



**Arbuscular mycorrhizal fungi provides enhanced development and reduced mite incidence in *Phaseolus vulgaris* L. by direct root colonization and via the common mycorrhizal network**

**Fungos micorrízicos arbusculares proporciona maior desenvolvimento e reduz a incidência de ácaros em *Phaseolus vulgaris* L. pela colonização direta das raízes e via rede micorrízica comum**

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#### **ABSTRACT**

Arbuscular mycorrhizal fungi (AMF) are important to agriculture due to positive effects on development and resistance to attack by herbivores in agricultural crops. AMF can affect a single plant by directly colonizing its roots and two or more plants through the common mycorrhizal network (CMN). This work aimed to analyze the influence of AMF on the development of *Phaseolus vulgaris* and on the incidence of the mite *Tetranychus urticae* in bean plants colonized directly by mycorrhizal propagules or linked by the CMN. The root systems of two plants were either separated by an impassable barrier so that one or both plants could be colonized by the mycorrhizal propagules without forming the RMC, or they were separated by a steel mesh that allowed the outer hyphae of the AMF on the roots of one of the plants to colonize the non-mycorrhizal roots of the other plant and form the RMC. The mite infestation occurred first on one plant and at the end of the experiment on all. Percentage of mycorrhizal colonization, dry mass of aerial part and roots, number of reproductive structures and mite incidence were analyzed. The colonization by the mycorrhizal propagules and the hyphae of the MRC positively influenced the vegetative and reproductive development of *P. vulgaris* and resulted in lower mite incidence on the leaflets of the plants. Therefore, direct colonization of *P. vulgaris* roots by AMFs and via CMR improves plant development and resistance of bean plants to *T. urticae* mite infestation.

**Keywords:** herbivory, arbuscular mycorrhiza, plant defense mechanism, priming effect.

#### **RESUMO**

Os fungos micorrízicos arbusculares (FMA) são importantes para a agricultura pelos efeitos positivos no desenvolvimento e na resistência ao ataque por herbívoros em culturas agrícolas. Os FMA podem afetar uma única planta ao colonizar diretamente suas raízes e também duas ou mais plantas por meio da rede micorrízica comum (RMC). Este trabalho teve o objetivo de analisar a influência dos FMA no desenvolvimento de *Phaseolus vulgaris* e na incidência do ácaro *Tetranychus urticae* em feijoeiros colonizados diretamente pelos propágulos micorrízicos ou interligados pela RMC. Os sistemas radiculares de duas plantas estavam separados por uma barreira intransponível para que uma ou as duas plantas pudessem ser colonizadas pelos propágulos micorrízicos sem a formação da RMC, ou estavam separados por uma malha de aço que permitia que

as hifas externas dos FMA nas raízes de uma das plantas pudessem colonizar as raízes não micorrizadas da outra planta e formar a RMC. A infestação pelo ácaro ocorreu primeiro em uma planta e ao final do experimento em todas. Foram analisados porcentagem de colonização micorrízica, massa seca da parte aérea e das raízes, número de estruturas reprodutivas e incidência dos ácaros. A colonização pelos propágulos micorrízicos e pelas hifas da RMC influenciou positivamente o desenvolvimento vegetativo e reprodutivo de *P. vulgaris* e resultou em menor incidência de ácaros nos folíolos das plantas. Portanto, a colonização direta das raízes de *P. vulgaris* pelos FMA e via RMC melhora o desenvolvimento das plantas e a resistência dos feijoeiros à infestação pelo ácaro *T. urticae*.

**Palavras-chave:** herbívoros, micorriza arbuscular, mecanismo de defesa vegetal, efeito priming.

## 1 INTRODUCTION

Arbuscular mycorrhizal (AM) symbiosis occurs in the roots of over 65% of terrestrial plant species (Bücking et al., 2016), including important agricultural crops (Verbruggen et al., 2013), such as common bean (*Phaseolus vulgaris* L.), maize (*Zea mays* L.), and soybean (*Glycine max* L.) (Naher et al., 2013). Arbuscular mycorrhizal fungi (AMF) can influence the development of colonized plants through their positive effects on host nutrition and resistance to biotic and abiotic stresses (Lekberg & Koide, 2005).

The acquisition of mineral nutrients via AM has a positive impact on the vegetative (shoot and root) and reproductive development of a lot of ecologically and agriculturally important plant species (Rondina et al., 2014; Chandrasekaran, 2020). In mycorrhizal roots, fungal external hyphae extend from the root epidermis, absorbing and transferring nutrients from the soil to the roots that would not be available to the plant without AMF colonization (Leake et al., 2004).

The external mycelium of AMF absorbs from the soil and transfers to its host, mainly nitrogen (Govindarajulu et al., 2005) and phosphorus (Chandrasekaran, 2020). In exchange, the plant supplies the AMF with carbon, which is used to form and maintain the extensive external mycelium in the soil and the mycorrhizal structures present in the root cortex (Smith & Read, 2008).

AM can positively influence plant defense mechanisms against herbivore attacks, such as aphids, caterpillars, and mites (Cameron et al., 2013; Shrivastava et al., 2015, Sharma et al., 2017). In direct defense, mycorrhizal colonization increases the concentration or induces the production of repellent volatile compounds released by plants and of secondary metabolites in the plant tissue after herbivore attack (Shrivastava et al., 2015; Meier & Hunter, 2019).

In indirect plant defense, colonization by AMF can alter the chemical composition of the volatile organic compounds emitted by leaves and increase the attractiveness to the natural enemies of herbivores. Schausberger et al. (2012) demonstrated that a greater number of individuals of the predatory mite *Phytoseiulus persimilis* were attracted to leaves of mycorrhizal *P. vulgaris* plants than to leaves taken from non-mycorrhizal plants.

Root colonization by AMF can occur from different inoculate present in the soil, including mycelium, spores, and fragments of mycorrhizal roots (Klironomos & Hart 2002; Leake et al., 2004). Spores and fragments of mycorrhizal roots are considered the most important mycorrhizal inoculate for persistence in the soil in the absence of roots and for the establishment of new symbiotic relationships (Smith & Read, 2008; Hage-Ahmed et al., 2018).

However, research has shown that in many habitats and for certain FMA species, the mycelium is a mycorrhizal propagule as efficient as spores in colonizing roots (Brundett et al., 1999; Klironomos & Hart, 2002). Different agricultural practices can negatively affect mycelial viability and growth in soil, thus reducing the mycorrhizal potential of the soil (Novais et al., 2019).

The lack of specificity of FMA in relation to hosts allows their external hyphae to simultaneously colonize the root system of two or more plants of the same or different species, forming a common mycorrhizal network (CMN) in the soil (Bücking et al., 2016; Gilbert & Johnson 2015). The CMN can influence the acquisition of nutritional resources by mycorrhizal plants (Selosse et al., 2006), due to the ability of the hyphae to transfer mineral nutrients (nitrogen and phosphorus), carbon (He et al., 2009; Ren et al., 2013), and water between interconnected plants (Egerton-Warburton et al., 2007).

The RMC formed by FMA has been shown to improve plant defense responses (Johnson & Gilbert 2015). The RMC's hyphae transfer signaling molecules synthesized by a plant after infection by phytopathogenic fungi (Song et al., 2010; Alaux et al., 2020) or herbivore infestation (Babikova et al., 2013; Song et al., 2019). These signaling molecules induce the expression of defense genes (Song et al., 2010; Song et al., 2019; Alaux et al., 2020) and the emission of repellent volatile compounds (Schausberger et al., 2012; Sharma et al., 2017) in the healthy receptor plant, even before contact with the pathogen or pest organism.

The initial objective of this study was to evaluate the effect of mycorrhizal colonization on the vegetative and reproductive development of *Phaseolus vulgaris* under two conditions: plants interconnected by the RMC and plants colonized by FMA without the formation of the mycorrhizal network. However, during the experiment, one of the plants was unintentionally infested by the cosmopolitan and pesticide-resistant agricultural pest *Tetranychus urticae* Koch.

Considering that AM symbiosis can affect plant defense mechanisms against herbivores that attack the aerial part (Hoffmann et al., 2011; Schausberger et al., 2012), in this study we chose not to interfere with the infestation of bean plants by the mite and to analyze its incidence in plants interconnected by the RMC, in mycorrhizal plants without RMC formation, and in non-colonized plants by AM fungi.

## 2 MATERIALS AND METHODS

The experiment was conducted in a greenhouse without temperature and light control, at the Luiz Meneghel campus of the State University of Northern Paraná (UENP-CLM) in Bandeirantes, Paraná, Brazil.

### 2.1 SOIL COLLECTION AND PREPARATION

The soil used for plant growth is classified as a typical Eutroferic Red Latosol, with a clay texture (Embrapa, 2006), collected within the UENP-CLM campus (S 23° 06' 24.7", W 50° 21' 37.3", altitude of 439 m). The vegetation in the collection area is dominated by three tree species: *Delonix regia* (Hook.) Raf. (flamboyant), *Caesalpinia*



*pluviosa* DC. (sibipiruna) and *Mangifera indica* L. (mango), and no management has been carried out in the area for the past six years. Soil samples were collected at two different times, each time from five points that were 10 m apart, at a depth of 0-10 cm. In both collections, the five sub-samples were mixed, homogenized and sieved through a 4 mm mesh.

The first soil sample collected was subjected to a heat treatment to inactivate viable FMA propagules before being placed in the pots. The second soil sample was not altered after collection. To inactivate viable mycorrhizal propagules, the soil was heated to 90°C for one hour in flowing steam. This procedure was repeated three times with 24-hour intervals between the heatings. To restore the microbial community, except for FMA, in the heated soil, 100 ml of a filtrate from the unaltered soil, which did not contain mycorrhizal propagules, was added after plant emergence. To prepare the filtrate, 500 grams of soil were suspended in two liters of water and the supernatant was sieved through a 53 µm mesh.

## 2.2 ASSEMBLING THE POTS AND DESCRIPTION OF TREATMENTS

Twenty rectangular pots were divided into two equal compartments, named C1 and C2. In each pot, two *P. vulgaris* plants grew, one in each compartment, whose root systems were separated by a barrier. In ten pots, the barrier that separated C1 and C2 was a stainless-steel mesh with pores of 25 µm diameter that allowed FMA hyphae but not roots to pass through it. In ten more pots, an impenetrable barrier for both roots and FMA hyphae was used to separate the root systems of the two plants.

The compartments were filled with four liters of soil containing viable FMA propagules or that had been inactivated by the heat treatment. Five beans were placed to germinate in each compartment, whose surface had been disinfected in 1% sodium hypochlorite for 10 minutes and then washed in sterile distilled water. Five days after the emergence of the seedlings, thinning was performed, leaving one plant per compartment. The maximum and minimum distances between the stems of the two plants in the same pot were 10.9 cm and 7.8 cm, respectively.



Four treatments were developed and named as follows: 1) C1-MA/C2-MA: both compartments were filled with soil without viable mycorrhizal propagules (-MA) and were separated by the stainless-steel mesh. In this treatment, throughout the plant growth period, there was no mycorrhizal colonization in their roots to enable the comparison between plants under mycorrhizal conditions (+MA and +RMC) and non-mycorrhizal conditions; 2) C1+MA/C2+MA: both compartments were filled with soil containing viable mycorrhizal propagules (+MA). Both were separated by an impenetrable barrier to prevent the mycorrhizal roots of the plants in C1 and C2 from being interconnected by FMA hyphae and forming an RMC. 3) C1+MA/C2-MA: compartment C1 was filled with soil containing viable FMA propagules, and compartment C2 had non-viable mycorrhizal propagules and both were separated by an impenetrable barrier; 4) C1+MA/C2+RMC: compartment C1 had viable mycorrhizal propagules, and compartment C2 contained non-viable propagules. Both were separated by the stainless-steel mesh to allow the hyphae that grew in the soil from the mycorrhizal roots of the plant in C1 to colonize the non-mycorrhizal root system of the plant in the other compartment (in a local without viable mycorrhizal propagules) and form the RMC (+RMC). Each treatment consisted of five pots.

### 2.3 MITE INFESTATION OF PLANTS

Twenty-three days after the emergence of *P. vulgaris* seedlings, the presence of *T. urticae* mites was observed on the adaxial and abaxial surfaces of five leaflets of a plant in the C1(+MA)/C2(+MA) treatment. At this point, the presence of the mite and signs of infestation (white-silver, reddish, and bronzed-colored spots, chlorotic or necrotic areas) were not observed in the other plants of the experiment, after careful inspection of both leaf surfaces of the bean plants in all treatments.

During the experiment, infestation in the other bean plants occurred naturally, and seven days after the first plant was infested, the presence of mites was observed on the leaflets of at least one plant in each of the treatments. At the end of the experiment, *T. urticae* mites were present or signs of their presence were observed in all the plants of the experiment. However, at the end of the experiment, the number of leaflets in which the

mite was or had been present differed among the treatments. Therefore, the incidence of mites in the plants among the four treatments was analyzed and compared, as described in the section below.

#### 2.4 ANALYSIS OF THE PLANTS

After 60 days of growth, the number of reproductive structures (flower buds, flowers, and pods) and the incidence of mites were determined, and then the plants were removed from the pots. The aboveground and root systems were separated and kept in an oven at 60°C to determine dry masses. The incidence of mites (IM) was determined by calculating the percentage of leaflets where the mite was present or showed signs caused by its presence (Nishida et al., 2010):

$$IM = \frac{(\text{number of leaves with mite signs or with mites})}{(\text{total number of leaves})} \times 100$$

The presence and signs (white-silvery, reddish and bronzed-colored spots, chlorotic or necrotic areas) of mite attack were analyzed on both sides of the leaflets. A fresh root sample was collected from the root system of each plant for determination of mycorrhizal colonization. For this procedure, the AMF structures in the roots were stained according to the methodology described by Phillips & Hayman (1970): clarification (KOH 10%), acidification (HCl 1%), and staining of fungal structures (0.05% Trypan Blue). The percentage of mycorrhizal colonization was determined by the intersection method of quadrants (Mcgonigle et al., 1990) on 100 root fragments, where the presence of cenocytic hyphae, arbuscules, and vesicles in the cortex was analyzed at a magnification of 100×.

#### 2.5 STATISTICAL ANALYSIS

The normality and homoscedasticity of the data were assessed using the Shapiro-Wilk and Levene tests, respectively. The percentage values of mycorrhizal colonization and mite incidence in the plants were transformed to arcsine ( $x/100$ ) prior to statistical

analysis. The means of shoot and root dry masses, plant height, number of reproductive structures, mycorrhizal colonization percentage, and mite incidence in the plants were compared among the four treatments using one-way ANOVA, followed by Tukey's post hoc test. Pearson's correlation analysis was performed between the mycorrhizal colonization percentage and the values of shoot dry mass, root dry mass, and number of reproductive structures per plant. In all statistical analyses,  $p < 0.05$  was considered significant (95% significance level). The statistical analyses were performed using the statistical software Past version 4.03.

### 3 RESULTS AND DISCUSSION

As shown in Table 1, mycorrhizal colonization (MC), shoot dry mass (SDM), root dry mass (RDM), and number of reproductive structures (NRS) per plant differed significantly among plants that were colonized from active mycorrhizal propagules in the soil (+MA), plants colonized by hyphae that formed the common mycorrhizal network (+RMC), and plants not colonized by AMF (-MA). In treatment C1+MA/C2+RMC, the presence of AMF structures on the roots of compartment C2 indicates that in these plants, mycorrhizal colonization started from the external hyphae of AMF present on the roots of the plant in compartment C1 and led to the formation of RMC.

In this treatment, the percentage (57%) of colonization on the roots of plants in compartment 2 was significantly lower than the percentage (88% to 97%) of colonization of plants colonized from viable AMF propagules in the soil (+MA). In both compartments of treatment C1-MA/C2-MA and in compartment C2 of treatment C1+MA/C2-MA, which were filled with soil containing inactivated mycorrhizal propagules, the presence of AMF structures on the roots was not observed.

All plants colonized by FMA (+MA and +RMC) had significantly higher dry shoot mass (1.04 g to 6.79 g) and root mass (0.54 g to 1.69 g) than non-colonized plants (MSPA = 0.19 g to 0.39 g and MSR = 0.07 g to 0.20 g). In the absence of viable mycorrhizal propagules in the soil, in the C1-MA/C2-MA treatment, plants did not produce reproductive structures, and in the C1+MA/C2-MA treatment, the total number

of flower buds, flowers, and pods per plant (2.2) in compartment C2 was significantly lower than in mycorrhizal plants in this (14.2) and other treatments (6.0 to 13.8).

Dry shoot mass ( $r = 0.70$ ;  $p < 0.0001$ ;  $n = 30$ ), root mass ( $r = 0.82$ ;  $p < 0.0001$ ;  $n = 30$ ), and number of reproductive structures per plant ( $r = 0.45$ ;  $p < 0.0216$ ;  $n = 30$ ) correlated positively with the percentage of mycorrhizal colonization.

Table 1. Mean and standard deviation of percentage of mycorrhizal colonization, dry shoot mass, dry root mass, and number of reproductive structures per plant of *P. vulgaris* plants grown in pots divided into compartment 1 and compartment 2, separated by steel mesh or impermeable barrier and colonized or not by arbuscular mycorrhizal fungi.

Treatments	MC (%)	SDM (g)	RDM (g)	NRS
C1-MA	Absent	0.21±0.12d	0.07±0.05d	Absent
C2-MA	Absent	0.19±0.08d	0.10±0.04d	Absent
C1+MA	88.0±0.14a	3.89±1.74b	0.94±0.52bc	11.50±5.80a
C2+MA	97.0±0.03a	4.89±0.91ab	1.69±0.36a	8.20±4.00a
C1+MA	96.0±0.01a	6.25±2.38a	1.19±0.14ab	14.20±6.80a
C2-MA	Absent	0.39±0.07d	0.20±0.09d	2.20±1.60b
C1+MA	94.0±0.06a	6.79±1.53a	1.42±0.44ab	0.54±0.17c
C2+RMC	57.0±0.03b	1.04±0.57c	13.8±6.10a	6.00±4.00a

MC: mycorrhizal colonization. SDM: shoot dry mass. RDM: root dry mass. NRS: number of reproductive structures. C1: compartment 1. C2: compartment 2. MA-: soil without viable mycorrhizal propagules. MA+: soil with viable mycorrhizal propagules. RMC+: common mycorrhizal network formation. Means followed by different letters in the column are significantly different (Tukey's test,  $p \leq 0.05$ ).

These results demonstrate that colonization of roots from viable mycorrhizal propagules or by the hyphae that formed the RMC positively influenced the vegetative and reproductive development of plants, and this benefit was more significant in the former condition. In various plant species, the main benefit of colonization by AMF is the increase in nutrient uptake and biomass production of shoots and roots (Smith & Read 2008; Chandrasekaran, 2020).

In this study, the strong positive and significant relationship between the percentage of mycorrhizal colonization and the values of SDM ( $r = 0.70$ ) and RDM ( $r = 0.82$ ) indicate that the higher intensity of colonization (88% to 97%) of roots from viable mycorrhizal propagules present in the soil allowed the plants to obtain the necessary nutrients to produce a greater amount of aboveground and root biomass (Lu & Koide 1994, Rondina et al., 2014).



The lower vegetative and reproductive development of plants colonized by the RMC, compared to those colonized by viable mycorrhizal propagules, can be explained by the later and less intense mycorrhizal colonization (57%). The colonization of roots in compartment C2 by the RMC only occurred after the AM symbiosis had been established in the cortex of the roots in compartment C1, so that the external AM fungal hyphae could grow and colonize the roots of plants in compartment C2, where there were no viable mycorrhizal propagules, forming the RMC. During the period between seedling establishment and root colonization in compartment C2, the vegetative development of *P. vulgaris* depended on nutrients absorbed only by the roots (Smith & Read 2008; Rondina et al., 2014).

The results demonstrate that plants colonized by RMC hyphae showed higher production of vegetative biomass and number of reproductive structures compared to non-mycorrhizal plants. Therefore, the external mycelium that grew from the plant roots in compartment C1 and colonized the roots in C2 was important for the development of *P. vulgaris*. FMA can use three types of propagules to colonize plant roots (Hart & Reader, 2001; Klironomos & Hart, 2002): spores, colonized root fragments, and external FMA mycelium.

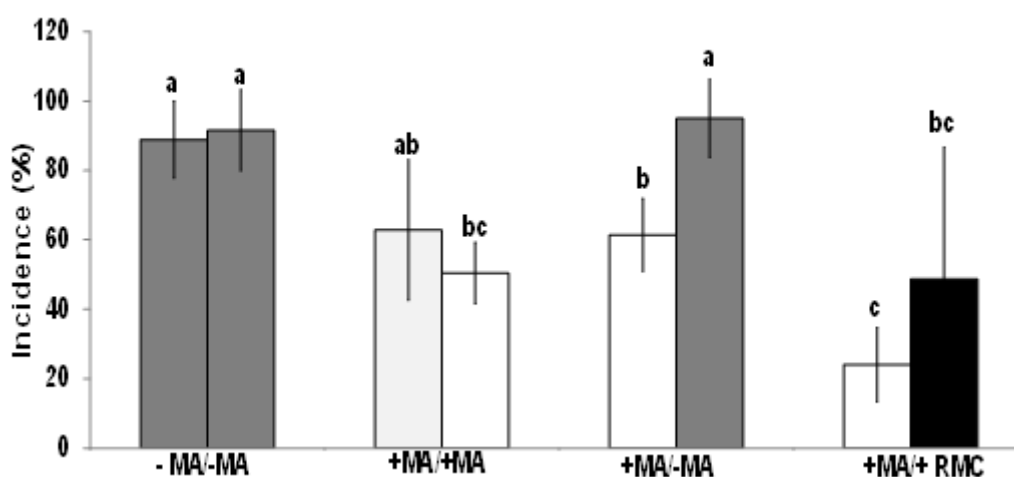
The results indicate that the external mycelium attached to the roots not only functions as a structure to absorb nutrients from the soil (Leake et al., 2004) but also as a source of inoculum for the roots of other plants. Although in field conditions, plants can be colonized by external FMA hyphae present in the roots of another plant, few studies analyze and discuss the importance of this form of colonization (Read et al., 1976).

Certain agricultural practices, including prolonged use of pesticides and chemical fertilizers (Hage-Ahmed et al., 2018; Novais et al., 2019) and soil tillage (Jansa et al., 2002; Alaux et al., 2020), can reduce the diversity of FMA species and the abundance of viable mycorrhizal propagules (primarily spores and infective mycelia), which can cause a reduction in the intensity of mycorrhizal colonization and compromise the benefits provided by the AM symbiosis (Schonnor et al., 2011; Verbruggen et al., 2013; Novais et al., 2019). In these conditions, root colonization from hyphae originating from

mycorrhizal roots may be important for achieving higher levels of mycorrhizal colonization (Read et al., 1976).

The infestation of *T. urticae* mites on plants allowed for the evaluation of the effect of AM symbiosis on the incidence of this pest in bean plants (Razmjou et al., 2009). Except for plants in compartment C1 of the C1+MA/C2+MA treatment, a lower incidence of mites was observed in plants colonized by FMA, either from viable mycorrhizal propagules (+MA) or from RMC hyphae (+RMC) (Figure 1). In non-mycorrhizal plants, the incidence varied from 88.9% to 95.0%, while in mycorrhizal plants it varied from 62.9% to 24.1% (Figure 1). There was no significant difference in mite incidence between plants colonized by viable mycorrhizal propagules (62.87% to 24.09%) and those colonized by RMC hyphae (48.67%).

Figure 1. Incidence of mites (percentage of leaflets per plant with presence or signs of infestation by *T. urticae* mite) in *P. vulgaris* plants whose roots were not colonized by AMF (-MA and gray bars) or were colonized by AMF from viable mycorrhizal propagules present in the soil of the compartments (+MA and white bars) or by the hyphae that formed the common mycorrhizal network (+RMC and black bar). Different letters indicate significant differences (Tukey's test,  $p \leq 0.05$ ) between the columns.



Source: The authors

In a study conducted by Hoffmann et al. (2009) solely with leaves of *P. vulgaris*, it was observed that the growth rate of the *T. urticae* population was higher in leaves taken from plants colonized by AMF than in leaves from non-colonized plants. However, in the present study, the incidence of mites on mycorrhizal plants was lower than on non-

mycorrhizal plants. This result suggests that plants associated with AMF may have controlled the growth of mite populations on leaves (Schausberger et al., 2012; Meier and Hunter, 2018), and consequently, the percentage of infested leaves per plant was lower than in the non-mycorrhizal condition. Moreover, the lower incidence of mites on AMF-colonized plants may have also contributed to the better vegetative and reproductive development of *P. vulgaris* by resulting in less damage to the photosynthetic tissue.

Although several studies have focused on the effect of AMF on the emission of volatile organic compounds by plants that are attractive to the predatory mite *Phytoseiulus persimilis*, which feeds on *T. urticae* (Schausberger et al., 2012; Sharma et al., 2017), in the present study, careful inspection of the leaflets of all plants revealed only the presence of the *T. urticae* mite. Therefore, other factors may be associated with the lower incidence of mites on *P. vulgaris* plants colonized by AMF. Although AMF can increase their host's resistance to attack by herbivores and phytopathogens through various mechanisms (Cameron et al., 2013), in this study, we suggest that the better nutritional condition of the plants and the induction of "priming" in mycorrhizal plants are the two factors that can better explain the lower percentage of mites on plants with AMF.

In the present study, the better nutritional condition of the colonized plants (evidenced by greater vegetative biomass compared to plants without AMF) by viable propagules in the soil and by the RMC hyphae may have contributed to the better defense response of *P. vulgaris* to mite infestation (Pozo & Azcón-Aguillar, 2007; Fontanna et al., 2009). The lower vegetative biomass and absence of reproductive structures in plants without AMF demonstrate the importance of nutrients obtained through AM for the development of *P. vulgaris*. The absence of these nutrients may have also limited the ability of non-mycorrhizal plants to direct resources towards defense mechanisms against mites (Fontanna et al., 2009; Kempel et al., 2010).

Studies have shown that, regardless of the nutritional condition of the plant (Kempel et al., 2010; Song et al., 2013; Meier & Hunter, 2018), the protective effect of AMF against herbivore attacks in the aboveground part has been explained by the positive influence of AMF on the host's defense mechanisms (Schausberger et al., 2012; Shrivastava et al., 2015; Meier & Hunter, 2019). Song et al. (2013) observed that the



weight gain of the herbivorous larvae *Helicoverpa armigera* on tomato leaves colonized by the AMF *Funneliformis mosseae* was lower than in the non-mycorrhizal condition. The authors attributed the greater resistance of mycorrhizal plants to the strong and rapid expression of defense genes in response to the caterpillar attack. Meier & Hunter (2018) and Rivero et al. (2020) also reported a higher concentration of secondary defense metabolites produced in response to herbivore attack in the aboveground part of plants colonized by AMF compared to those without AMF.

In the studies by Song et al. (2013), Meier & Hunter (2018), and Rivero et al. (2020), the authors considered that the priming state induced after root colonization by AMF allowed mycorrhizal plants to activate their defense mechanisms more quickly and efficiently. Consequently, plants in a primed state induced by colonization by AMF are capable of negatively affecting the survival and development of the herbivore more efficiently and are more resistant to infestation than without colonization by AMF (Kempel et al., 2010; Song et al., 2013; Rivero et al., 2020). This type of resistance has been called mycorrhiza-induced resistance (Pozo & Azcón-Aguilar, 2007; Jung et al., 2012; Cameron et al., 2013).

In study, mite infestation began 23 days after seedling emergence, sufficient time for roots to have been colonized by viable mycorrhizal propagules present in the soil (+MA) and by AMF hyphae (+RMC). Considering that colonization by AMF can induce priming and strengthen defense responses against herbivore attacks (Song et al., 2013; Pozo et al., 2020). in the present study as well, *P. vulgaris* plants colonized by AMF may have been able to activate defense mechanisms more quickly and efficiently compared to non-mycorrhizal plants, negatively affecting mite survival and/or development. Consequently, *P. vulgaris* plants with AMF showed greater resistance to *T. urticae* infestation and a lower percentage of infested leaflets per plant.

Priming can also be induced in non-infested plants, which recognize signaling molecules synthesized and emitted by nearby plants in response to aerial herbivore attack (Song et al., 2010; Babikova et al., 2013; Song et al., 2019). In plants connected via AMF, signaling molecules can be transferred from the infested plant to the healthy plant through the hyphae that form the mycorrhizal network, preparing the activation of defense

mechanisms before contact with the pathogen (Song et al., 2010; Babikova et al., 2013; Song et al., 2019; Alaux et al., 2020).

Although, in the C1+MA/C2+RMC treatment of this study, colonization via RMC resulted in a lower percentage of infested folioles per plant in compartment C2 compared to non-mycorrhizal plants, it is not possible to affirm if the greater resistance of *P. vulgaris* plants was induced by root colonization and/or by transfer of signaling molecules via RMC. However, the greater resistance of plants colonized by RMC hyphae to herbivore infestation, possibly due to the induction of priming state, again demonstrates the benefit provided to the plant through colonization by RMC hyphae. Thus, we emphasize its importance as a source of colonization so that FMA can provide their benefits in plant development and resistance to herbivore attack.

#### 4 CONCLUSIONS

Root colonization by FMA, both from viable mycorrhizal propagules and from the common mycorrhizal network hyphae, provided greater vegetative growth (shoot and root dry mass), higher number of reproductive structures (number of floral buds, flowers, and pods) per plant, and lower incidence of mites (percentage of leaflets with presence or signs of infestation by the mite) in *P. vulgaris* plants. Therefore, in the present study, the two forms of FMA colonization benefited the plants in their vegetative and reproductive development and in resistance to mite infestation. Despite the infestation occurring in a non-controlled manner, the difference in mite incidence between mycorrhizal and non-mycorrhizal plants strongly indicates that those colonized by FMA had a greater ability to control infestation. We suggest that two factors were responsible for the greater resistance to the *T. urticae* mite: better nutritional condition and induction of the priming effect, resulting in more efficient activation of the defense mechanisms of *P. vulgaris*.



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