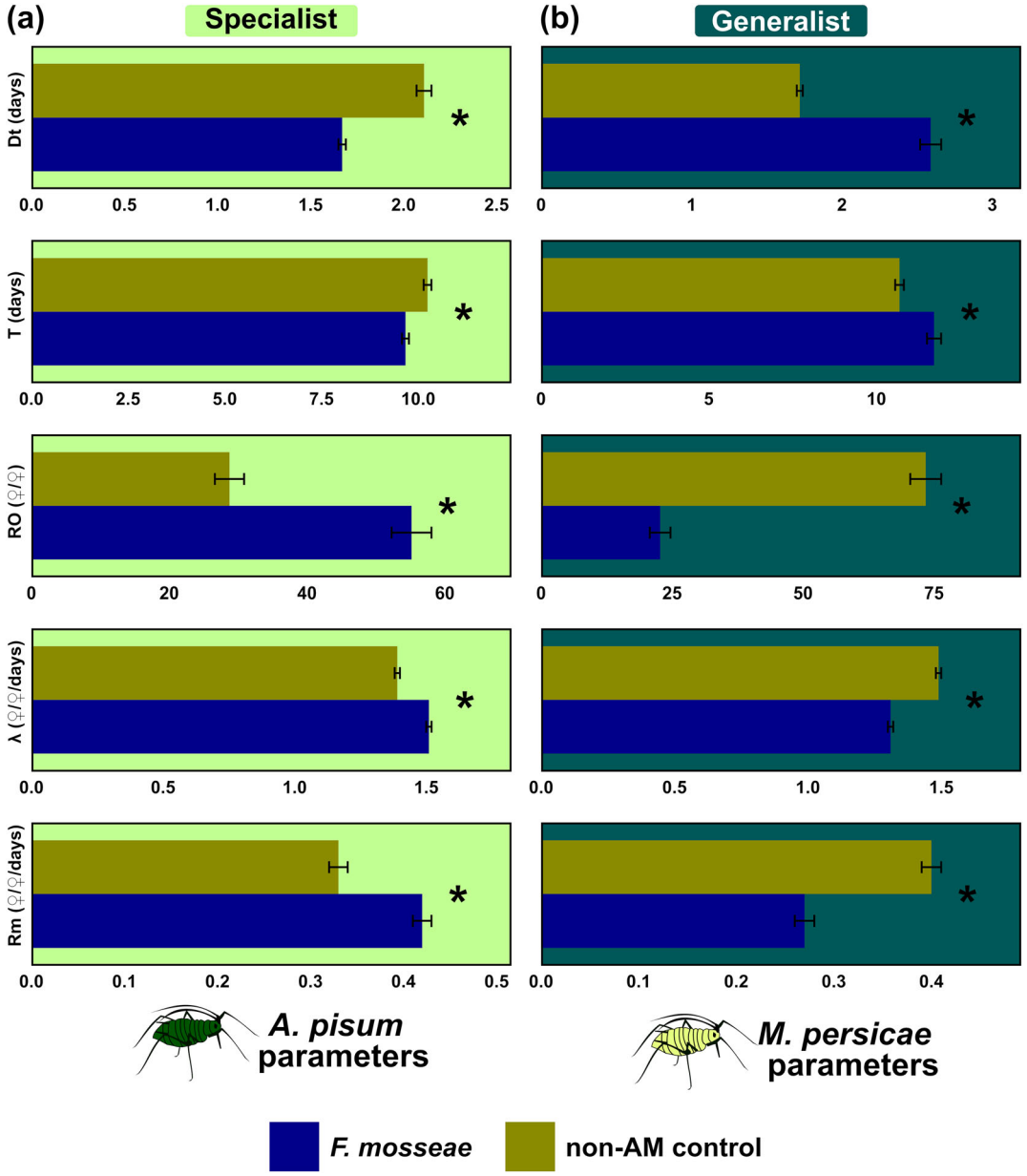

FIGURE 3 The effect of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* colonization on the development and population growth of the aphids *Acyrthosiphon pisum* and *Myzus persicae*. Aphids' associated life-table parameters and standard error, represented by error bars, were estimated by jackknife technique for *A. pisum* (column a, specialist, lime green background) and *M. persicae* (column b, generalist, sky blue background) reared on *Vicia faba* plants inoculated with *F. mossae* (Fm) or uninoculated controls (Nm). *Dt*, doubling time (days); λ, finite rate of increase (female/female/days); r_m, intrinsic rate of increase (female/female/days); R_O, net reproductive rate (female/female); T, mean generation time (days). Statistically significant differences between treatments (Nm, Fm) were labeled by asterisks (*$p < .001$, jack-knife test).

3.2 | Mycorrhizal impacts on aphid parasitoids' behavior

The effect of AMF on the flight behavior of *A. ervi* and *A. colemani*, parasitoids of *A. pisum* and *M. persicae*, respectively, was tested for aphid-infested and un-infested broad bean plants in wind tunnel bioassay (Experiment 2). Figure 4a,c shows that root colonization affected the foraging behavior of *A. ervi* by inducing a significant increase of the oriented flights (Figure 4a, FmNi vs. NmNi, G test, $\chi2 = 8.985$ $p = .003$) and landings (Figure 4c, FmNi vs. NmNi, G test, $\chi2 = 6.17$ $p = .013$). Concerning the oriented flights of *A. ervi*, we also recorded a synergic effect of aphid infestation and mycorrhizal inoculation (Figure 4a, FmAp vs. FmNi, G test, $\chi2 = 4.855$ $p = .028$). Conversely, no effect was recorded for *A. colemani* due to AMF in terms of oriented flights (Figure 4b, FmNi vs. NmNi, G test, $\chi2 = 0.262$ $p = .609$) and landings (Figure 4d, FmNi vs. NmNi, G test, $\chi2 = 0.059$

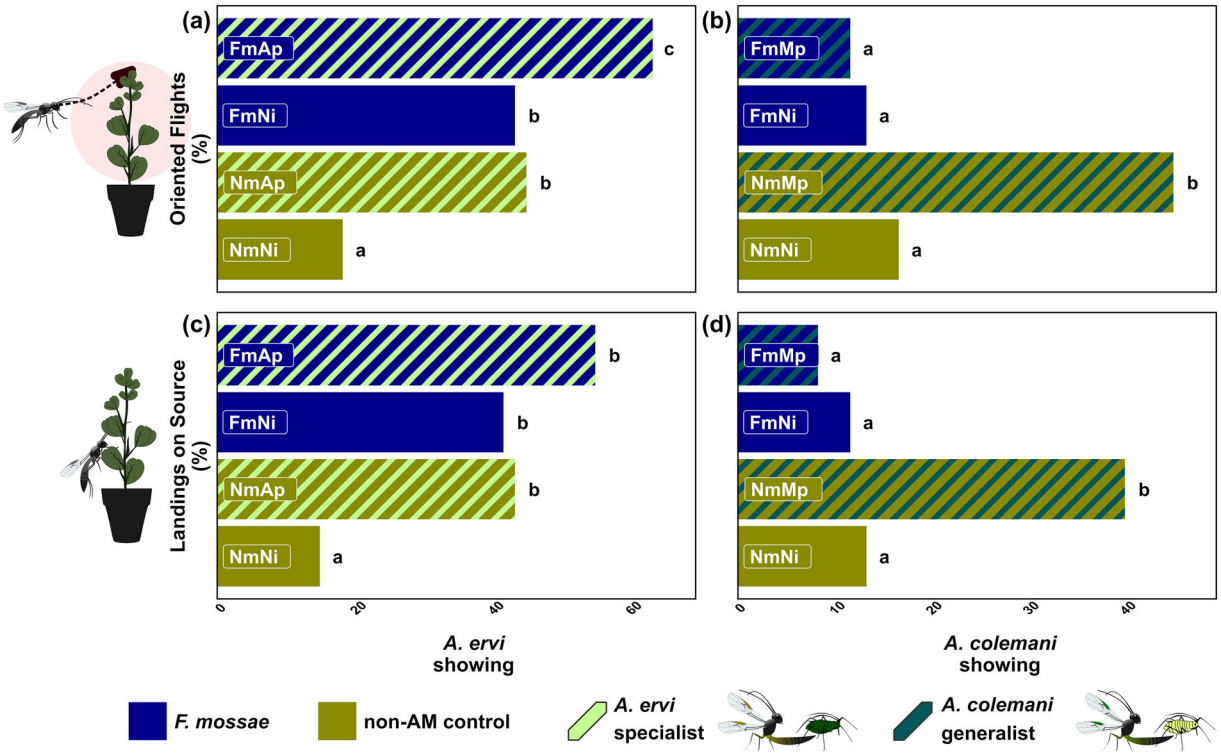

FIGURE 4 The effects of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* colonization and aphids *Acyrthosiphon pisum* and *Myzus persicae* infestations on relative parasitoids. Flight behavior (in terms of oriented flights - panels a and b - and landings on source - panels c and d - percentages towards *Vicia faba* plants) of the specialist parasitoid *Aphidius ervi* (panels a and c - lime green stripes pattern) and generalist parasitoid *Aphidius colemani* (panels b and d - sky blue stripes pattern). NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant. Different letters indicate significant differences between means (G-test, $p < .05$).

$p = .808$). For both parasitoids, a natural attraction towards plants infested by the relative host aphid was recorded in terms of oriented flights (Figure 4a, NmAp vs. NmNi, G test, $\chi2 = 10.094$ $p = .001$; Figure 4b, NmMp vs. NmNi, G test, $\chi2 = 11.619$ $p = .001$) and landings (Figure 4c, NmAp vs. NmNi, G test, $\chi2 = 6.749$ $p = .009$; Figure 4d, NmMp vs. NmNi, G test, $\chi2 = 6.685$ $p = .01$). The exception was for the plants infested by *M. persicae* and inoculated with *F. mosseae*, which resulted in a dramatic loss of attractiveness towards the parasitoid *A. colemani* (Figure 4d, FmMp vs. NmNi, G test, $\chi2 = 0.076$ $p = .782$).

3.3 | Mycorrhizal and aphids impact on photosynthesis and VOC emissions

In order to support the differential parasitoid behavior noted in the second experiment, on the same broad bean plant we collected and analyzed the photosynthetic parameters and VOC emitted for all treatments. We recorded a significative lowered net assimilation rate (A) (Kruskal–Wallis test, $\chi2 = 6.896$ $p = .032$) and photosystem II efficiency (PhiPSII) (Kruskal–Wallis test, $\chi2 = 6.062$ $p = .048$) physiological parameters due to aphids (Figure 5), in line with literature (Pincebourde & Ngao, 2021), while no effect was recorded on these parameters by mycorrhization and aphid feeding (Figure 5).

Regarding the volatilome, a total of 144 metabolites were collected and 70 were tentatively identified. Fourteen were significantly influenced by AMF, while aphid infestations changed the release rate of 80 metabolites with 18 differentially released by *A. pisum* and *M. persicae* infested plants (Table S2).

Two models were built on VOC data emitted by broad bean plants with partial least-squares-discriminant analysis (PLS-DA), using the treatments (NmAp, NmMp, NmNi) and (FmAp, FmMp, FmNi) as categorical variable (y) for the uninoculated control and mycorrhizal plants, respectively (Figure 6a,b), and VOC emission as independent variable (x). Each model explained more than 60% of the total variability and resulted in a clear separation between the treatments, with all components being significant (Figure 6a: p1-$R^2X = 0.524$, $R^2Y = 0.489$, $Q^2 = 0.388$; p2-$R^2X = 0.106$, $R^2Y = 0.487$, $Q^2 = 0.698$; Figure 6b: p1-$R^2X = 0.430$, $R^2Y = 0.494$, $Q^2 = 0.235$; p2-$R^2X = 0.254$, $R^2Y = 0.486$, $Q^2 = 0.917$).

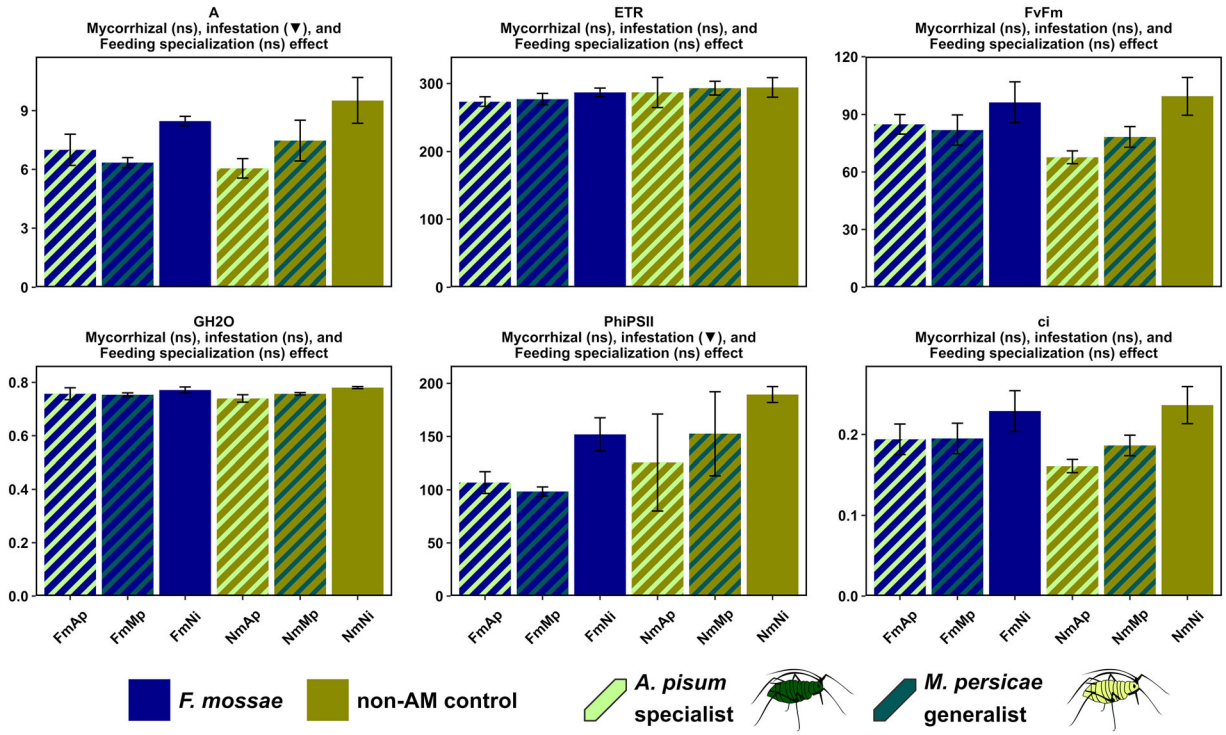

FIGURE 5 The effects of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* colonization and aphids *Acyrthosiphon pisum* and *Myzus persicae* infestations on photosynthetic parameters. Values of net assimilation rate (A), electron transport rate (ETR), the maximum quantum yield of photosystem II (Fv/Fm), stomatal conductance to water vapor (gH2O), photosystem II efficiency (PhiPSII) and intercellular CO_2 concentration (ci) and SE (error bars) are reported. The effect of AMF *F. mosseae*, aphid infestation, and feeding specialization (*A. pisum*/*M. persicae*) on the parameters are reported as subtitle (▼/▲ indicate significant lowering/increasing effects $p < .05$ Kruskal–Wallis or one way ANOVA). NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant.

3.4 | Mycorrhizal and aphids impact on the transcriptome

In order to support the differential parasitoid behavior noted in the second experiment and to evaluate the metabolic pathways involved in these multitrophic interactions, we used NmNi as a reference for NmAp and NmMp treatments, and FmNi as a reference for FmAp and FmMp treatments. As shown in Figure 7a, compared with NmNi, there were: 71% different DETs in NmAp treatment of which 2841 were upregulated and 1646 were downregulated, 13% DETs in NmMp treatment of which 359 were upregulated and 485 were downregulated, and 16% DETs shared by these treatments. Figure 7b shows, compared with FmNi, that there were: 68% DETs in FmAp treatment of which 4908 were upregulated and 5924 were downregulated, 6% DETs in FmMp treatment of which 764 were upregulated and 273 were downregulated, and 26% DETs were shared by these treatments.

In order to acquire a comprehensive insight into what pathways were influenced by *V. faba* treatments, DETs were annotated based on KEGG pathway enrichment via the ClusterProfiler package. A total of 120 pathways were enriched (Table S3). Among these enriched KEGG pathways, 27 were the most significant ones ($p \leq .01$) and are shown in Figure 8. The highest number of DETs (886, 592 upregulated and 294 downregulated) were enriched for FmAp, followed by DETs enriched by FmMp treatment (576, 308 upregulated and 268 downregulated), NmAp (238, 93 upregulated and 245 downregulated) and NmMp (264, 34 upregulated and 230 downregulated). Remarkable pathways are involved in VOC biosynthesis (phenylpropanoids, isoflavonoid, flavonoid biosynthesis; alpha-linolenic, linolenic acid metabolism, and phenylalanine metabolism) or in interaction with plant pathogen (Figure 8). All enriched DETs are mapped on the significative enriched metabolic pathways as shown in Figure S2–S28.

3.5 | Mycorrhizal impact on aphid parasitoids' fitness

To investigate whether the better performance of the specialist aphid *A. pisum* on mycorrhizal plants which was possibly linked to better plant quality, could also reflect on host quality for the development of the parasitoid *A. ervi*, we measured some morphological traits and development duration across one parasitoid generation (mother and F1 progeny). All considered morphological parameters, that is, head width (Welch Two Sample *t*-test, $t = -5.71$, $df = 87.92$,

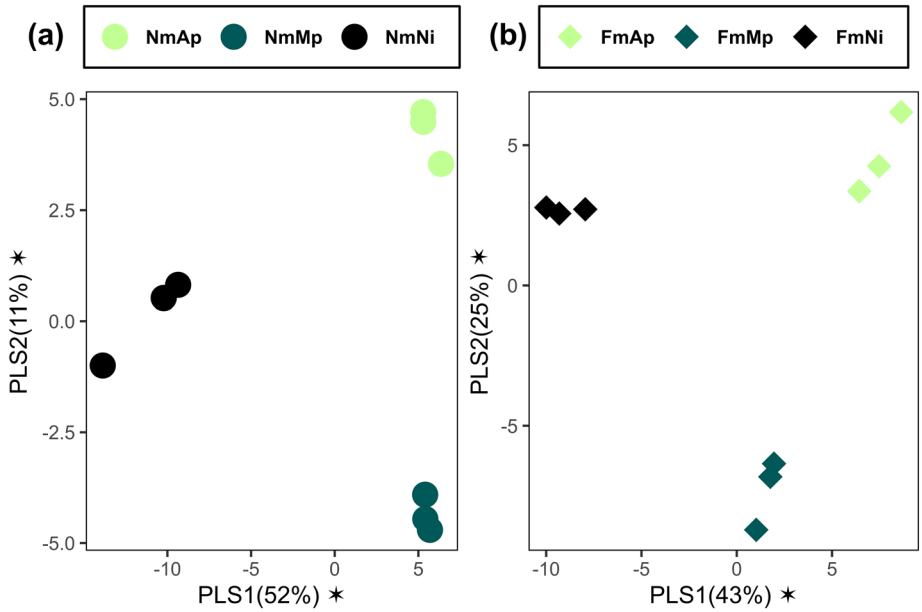

FIGURE 6 The effects of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* colonization and aphids *Acyrthosiphon pisum* and *Myzus persicae* infestations on volatile organic compounds (VOCs) emitted by plants. Partial least squares-discriminant analyses on VOC emitted by non-AM controls (a) and plants colonized by AMF (b) *Vicia faba* plants infested by specialist (*A. pisum*, lime green) and generalist (*M. persicae*, sky blue) aphids and relative un-infested controls (black). The asterisk indicates a significant component ($p < .05$). NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant.

$p < .001$), mid tibia length (Welch Two Sample *t*-test, $t = -3.53$, $df = 87.23$, $p < 0.001$), hind tibia length (Welch Two Sample *t*-test, $t = 2.44$, $df = 82.53$, $p = .017$), and the ratio head width:frontovertex width (Kruskal–Wallis $\chi^2 = 57.381$, $df = 1$, *p*-value < 0.001) significantly increased in the progeny (F1) from aphids reared on mycorrhizal plants (Figure 9). Conversely, no effect of *F. mosseae* was noted on the parasitoid development time.

4 | DISCUSSION

In this study, we examined the effect of AM symbiosis on two sap suckers, the aphids *A. pisum* and *M. persicae*, which are characterized by a thorough difference in plant preference. *A. pisum* is a clear specialist on Fabaceae while *M. persicae* is a wide generalist attacking hundreds of plant species across several plant families.

In line with what was generally observed for chewers in previous studies, we found that the AM symbiosis stimulated and improved the development and the reproduction of the specialist aphid, but was highly detrimental for the generalist one (Figures 2 and 3). In a similar multitrophic system including a Fabaceae plant, a specialist aphid, a mycorrhizal symbiont and a nitrogen-fixing bacteria, there was no effect of AM colonization on aphid development (Dabré et al., 2022). This apparent discrepancy could be partly due to the different mycorrhizal species used and the different protocols used for the assessment of aphid development. In fact, it has been shown that association with two different AMFs differently affects the water stress tolerance in tomatoes (Volpe et al., 2018). Moreover, the colonization of *Phleum pratense* by *F. mosseae* (or *R. irregularis*) had no effect on the performance of the specialist aphid *Rhopalosiphum padi* (Hempel et al., 2009) strengthening the hypothesis that there is a species-specificity regulating the final outcome of these complex interactions.

The AM symbiosis also had positive effects on the specific antagonist of the specialist aphid, the parasitoid *A. ervi* as shown in Figure 4, where a significant synergic effect of aphid infestation and AM colonization on the attraction of the aphid specialist parasitoid *A. ervi* was also recorded. This positive effect was likely regulated by the release of specific VOC (Table S2). As reported for other systems (Guerrieri et al., 2004), we hypothesized that the AM symbiosis mimicked the effect of aphid infestation in a broad bean plant making it as attractive as an infested one towards the parasitoid (Figure 4). However, only two tentatively identified compounds (2-methoxyphenol—guaiacol and 4-Methyl-1,3-heptadiene) were released at a significantly higher rate by mycorrhizal plants with respect to control ones (Table S2), and no indications about their effect on the foraging behavior of aphid parasitoids are available. Conversely, aphid infestation altered the release of 80 VOCs among which were 13 compounds differentially elicited by *A. pisum* that could play a role in the foraging behavior of *A. ervi* (terperns, monoterpens, sesquiterpens) (Table S2). The synergy may be attributed to the VOC emitted, as some are influenced solely by mycorrhizal colonization or aphid infestation. Additionally, certain emissions decrease in the presence of combined treatments and are eliminated in mycorrhizal and infested plants. One

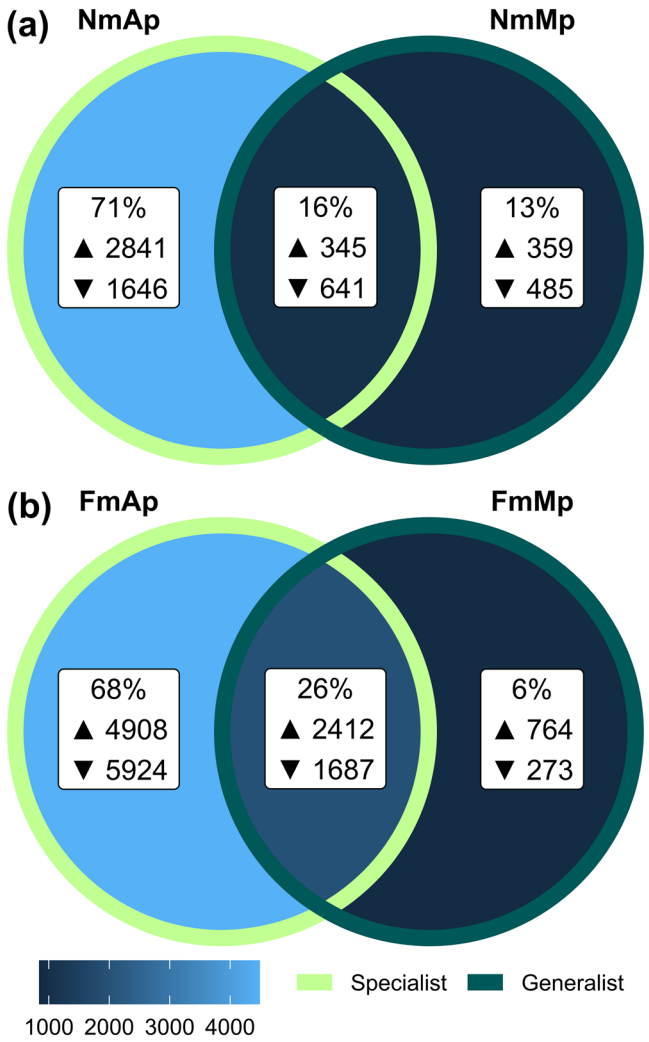

FIGURE 7 Overlap of differentially expressed transcripts (DETs) shared by plants infested by aphids *Acyrthosiphon pisum* and *Myzus persicae* and colonized by arbuscular mycorrhizal fungi (AMF) *Funneliformis mosseae*, and relative control. Venn diagrams of significant DETs among the uninoculated control (a) or mycorrhizal (b) *Vicia faba* plants infested by specialist (*A. pisum*, diagram border lime greed) or generalist (*M. persicae*, diagram border sky blue) aphids. The filled triangle refers to upregulation and an inverted filled triangle refers to downregulation. The overlapping sections of the diagrams represent the common DET between specialist and generalist aphids. NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant.

example is geranyl acetone, recognized as a repellent for aphids (Dardouri et al., 2019; Wróblewska-Kurdyk et al., 2022; Zhang et al., 2017), which potentially acts as a kairomone for *A. ervi*. Notably, FmNi and NmAp plants exhibited similar levels of geranyl acetone, while FmAp plants did not emit it at all. This, considering unidentified VOCs, could provide an explanation for the observed synergy. Furthermore, it is worth noting that the performance of *A. pisum* is enhanced on mycorrhizal plants, raising the possibility that the increased attractiveness to *A. ervi* is a result of a higher number of aphids. Here, we found that the specialist *A. pisum* was able to suppress plant defense by downregulating genes involved in the plant-pathogen interaction pathway (Figure S18), particularly those active against the aphids including PR1, Pti6, KCS1/10, and elf18 (Lewandowska et al., 2020; Prince et al., 2014; Sun et al., 2022; Zhang et al., 2020). Genes involved in the production of phenolic compounds were also downregulated by *A. pisum*, thereby hampering plant defenses against its infestation. Hence, *A. pisum is* confirmed to be highly adapted to its natural host plant being also able to exploit plant toxic compounds. For example, the leaves and the pods of *V. faba* plants contain high quantities of DihydrOxyPhenylAlanine (L-DOPA) (Burbano et al., 1995), which is detrimental for most generalist herbivores, while it is exploited by the pea aphid for wound healing and protection against UVA-radiation (Huang et al., 2011). However, *A. pisum*-infested plants release this same compound belowground alerting uninfested conspecific neighboring plants and making them more attractive to the aphid parasitoid *A. ervi* (Cascone et al., 2023).

The very opposite situation was recorded for the "generalist aphid" system where only aphid-infested plants were found to be attractive towards the aphid parasitoid *A. colemani*. Moreover, even though we cannot directly demonstrate a negative impact on a third trophic level, indirect evidence suggests that AM significantly impairs the fitness of *M. persicae* aphids to the extent that assessing the impact on the fitness of their parasitoid *A. colemani* becomes impractical due to the elevated mortality of aphids. Conversely, AM symbiosis in combination with *M. persicae* attack suppressed this attraction (Figure 4b,d). In support of these results was the characterization of VOC released by the tested plants analyzed by the PLS-DA. This analysis clearly separated the treatments indicating how the mycorrhizal symbiosis and aphid species differentially alter the release of VOC (Figure 6).

Volatilome data obtained in Experiment 2 also provided an explanation for the better performance of *A. pisum* on mycorrhizal plants (Experiment 1). For example, geranyl acetone, which is reported to be a powerful repellent for aphids including *A. pisum* (Zhang et al., 2017) is significative reduced upon *A. pisum* infestation (NmAp) with respect to control plants (NmNi) and a total depletion when aphid infestation was combined with *F. mossae* colonization (FmAp) (Table S2). Also, *A. pisum* infestation, in combination or not with mycorrhizal symbiosis, resulted in the upregulation of two enzymes, phospholipase A and A1, involved in the biosynthesis of linolenic acid (Figure S17) a precursor of several VOCs, including jasmonates and green life volatiles (Gosset et al., 2009), reported to play a crucial role in the foraging behavior of *A. ervi* (Sasso et al., 2009; Takemoto & Takabayashi, 2015) (Figure 4).

The positive effect of the AM symbiosis by *F. mosseae* on *A. ervi* performance extended to its fitness as shown in Figure 9, derived from Experiment 3. Larger dimensions, particularly for parasitoid species, are considered linked to better fitness and performance (Ellers & Jervis, 2003; Wang & Keller, 2020). Contrary to what was reported in the system composed of *Glycine max*, its specialist aphid (*Aphis glycines*), and the relative parasitoid (*Aphelinus certus*) (Dabré et al., 2022), we suppose an enhanced nutritional quality resulting

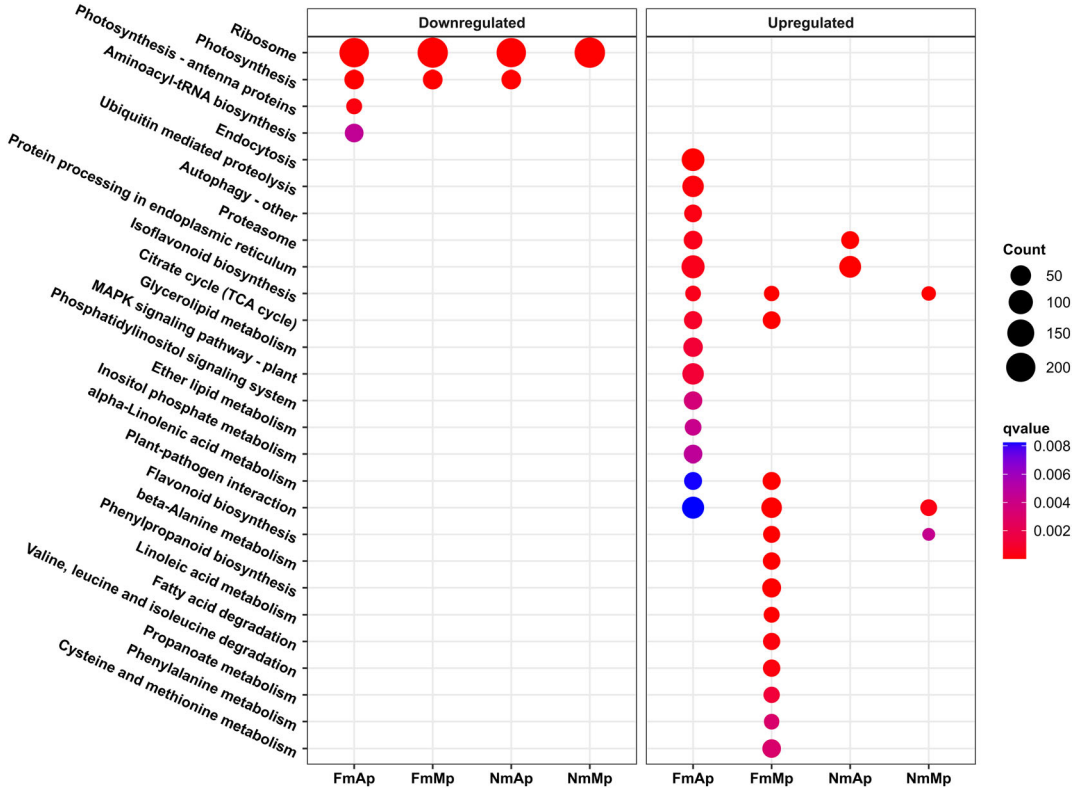

FIGURE 8 Functional annotation and KEGG enrichment analysis of significantly downregulated and upregulated expressed transcripts in plants colonized by arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae*, infested by aphids *Acyrthosiphon pisum* and *Myzus persicae* and relative control. The ordinate shows the pathway names. The following treatments were applied to *Vicia faba* plants: NmMp: non-AM control plant infested by *M. persicae*, NmAp: non-AM control plant infested by *A. pisum*; NmNi: non-AM control and un-infested plant, FmMp: mycorrhizal plant infested by *M. persicae*, FmAp: mycorrhizal plant infested by *A. pisum*, FmNi: mycorrhizal and un-infested plant, are reported in the abscissa. The size and the color of each point represent the number of genes enriched in each pathway and the q-value, respectively.

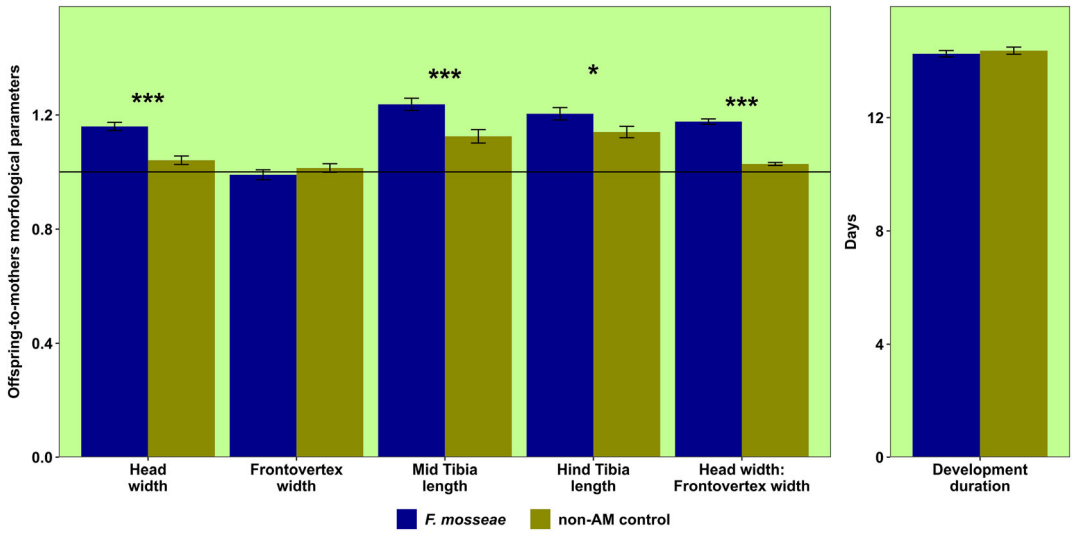

FIGURE 9 The effect of arbuscular mycorrhizal fungi (AMF) *Funneliformis mossae* on morphological parameters and development duration of the aphid parasitoid *Aphidius ervi*. Error bars represent the standard errors. Asterisks indicate a significant difference (Welch Two Sample or Kruskal–Wallis test, * $p < .05$; *** $p < .001$).

from the AM symbiosis leading to better development and reproductive response of the aphid *A. pisum*, passed on to the higher trophic level as shown by the larger dimensions of parasitoid progeny obtained by aphids reared on mycorrhizal plants in respect to those reared on control ones (Figure 9). As far as we know, this is the first study to investigate the impact of AM symbiosis on the fitness of a parasitoid.

There are interesting ecological considerations emerging from the results of this research. Plants are the sessile substrate for different organisms above and belowground that compete for the common source (i.e., carbon). In our experimental system comprising both the generalists, *F. mosseae* belowground and the aboveground *M. persicae*, it appears that the AM fungus clearly prevails over aphids by reducing dramatically the infestation ability of the aboveground competitor. This also reflects on the higher trophic level, the one represented by the parasitoid *A. colemani*, whose foraging activity is highly hampered by *F. mosseae* colonization even if in the presence of its host aphid. This phenomenon can be explained by a trade-off between direct and indirect defense in which the plants allocate more direct resources to fight against the aphid *M. persicae* instead of attracting the relative natural enemies by activating indirect defense (Ballhorn et al., 2008). Conversely, for the specialist species *A. pisum*, the AM symbiosis plays a significant role in boosting and enhancing the aphid performance. At this stage comes the plant response to herbivory that adds to that induced by the presence of the mycorrhizal symbiont both in terms of attractiveness towards the antagonist and of its fitness (as represented by the larger size).

Even if we focused on the bottom-up effects due to the AM symbiosis on aboveground interactions, we also had little evidence that aphid feeding did not influence the fitness of the AM fungi. Indeed, no significant differences in mycorrhizal colonization have been observed among FmNm (15.6 ± 1.0%), FmAp (21.0 ± 1.7%), and FmMp (17.4 ± 1.8%) treatments. This suggests that aphids did not affect AMF plant colonization, as previously reported in other experimental systems (Babikova et al., 2014; Cabral et al., 2018).

5 | CONCLUSION

The results of this study contribute to reinforcing the concept of the species-specificity of multitrophic (plant-herbivore-antagonist) and multilevel (belowground-aboveground) interactions. The final outcome of the involved species needs to be studied case by case. Nonetheless, their thorough characterization renders these interactions a source of powerful tools that can be potentially exploited for the sustainable protection of agricultural crops and forests. Furthermore, this work suggests the importance of investigating the physiological mechanisms (e.g., sodium and phosphorus uptake) influenced by mycorrhizae which lead to an improved nutritional status of plants and can be exploited in more sustainable agriculture. Moreover, the effect of multiple mycorrhizal fungal species (e.g., a mixed assemblage) needs also be studied next, as this would replicate a field/real-world scenario.

AUTHOR CONTRIBUTIONS

Emilio Guerrieri, Guido Lingua, Michelina Ruocco, and Pasquale Cascone designed the research, and Pasquale Cascone, Luigi Iodice, Liberata Gualtieri, Assunta Russo, Patrizia Cesaro, Maurilia Maria Monti, Nadia Massa, and Zekun Yang performed the research. Pasquale Cascone, Nadia Massa, Patrizia Cesaro, Emilio Guerrieri, and Guido Lingua interpreted the results. Pasquale Cascone, Guido Lingua, and Emilio Guerrieri wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

None declared.

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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REFERENCES

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*, 293–302. https://doi.org/10.1016/j.tplants.2012.02.006

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ [accessed 2021-07-30].

Babikova, Z., Gilbert, L., Bruce, T., Dewhirst, S. Y., Pickett, J. A., & Johnson, D. (2014). Arbuscular mycorrhizal fungi and aphids interact by changing host plant quality and volatile emission. *Functional Ecology*, *28*, 375–385. https://doi.org/10.1111/1365-2435.12181

Ballhorn, D. J., Kautz, S., Lion, U., & Heil, M. (2008). Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). *Journal of Ecology*, *96*, 971–980. https://doi.org/10.1111/j.1365-2745.2008.01404.x

Balog, A., Loxdale, H. D., Bálint, J., Benedek, K., Szabó, K.-A., Jánosi-Rancz, K.-T., & Domokos, E. (2017). The arbuscular mycorrhizal fungus *Rhizophagus irregularis* affects arthropod colonization on sweet pepper in both the field and greenhouse. *Journal of Pest Science*, *90*, 935–946. https://doi.org/10.1007/s10340-017-0844-1

Bennett, A. E., Millar, N. S., Gedrovics, E., & Karley, A. J. (2016). Plant and insect microbial symbionts alter the outcome of plant–herbivore–parasitoid interactions: Implications for invaded, agricultural and natural systems. *Journal of Ecology*, *104*, 1734–1744. https://doi.org/10.1111/1365-2745.12620

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*, *34*, 525–527. https://doi.org/10.1038/nbt.3519

Burbano, C., Cuadrado, C., Muzquiz, M., & Cubero, J. I. (1995). Variation of favism-inducing factors (vicine, convicine and L-DOPA) during pod development in *Vicia faba* L. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *47*, 265–275. https://doi.org/10.1007/BF01088335

Bushnell, B., Rood, J., & Singer, E. (2017). BBMerge—Accurate paired shotgun read merging via overlap. *PLoS ONE*, *12*, e0185056. https://doi.org/10.1371/journal.pone.0185056

Cabral, C., Wollenweber, B., António, C., Rodrigues, A. M., & Ravnskov, S. (2018). Aphid infestation in the phyllosphere affects primary metabolic profiles in the arbuscular mycorrhizal hyphosphere. *Scientific Reports*, *8*, 14442. https://doi.org/10.1038/s41598-018-32670-1

Cascone, P., Radkova, M., Arpaia, S., Errico, S., Lotz, L. A. P., Magarelli, R. A., Djilianov, D., & Guerrieri, E. (2018). Unintended effects of a Phytophtora-resistant cisgenic potato clone on the potato aphid *Macrosiphum euphorbiae* and its parasitoid *Aphidius ervi*. *Journal of Pest Science*, *91*, 565–574. https://doi.org/10.1007/s10340-017-0941-1

Cascone, P., Vuts, J., Birkett, M. A., Dewhirst, S., Rasmann, S., Pickett, J. A., & Guerrieri, E. (2023). L-DOPA functions as a plant pheromone for belowground anti-herbivory communication. *Ecology Letters*, *26*, 460–469. https://doi.org/10.1111/ele.14164

Cesaro, P., Massa, N., Cantamessa, S., Todeschini, V., Bona, E., Berta, G., Barbato, R., & Lingua, G. (2020). Tomato responses to *Funneliformis mosseae* during the early stages of arbuscular mycorrhizal symbiosis. *Mycorrhiza*, *30*, 601–610. https://doi.org/10.1007/s00572-020-00973-9

Cesaro, P., van Tuinen, D., Copetta, A., Chatagnier, O., Berta, G., Gianinazzi, S., & Lingua, G. (2008). Preferential colonization of *Solanum tuberosum* L. roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. *Applied and Environmental Microbiology*, *74*, 5776–5783. https://doi.org/10.1128/AEM.00719-08

Charters, M. D., Durant, E. K., Sait, S. M., & Field, K. J. (2022). Impacts of aphid herbivory on mycorrhizal growth responses across three cultivars of wheat. *Plants, People, Planet*, *4*, 655–666. https://doi.org/10.1002/ppp3.10302

Coppola, M., Cascone, P., Bossi, S., Corrado, G., Garonna, A. P., Maffei, M., Rao, R., & Guerrieri, E. (2018). TPS genes silencing alters constitutive indirect and direct defense in tomato. *International Journal of Molecular Sciences*, *19*, 2748. https://doi.org/10.3390/ijms19092748

Coppola, M., Cascone, P., Lelio, I. D., Woo, S. L., Lorito, M., Rao, R., Pennacchio, F., Guerrieri, E., & Digilio, M. C. (2019). *Trichoderma atroviride* P1 colonization of tomato plants enhances both direct and indirect defense barriers against insects. *Frontiers in Physiology*, *10*, 813. https://doi.org/10.3389/fphys.2019.00813

Coppola, M., Di Lelio, I., Romanelli, A., Gualtieri, L., Molisso, D., Ruocco, M., Avitabile, C., Natale, R., Cascone, P., Guerrieri, E., Pennacchio, F., & Rao, R. (2019). Tomato plants treated with systemin peptide show enhanced levels of direct and indirect defense associated with increased expression of defense-related genes. *Plants*, *8*, 8. https://doi.org/10.3390/plants8100395

Dabré, É. E., Hijri, M., & Favret, C. (2022). Influence on soybean aphid by the tripartite interaction between soybean, a rhizobium bacterium, and an arbuscular mycorrhizal fungus. *Microorganisms*, *10*, 1196. https://doi.org/10.3390/microorganisms10061196

Dardouri, T., Gomez, L., Schoeny, A., Costagliola, G., & Gautier, H. (2019). Behavioural response of green peach aphid *Myzus persicae* (Sulzer) to volatiles from different rosemary (*Rosmarinus officinalis* L.) clones. *Agricultural and Forest Entomology*, *21*, 336–345. https://doi.org/10.1111/afe.12336

Ellers, J., & Jervis, M. (2003). Body size and the timing of egg production in parasitoid wasps. *Oikos*, *102*, 164–172. https://doi.org/10.1034/j.1600-0706.2003.12285.x

Frew, A., Antunes, P. M., Cameron, D. D., Hartley, S. E., Johnson, S. N., Rillig, M. C., & Bennett, A. E. (2022). Plant herbivore protection by arbuscular mycorrhizas: A role for fungal diversity? *New Phytologist*, *233*, 1022–1031. https://doi.org/10.1111/nph.17781

Gange, A. C. (2001). Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist*, *150*, 611–618. https://doi.org/10.1046/j.1469-8137.2001.00137.x

Gange, A. C., Bower, E., & Brown, V. K. (1999). Positive effects of an arbuscular mycorrhizal fungus on aphid life history traits. *Oecologia*, *120*, 123–131. https://doi.org/10.1007/s004420050840

García-Garrido, J. M., & Ocampo, J. A. (2002). Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. *Journal of Experimental Botany*, *53*, 1377–1386. https://doi.org/10.1093/jxb/53.373.1377

Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Alaoui, A. T., & Gianinazzi, S. (1996). Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytologist*, *133*, 45–57. https://doi.org/10.1111/j.1469-8137.1996.tb04340.x

Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, *84*, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Gosset, V., Harmel, N., Göbel, C., Francis, F., Haubruge, E., Wathelet, J.-P., du Jardin, P., Feussner, I., & Fauconnier, M.-L. (2009). Attacks by a piercing-sucking insect (*Myzus persicae* Sultzer) or a chewing insect (*Leptinotarsa decemlineata* Say) on potato plants (*Solanum tuberosum* L.) induce differential changes in volatile compound release and oxylipin synthesis. *Journal of Experimental Botany*, *60*, 1231–1240. https://doi.org/10.1093/jxb/erp015

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, *29*, 644–652. https://doi.org/10.1038/nbt.1883

Guerrieri, E., Lingua, G., Digilio, M. C., Massa, N., & Berta, G. (2004). Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecological Entomology*, *29*, 753–756. https://doi.org/10.1111/j.0307-6946.2004.00644.x

Hempel, S., Stein, C., Unsicker, S. B., Renker, C., Auge, H., Weisser, W. W., & Buscot, F. (2009). Specific bottom–up effects of arbuscular mycorrhizal fungi across a plant–herbivore–parasitoid system. *Oecologia*, *160*, 267–277. https://doi.org/10.1007/s00442-009-1294-0

Huang, T., Jander, G., & De Vos, M. (2011). Non-protein amino acids in plant defense against insect herbivores: Representative cases and opportunities for further functional analysis. *Phytochemistry*, *72*, 1531–1537. https://doi.org/10.1016/j.phytochem.2011.03.019

Jung, S. C., Martinez-Medina, A., Lopez-Raez, J. A., & Pozo, M. J. (2012). Mycorrhiza-induced resistance and priming of plant defenses. *Journal of Chemical Ecology*, *38*, 651–664. https://doi.org/10.1007/s10886-012-0134-6

Koricheva, J., Gange, A. C., & Jones, T. (2009). Effects of mycorrhizal fungi on insect herbivores: A meta-analysis. *Ecology*, *90*, 2088–2097. https://doi.org/10.1890/08-1555.1

Lewandowska, M., Keyl, A., & Feussner, I. (2020). Wax biosynthesis in response to danger: Its regulation upon abiotic and biotic stress. *New Phytologist*, *227*, 698–713. https://doi.org/10.1111/nph.16571

Li, Y., Nan, Z., & Duan, T. (2019). *Rhizophagus intraradices* promotes alfalfa (*Medicago sativa*) defense against pea aphids (*Acyrthosiphon pisum*) revealed by RNA-Seq analysis. *Mycorrhiza*, *29*, 623–635. https://doi.org/10.1007/s00572-019-00915-0

Manni, M., Berkeley, M. R., Seppey, M., Simão, F. A., & Zdobnov, E. M. (2021). BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Molecular Biology and Evolution*, *38*, 4647–4654. https://doi.org/10.1093/molbev/msab199

Maurya, A. K., Kelly, M. P., Mahaney, S. M., & Gomez, S. K. (2018). Arbuscular mycorrhizal symbiosis alters plant gene expression and aphid weight in a tripartite interaction. *Journal of Plant Interactions*, *13*, 294–305. https://doi.org/10.1080/17429145.2018.1475020

Peña, E. A., & Slate, E. H. (2006). Global validation of linear model assumptions. *Journal of the American Statistical Association*, *101*, 341–354. https://doi.org/10.1198/016214505000000637

Pincebourde, S., & Ngao, J. (2021). The impact of phloem feeding insects on leaf ecophysiology varies with leaf age. *Frontiers in Plant Science*, *12*, 625689. https://doi.org/10.3389/fpls.2021.625689

Prince, D. C., Drurey, C., Zipfel, C., & Hogenhout, S. A. (2014). The leucine-rich repeat receptor-like kinase BRASSINOSTEROID INSENSITIVE1-ASSOCIATED KINASE1 and the cytochrome p450 PHYTOALEXIN DEFICIENT3 contribute to innate immunity to aphids in arabidopsis. *Plant Physiology*, *164*, 2207–2219. https://doi.org/10.1104/pp.114.235598

Robertson, G., Schein, J., Chiu, R., Corbett, R., Field, M., Jackman, S. D., Mungall, K., Lee, S., Okada, H. M., Qian, J. Q., Griffith, M., Raymond, A., Thiessen, N., Cezard, T., Butterfield, Y. S., Newsome, R., Chan, S. K., She, R., Varhol, R., … Birol, I. (2010). De novo assembly and analysis of RNA-seq data. *Nature Methods*, *7*, 909–912. https://doi.org/10.1038/nmeth.1517

Sasso, R., Iodice, L., Woodcock, C. M., Pickett, J. A., & Guerrieri, E. (2009). Electrophysiological and behavioural responses of *Aphidius ervi* (Hymenoptera: Braconidae) to tomato plant volatiles. *Chemoecology*, *19*, 195–201. https://doi.org/10.1007/s00049-009-0023-9

Simon, A. L., Wellham, P. A. D., Aradottir, G. I., & Gange, A. C. (2017). Unravelling mycorrhiza-induced wheat susceptibility to the English grain aphid *Sitobion avenae*. *Scientific Reports*, *7*, 46497. https://doi.org/10.1038/srep46497

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis*. Academic Press.

Stallmann, J., & Schweiger, R. (2021). Effects of arbuscular mycorrhiza on primary metabolites in phloem exudates of *Plantago major* and *Poa annua* and on a generalist aphid. *International Journal of Molecular Sciences*, *22*, 13086. https://doi.org/10.3390/ijms222313086

Sun, M., Qiu, L., Liu, Y., Zhang, H., Zhang, Y., Qin, Y., Mao, Y., Zhou, M., du, X., Qin, Z., & Dai, S. (2022). Pto interaction proteins: Critical regulators in plant development and stress response. *Frontiers in Plant Science*, *13*, 774229. https://doi.org/10.3389/fpls.2022.774229

Sun, R., Jiang, X., Reichelt, M., Gershenzon, J., & Vassão, D. G. (2021). The selective sequestration of glucosinolates by the cabbage aphid severely impacts a predatory lacewing. *Journal of Pest Science*, *94*, 1147–1160. https://doi.org/10.1007/s10340-020-01319-2

Takemoto, H., & Takabayashi, J. (2015). Parasitic wasps *Aphidius ervi* are more attracted to a blend of host-induced plant volatiles than to the independent compounds. *Journal of Chemical Ecology*, *41*, 801–807. https://doi.org/10.1007/s10886-015-0615-5

Tarazona, S., Furió-Tarí, P., Turrà, D., Pietro, A. D., Nueda, M. J., Ferrer, A., & Conesa, A. (2015). Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. *Nucleic Acids Research*, *43*, e140.

Thévenot, E. A., Roux, A., Xu, Y., Ezan, E., & Junot, C. (2015). Analysis of the human adult urinary metabolome variations with age, body mass index, and gender by implementing a comprehensive workflow for univariate and OPLS statistical analyses. *Journal of Proteome Research*, *14*, 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354

Tomczak, V. V., & Müller, C. (2017). Influence of arbuscular mycorrhizal stage and plant age on the performance of a generalist aphid. *Journal of Insect Physiology*, *98*, 258–266. https://doi.org/10.1016/j.jinsphys.2017.01.016

Trotta, A., Varese, G. C., Gnavi, E., Fusconi, A., Sampò, S., & Berta, G. (1996). Interactions between the soilborne root pathogen *Phytophthora nicotianae* var.parasitica and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant and Soil*, *185*, 199–209. https://doi.org/10.1007/BF02257525

van Emden, H., & Harrington, R. (2007). In H. F. van Emden & R. Harrington (Eds.), *Aphids as crop pests*. CABI.

Volpe, V., Chitarra, W., Cascone, P., Volpe, M. G., Bartolini, P., Moneti, G., Pieraccini, G., di Serio, C., Maserti, B., Guerrieri, E., & Balestrini, R. (2018). The association with two different arbuscular mycorrhizal fungi differently affects water stress tolerance in tomato. *Frontiers in Plant Science*, *9*, 1480. https://doi.org/10.3389/fpls.2018.01480

Wang, T., & Keller, M. A. (2020). Larger is better in the parasitoid *Eretmocerus warrae* (Hymenoptera: Aphelinidae). *Insects*, *11*, 39. https://doi.org/10.3390/insects11010039

Wang, Y., Li, Y., & Duan, T. (2023). Arbuscular mycorrhizal fungus changes alfalfa response to pathogen infection activated by pea aphid infestation. *Frontiers in Microbiology*, *13*, 1074592. https://doi.org/10.3389/fmicb.2022.1074592

Wróblewska-Kurdyk, A., Dancewicz, K., Gliszczyńska, A., & Gabryś, B. (2022). Antifeedant potential of geranylacetone and nerylacetone and their epoxy-derivatives against *Myzus persicae* (Sulz.). *Molecules*, *27*, 8871. https://doi.org/10.3390/molecules27248871

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovations*, *2*, 100141. https://doi.org/10.1016/j.xinn.2021.100141

Yu, L., Zhang, W., Geng, Y., Liu, K., & Shao, X. (2022). Cooperation with arbuscular mycorrhizal fungi increases plant nutrient uptake and improves defenses against insects. *Frontiers in Ecology and Evolution*, *10*, 833389. https://doi.org/10.3389/fevo.2022.833389

Zeng, T., Holmer, R., Hontelez, J., te Lintel-Hekkert, B., Marufu, L., de Zeeuw, T., Wu, F., Schijlen, E., Bisseling, T., & Limpens, E. (2018). Host- and stage-dependent secretome of the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *The Plant Journal*, *94*, 411–425. https://doi.org/10.1111/tpj.13908

Zhang, R., Wang, B., Grossi, G., Falabella, P., Liu, Y., Yan, S., Lu, J., Xi, J., & Wang, G. (2017). Molecular basis of alarm pheromone detection in aphids. *Current Biology*, *27*, 55–61. https://doi.org/10.1016/j.cub.2016.10.013

Zhang, Y., Fu, Y., Wang, Q., Liu, X., Li, Q., & Chen, J. (2020). Transcriptome analysis reveals rapid defence responses in wheat induced by phytotoxic aphid *Schizaphis graminum* feeding. *BMC Genomics*, *21*, 339. https://doi.org/10.1186/s12864-020-6743-5

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