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The beneficial role of arbuscular mycorrhizal fungi on population rates of aboveground herbivory: *Zyginella pulchra* (Hemiptera, Cicadellidae) in plane trees

Hamed Aalipour[1]*, *Ali Nikbakht*[1], *Jahangir Khajehali*[2], *Mohammad Taghizadeh*[3]

[1]*Department of Horticulture, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*
[2]*Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*
[3]*Agricultural and Natural Resources Research Center of Fars, Iran*

**Corresponding author: h.ali@ag.iut.ac.ir*

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Abstract: Herbivorous pests and arbuscular mycorrhizal fungi (AMF) coexist on the same host plant, having an indirect effect on one another. We established an experiment in a randomised complete block design with four treatments and six replications to examine the impact of AMF on the population and the damage caused to plane trees by the leafhopper *Zyginella pulchra*. Manure, manure plus fertiliser, manure plus fertiliser plus AMF, and non-inoculated plants (control) were all of the treatments. The findings revealed that while the nutritional content and soluble carbohydrate content were significantly enhanced by all treatments, they largely reached their peak in the AMF-inoculated plants. When compared to control trees that were not inoculated, the concentrations of N, P, and Zn were boosted by 39%, 81%, and 425%, respectively. AMF inoculation increased the population of *Z. pulchra* nymphs and adults compared to the control. However, the plants with AMF inoculation eventually suffered greater leaf loss as a result of this rise in the pest population. The findings show that while AMF enhance nutrient absorption and are necessary to improve the nutritional state of the host trees, they also enhance the absorption of pests that are thought to be harmful to plane trees. However, AMF colonisation improved the potential attractiveness of *Z. pulchra* to plane trees.

Keywords: insect attractiveness; leaf damage; mycorrhizae; nutritional status; *Platanus orientalis*

Various species of *Platanus orientalis* L., commonly known as plane trees, are distributed across diverse geographical regions and exhibit adaptability to varying climatic conditions. The plane tree is a sizable and aesthetically pleasing tree with a broad and far-reaching canopy. The aforementioned characteristics have established the plane tree as a significant tree within the surrounding environment, as noted by Sabeti (1976). *Zyginella pulchra* (Cicadellidae, Typhlocybineae) is considered to be a pest of plane trees due to its impact on their foliage. According to Wilson and Muhlethaler (2010), *Acer* species (*Acer* spp.) are the primary host plants of the pest; however, it has the potential to impact a diverse array of plants, including but not limited to plane trees, cypress (*Cupressus* spp.), and yew trees (*Taxus baccata*). The feeding behaviour of pests on almond (*Prunus dulcis* Mill. DA Webb), plum (*Prunus domestica* L.), peach (*Prunus persica* L.), and apple (*Malus domestica* L.) trees has been documented in various provinces of Iran. The pest nymphs and adults

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exhibit frequent activity on the underside of leaves, while the resulting injury symptoms, such as discoloured spots, are observable on the upper surface of leaves (Radjabi, Mirzayans 1989).

The literature suggests that plants have evolved various defence mechanisms in response to herbivorous threats, as evidenced by studies conducted by Agrawal (2007) and Rasmann and Agrawal (2009). These defensive features are believed to have arisen through selective pressures imposed by herbivores (Agrawal 2007; Züst, Agrawal 2016). Nevertheless, while plant traits play a crucial role in pest resistance, recent research has also highlighted the importance of inter-species communication, such as with arbuscular mycorrhizal fungi (AMF), in enhancing plant defences (Hartley, Gange 2009; Simon et al. 2017a).

The symbiotic relationship between arbuscular mycorrhizal fungi and plants has the potential to enhance the host plant ability to absorb water and nutrients, specifically phosphorus (P) and nitrogen (N), ultimately leading to an increase in plant growth (Smith, Read 2008; Treseder 2013; Garcia et al. 2016; Aalipour et al. 2020, 2021). The quality of host plants is a predominant factor influencing the performance of insect herbivores. Hence, it is predictable that the plants inoculated with AMF exhibit an impact on the preference and performance of insect herbivores, as reported by Ng et al. (2022). The influence of plant nitrogen and phosphorus contents on insect performance has been demonstrated in previous studies (Elser et al. 2000; Currie et al. 2011). The growth of herbivorous pests is influenced by the nutritional status of plants, as evidenced by studies conducted by Schmid-Hempel (2005), Lee et al. (2008), and Triggs and Knell (2012). AMF inoculation influences the quality of host plants (Frew et al. 2017). Insect performance is largely determined by the nutritional status of their host plants. Interactions between insects and host plants that are mediated by AMF can be advantageous for both the host plant and the parasite (Pineda et al. 2010). The impact of AMF on herbivores is characterised by a high degree of variability, ranging from positive to negative effects (Bennett, Bever 2007). Nevertheless, these interactions are contingent upon numerous variables. These relationships can be modified by environmental factors, such as the availability of nutrients for the host plant. As demonstrated by Frew et al. (2017), alterations in plant resistance to herbivores can be attributed to nutrient limitation. Furthermore, the colonisation of arbuscular mycorrhizal fungi (AMF) has the potential to enhance plant nutrition and improve the concentration of soluble sugars, thereby promoting the invasion of insects (Cardoza et al. 2003).

The impact of AMF on plant resistance to pest herbivores was studied by Vannette and Hunter (2011), which in turn can affect pest population parameters as demonstrated by Wooley and Paine (2007). However, the effects on the fitness of the parasite vary based on the species characteristics of the AMF, the host plant, and the herbivore (Gange 2007; Vannette, Hunter 2011). The impact of AMF colonisation on herbivores can vary in terms of its effects, as has been demonstrated in previous research (Shrivastava et al. 2015; Gange et al. 1999; Gehring, Bennett 2009). It has been found that such colonisation can be either detrimental, advantageous or have no discernible impact on these organisms. However, a comprehensive understanding of the ultimate impact of arbuscular mycorrhizal fungal (AMF) colonisation on the population density of insect pests on their host plants remains elusive. A comprehensive investigation of the mechanisms underlying the tripartite interactions is yet to be conducted, as stated by Koricheva et al. (2009).

The primary objective of this investigation was to assess the potential impact of arbuscular mycorrhizal fungal (AMF) colonisation on the population density of *Z. pulchra* and the extent of leaf damage in plane trees. The correlation between the nutrient content of plants and the population of insects was also examined. Two hypotheses were examined in this study: (*i*) does the colonisation of AMF have an impact on the attractiveness of plane trees to *Z. pulchra*? and (*ii*) does this pest exhibit a preference for plants that have been inoculated with AMF?

MATERIAL AND METHODS

Experimental site and treatments. The study was carried out between 2014 and 2015 at Isfahan University of Technology, located in Isfahan, Iran (32°39'N, 51°40'E; 1 600 m a.s.l.). The research was conducted on sandy-clay soil with pH of 7.5 and electrical conductivity (EC) of 2.48 $dS \cdot m^{-1}$. The location is characterised by its arid climate, frigid winters, average annual precipitation of 122.8 mm, and

https://doi.org/10.17221/158/2022-JFS

the average annual temperature of 23.4 °C. A total of 24 specimens of plane trees (*P. orientalis* L.) that were 15 years old and uniform in size were chosen.

Manure (M), manure + fertiliser [MF; water-soluble 20 : 5 : 10 N : P : K compound fertiliser with 12.8% sulphur, 1.3% magnesium oxide (NovaTec Solub, Compo, Germany)], manure + fertiliser + AMF (MFA), and non-inoculated plants (control) were the four treatments in this study. There were six replications of each treatment. We inoculated *Rhizophagus irregularis* and *Funneliformis mosseae* as mycorrhizal treatments. Control plants were not inoculated.

Two identical holes (measuring 0.5 m × 0.5 m) were filled with three different types of filling materials, based on the treatment applied. In order to perform mycorrhizal inoculation, 250 g (80 spores per g) of mycorrhizal inoculum (obtained from the National Soil and Water Research Center located in Karaj, Iran) were introduced into each individual hole. The trees were subjected to M and MF treatments, wherein they were provided with 5 kg and 0.1 kg of manure and fertiliser per hole, respectively. The control group did not receive any intervention and instead underwent a procedure in which two identical holes were drilled. The trees received weekly irrigation. Table 1 displays the properties of soil and cow manure.

Plant analysis. The standard technique of dry ashing was used to determine the mineral nutrients (N, P, K, Fe, and Zn) in leaves. Four different tree sides were adopted to randomly choose leaf samples. In brief, the 48-h oven drying at 65 °C produced dried leaves. P, K, Fe, and Zn were extracted from the samples using 2 M HCl following 550 °C, 5.5 h of dry ashing (Aalipour et al. 2019). K was determined using a flame photometer, whereas Fe and Zn were measured using atomic absorption spectrometers (AA-670; Shimadzu, Japan). Phosphorus was measured using a spectrophotometer (UV-600A) at 460 nm using the vanadomolybdophosphoric acid colorimetric method (Eaton et al. 1995). Total N was determined using the Kjeldahl technique (Eaton et al. 1995).

Evaluation of the insect population and leaf damage. Digital image processing with MATLAB software was employed to assess the extent of leaf damage caused by insects. To accomplish this task, a sample of 100 leaves was randomly chosen from each tree and subjected to scanning of both leaf surfaces using a Canon 4410 scanner (Canon, Japan). Subsequently, a set of colours was established within the software to represent the various types of damage imposed by insects on leaves. The data pertaining to the pixel and colour attributes of individual leaves were exported in the form of '.csv' files, which could then be imported into MATLAB (Version 8.4, 2014) software to facilitate the computation of damaged areas, as described by Rathod et al. (2013).

Evaluation of adults and nymphs. In order to assess the population of leafhopper adults and nymphs, a random selection of four twigs measuring 20 cm in length was obtained from the central region of the crown, taken from four different directions. These twigs were promptly transferred to the laboratory in plastic bags on ice. Subsequently, the branches underwent a washing process utilising a combination of water and detergent. Following this, sifting was conducted, and the total amount of both adult and nymph specimens was recorded per 50 leaves.

In this experiment, in addition to leaf injury caused by *Z. pulchra* feeding, leaf chlorosis was also observed, most likely due to a deficiency of micronutrients such as Fe and Zn.

Statistical analysis. The data obtained from *P. orientalis* L. was analysed using the randomised complete block design (RCBD) and subjected to analysis of variance (ANOVA) using SAS (Version 9.1, 2003) with six replications. The statistical analysis involved performing means comparisons through the implementation of the least significant difference (LSD) test at a significance level of 0.05. Statistical Pearson's correlation coefficient (*r*) was used to establish the relationships between variables through a statistical correlation analysis.

Table 1. Soil and manure physico-chemical properties

Factors	Texture	pH	*EC*	*OM*	N	P available	K exchangeable	Fe	Zn
			(dS·m^{-1})	(%)		(mg·kg^{-1})			
Soil	clay	7.90	1.53	1.15	0.15	140	235	1 400	21
Manure	–	8.02	15.23	20.40	3.07	791	2 030	12 300	194

EC – electrical conductivity; *OM* – organic matter

https://doi.org/10.17221/158/2022-JFS

Table 2. Comparison of 2-year means of nutrients concentration under AMF and other treatments in plane tree leaves

Year	Treatment	N	P	Fe	Zn
		(g·kg⁻¹)		(mg·kg⁻¹)	
2014	control	7.69 ± 0.28^{d}	11.10 ± 0.85^{e}	44.70 ± 2.90^{c}	4.90 ± 0.90^{e}
	manure	8.57 ± 0.30^{c}	13.40 ± 1.60^{d}	57.60 ± 2.80^{c}	12.20 ± 1.10^{d}
	manure + fertiliser	9.44 ± 0.36^{b}	14.10 ± 0.95^{cd}	55.80 ± 3.60^{c}	14.90 ± 1.70^{c}
	AMF	9.94 ± 0.38^{b}	15.90 ± 1.00^{c}	67.90 ± 2.00^{c}	24.60 ± 2.40^{a}
2015	control	7.26 ± 0.17^{d}	10.70 ± 0.69^{e}	65.20 ± 8.00^{c}	4.73 ± 0.89^{e}
	manure	9.73 ± 0.82^{b}	19.10 ± 3.00^{b}	236.00 ± 16.00^{b}	18.00 ± 2.30^{b}
	manure + fertiliser	9.87 ± 0.43^{b}	20.50 ± 2.10^{b}	242.00 ± 18.00^{ab}	19.60 ± 2.80^{b}
	AMF	10.80 ± 0.71^{a}	23.50 ± 2.60^{a}	272.00 ± 29.00^{a}	26.00 ± 2.20^{a}

[a–e] Means (± SD) followed by similar letters within each column do not express significant differences at $P < 0.05$ according to the LSD test; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

RESULTS AND DISCUSSION

A significant interaction impact of year × treatments on nutritional levels was found using the analysis of variance (data not shown). The concentrations of N, P, Fe, and Zn in the leaves of plane trees were increased in mycorrhizal plants (Table 2). When compared to control plants, AMF-inoculated plants significantly increased P (by 43% and 119%) and Zn (by 402% and 449%, respectively) in 2014 and 2015. Regardless of the nature of the combination, all treatments successfully increased the Fe and N concentrations in the leaves. Although N appeared to be more dependent on the composition of the mixture, Fe could be increased by adding any type of fertiliser (Table 2).

The treatments that received AMF inoculation exhibited the highest peak in soluble sugar content. The results indicate that AMF-inoculated plants exhibited a significant increase in soluble sugar content, with a respective increase of 31.1% and 40.6% in 2014 and 2015, as depicted in Figure 1, when compared to the control plants.

The treatments had a significant impact on the population of nymphs and adults, as depicted in Figures 2 and 3. The inoculation of trees with AMF resulted in a significant increase in the num-

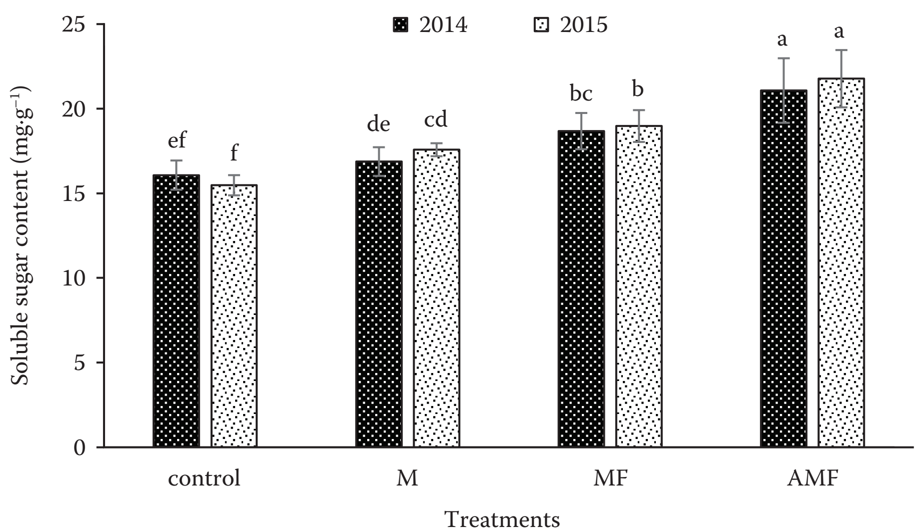

Figure 1. Influence of different treatments on soluble sugar content in plane tree

[a–f] Means followed by same letter do not differ significantly by LSD tests at $P \leq 0.05$; M – manure; MF – manure + fertiliser; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

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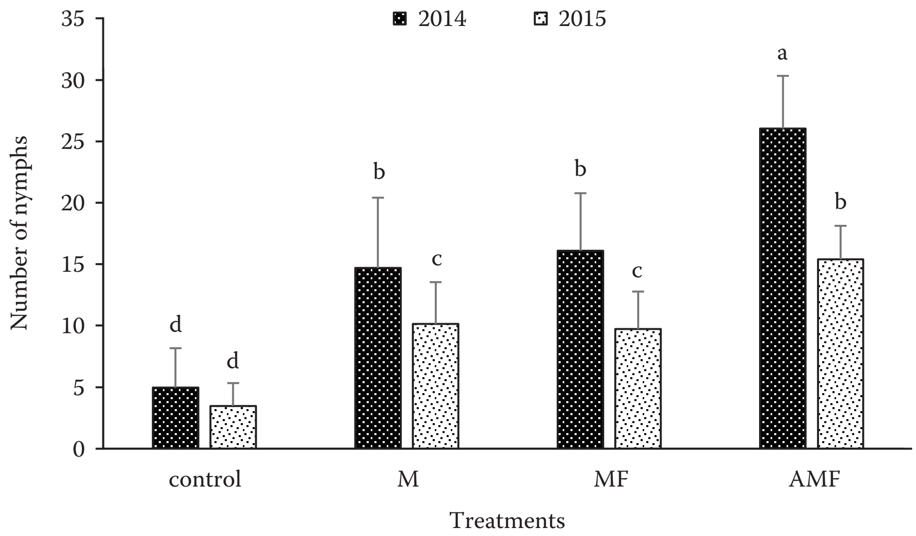

Figure 2. Influence of different treatments on the number of *Zyginella pulchra* nymphs in plane tree

[a–d] Means followed by same letter do not differ significantly by LSD tests at $P \leq 0.05$; M – manure; MF – manure + fertiliser; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

ber of nymphs and adults. Specifically, in 2014 and 2015, the number of nymphs increased by 424% and 342%, respectively, while the number of adults increased by 707% and 480%, respectively, when compared to control plants. The treatment with AMF also yielded the highest number of nymphs and adults recorded. Plants inoculated with AMF exhibited a significant increase in nymphs (62% and 58% for 2014 and 2015, respectively) and adults (72% and 90% for 2014 and 2015, respectively) compared to those that were solely treated with manure and fertiliser. The study revealed noteworthy associations between the number of nymphs and the elements N and Zn ($r = 0.53$ and 0.66, respectively, $P < 0.01$), as well as between the number of adults and N and Zn ($r = 0.57$ and 0.64 respectively, $P < 0.01$). The rise in the *Z. pulchra* population may be attributed to significant and consistent enhancements in the nutrient levels of the plant. The study revealed a significant and positive correlation between the quantity of soluble sugar and the count of adult and nymph insects, with correla-

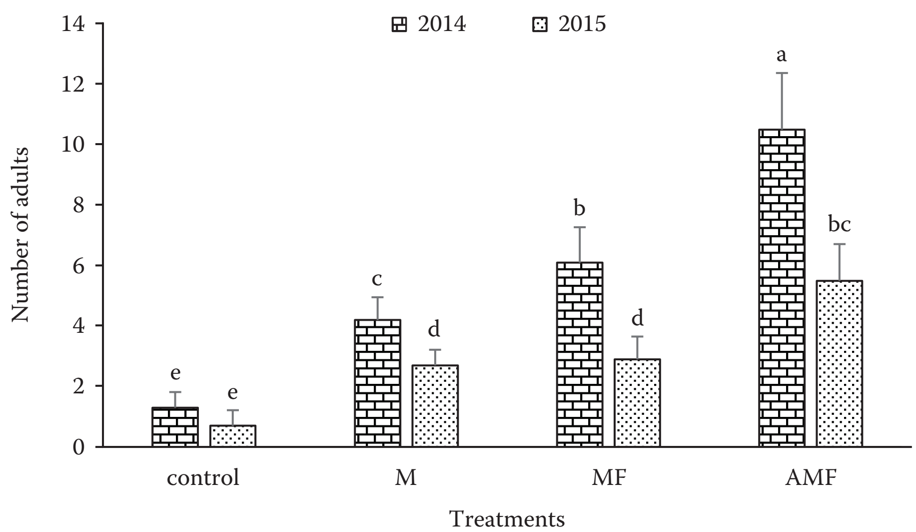

Figure 3. Influence of different treatments on the number of *Zyginella pulchra* adults in plane tree

[a–e] Means followed by same letter do not differ significantly by LSD tests at $P \leq 0.05$; M – manure; MF – manure + fertiliser; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

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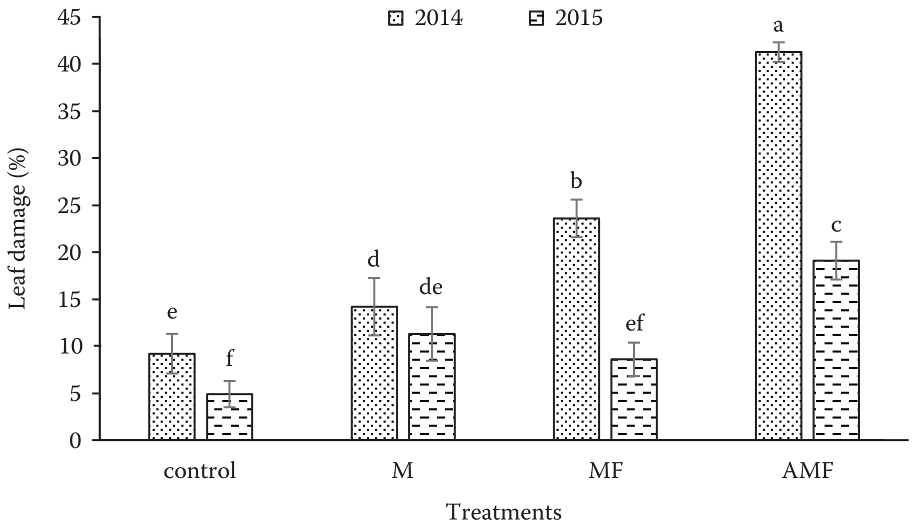

Figure 4. Influence of different treatments on leaf damage in plane tree.

[a–f] Means followed by same letter do not differ significantly by LSD tests at $P \leq 0.05$; M – manure; MF – manure + fertiliser; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

tion coefficients of 0.73 and 0.62, respectively, and a *P*-value of less than 0.01.

Based on the data, it was observed that the AMF-inoculated plants exhibited the highest percentage of leaf damage, while the control plants displayed the lowest amount of damage (Figure 4). The results indicate that leaf chlorosis showed a reverse trend, whereby the control group had the highest percentage of chlorosis, while the AMF-inoculated plants had the lowest percentage (Figure 5). There were negative correlations between leaf chlorosis and insect population ($r = -0.61$ and -0.64 for adults and nymphs, respectively, $P < 0.01$). An observation was made of a significant positive correlation between leaf damage and insect population, with correlation coefficients of 0.89 and 0.79 for adults and nymphs respectively, and a *P*-value of less than 0.01.

The presence of arbuscular mycorrhizal fungi can have both positive and negative impacts on the population of insect herbivores. Gehring and Bennett (2009) reported that the presence of AMF may not always have a consistent effect, and in certain

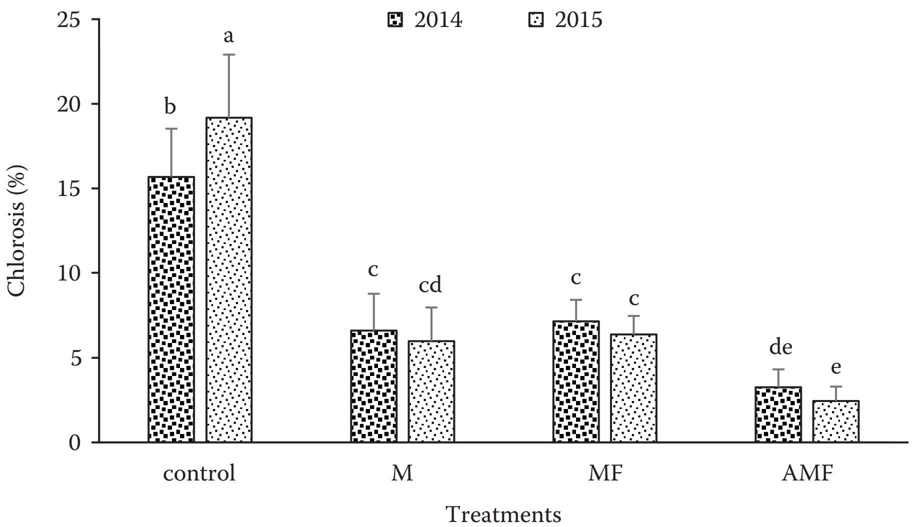

Figure 5. Influence of different treatments on chlorosis in plane tree

[a–e] Means followed by same letter do not differ significantly by LSD tests at $P \leq 0.05$; M – manure; MF – manure + fertiliser; AMF – arbuscular mycorrhizal fungi (mixture of *Rhizophagus irregularis* and *Funneliformis mosseae*)

https://doi.org/10.17221/158/2022-JFS

instances, the effects may be variable or negligible. According to Bennett and Bever's (2007) findings, the inoculation with three distinct species of AMF had varying effects on the resistance of *Plantago lanceolata* plants to feeding by *Junonia coenia* butterflies. The outcome was dependent on the specific AMF and plant species involved, with some instances resulting in positive effects, others in negative effects, and still others in neutral effects. According to Roger et al. (2013), various species of AMF evoke varying effects on plant growth and plant resistance to herbivory. According to Roger et al. (2013), larger and healthier plants are frequently targeted by sucking insects, irrespective of the arbuscular mycorrhizal fungi status of the plant. The results of this experiment indicate that insects exhibited a preference for consuming a greater quantity of leaves derived from plants that had been inoculated with AMF. Pests may be drawn to plants with larger leaves. The study conducted by Wurst and Forstreuter (2010) yielded results indicating the absence of any discernible influence of AMF colonisation on the preference of aphids (*Myzus persicae* Sulzer). However, it was observed that the plants inoculated with AMF exhibited greater biomass as compared to those that were not inoculated. The study observed that the application of AMF in *Phaseolus vulgaris* L. bean plants resulted in an increase in the population of *Tetranychus urticae* Koch, as compared to the non-inoculated plants, which is consistent with the findings of Hoffmann et al. (2011). The study conducted by Gange et al. (1999) demonstrated a rise in the performance of aphids, specifically *Myzus ascalonicus* and *M. persicae*, in *Plantago lanceolata* trees that were inoculated with AMF. The aphid population on the AMF-inoculated plants was observed to be seven times greater than that of the control plants.

According to Cornelissen et al. (2008), plants exhibiting accelerated growth rates tend to be more susceptible to a diverse range of pests, including Diptera, Homoptera, and Lepidoptera. The biological capacity of insect herbivores and their population can be influenced by the quality of the host plant. According to Vannette and Hunter (2009), herbivorous pests are primarily drawn toward plants that possess a greater concentration of nitrogen and phosphorus in their tissues. The level of nitrogen present in the plant is regarded as one of the key determinants of host plant quality. Research has indicated that insects with a sucking feeding mechanism exhibit a significant response to the presence of nitrogen in plants (Van Emden 1966). The alteration of nitrogen fertilisers, whether increased or decreased, has the potential to affect the quality of host plants for insect herbivores, thereby influencing their growth and fertility rates. This has been demonstrated in various studies, including those conducted by Larsson (1989), Nevo and Coll (2001), and Wang et al. (2006). Numerous studies have demonstrated that elevated levels of nitrogen are associated with elevated growth rates and population sizes of sap-sucking pests.

Several researchers (Wu et al. 2017; Aalipour et al. 2019, 2020, 2021) have reported an increase in the concentration of nitrogen (N) and phosphorus (P) in plants that were inoculated with AMF. It is noteworthy that AMF lack the direct ability to decompose organic matter. Nevertheless, it is worth noting that fungal hyphae exhibit a higher degree of efficiency in penetrating decomposing material compared to plant roots, as per Javaid's research (Javaid 2009). Consequently, the inoculation with AMF resulted in an increase in both the size and quality of the plant, potentially due to alterations in nutrient uptake mechanisms (Smith, Read 2008; Aalipour et al. 2021). Additionally, the changes in the populations of insect herbivores as a result of AMF have been linked to modifications in the quantity, quality, defence mechanisms, or tolerance to pests in host plants (Gange et al. 1999). The inoculation of tomato (*Lycopersicon esculentum* Mill.) plants with AMF resulted in a reduction in the feeding activity of beet armyworm (*Spodoptera exigua* Hübner) in comparison with those insects that fed on non-inoculated plants. According to Shrivastava et al. (2015), there is evidence to suggest that AMF plants exhibit a stronger resistance response to herbivore feeding compared to non-inoculated plants, which may partially be due to variations in terpenoid levels. According to Pineda et al. (2010), plants inoculated with AMF may exhibit an increase in the production of secondary metabolites that possess toxic properties to pests. These compounds are known to be synthesised through enzymatic hydrolysis. Chewing insects are known to employ enzymes that facilitate the release of cell contents upon contact with secondary metabolites, resulting in the production of hazardous combinations that are detrimental to their survival. However, sucking

https://doi.org/10.17221/158/2022-JFS

pests have developed a mechanism to counteract this challenge. According to Walling (2008), the sucking organs of these pests prevent the formation of a connection between hydrolyzate enzymes and secondary metabolites by penetrating the phloem. Consequently, the production of toxic metabolites is inhibited. According to Hartley and Gange (2009) and Gehring and Bennett (2009), there appears to be a reduced impact of the defence mechanism activated by AMF in the host plant on sucker pests.

Alterations in the primary metabolite composition, specifically the soluble carbohydrates, within plants inoculated with AMF have been observed to have an impact on insects that consume said plants (Gehring, Witham 1994). The symbiotic relationship between AMF and the host plant results in a modification of the carbon, nitrogen, and carbohydrate levels, as reported by Wu et al. (2015) and He et al. (2017). According to Gehring and Witham's (1994) findings, the inoculation with mycorrhizal fungi in plants can lead to an increase in soluble carbohydrate content, which may potentially enhance the growth of insects. According to Gange and West's research (Gange, West 1994), a decrease in leaf carbohydrates results in a decline in insect performance, whereas an elevation in nitrogen levels leads to an improvement in insect performance.

According to Gange et al. (1999), there is evidence to suggest that the colonisation of AMF may have a positive impact on the life history traits of phloem feeders, such as growth rate and reproductive success. The observed rise in feeding potential could be attributed to various factors such as increased leaf area, plant height, and biomass, as reported by Simon et al. (2017b). Our findings suggest that the enhanced nutritional status of the plant, facilitated by the fungi, is a probable contributing factor to the increased *Z. pulchra* population observed in AMF plants. However, the precise mechanisms through which arbuscular mycorrhizal fungi impact insect growth and population remain largely unexplored. Consequently, to comprehend the mechanism of AMF-insect interactions, it is necessary to conduct a comparative analysis of insect preference and performance on both AMF and non-AMF plants. To the best of our knowledge, there have been no similar findings regarding the impact of AMF inoculation on the population and the efficacy of *Z. pulchra* in plane trees.

CONCLUSION

The findings of this research indicate that the population of *Z. pulchra* and the resulting leaf damage can be increased by the addition of AMF. It is widely acknowledged that the performance of herbivorous insects is closely linked to the availability of food supplied by plants. In this regard, it has been observed that the presence of arbuscular mycorrhizal fungi with plane trees can enhance the nutrient and soluble sugar content, thereby improving the performance and population of *Z. pulchra*. Although nutritional strategies may account for certain triple responses observed among AMF, insects, and plants, further exploration is necessary to examine alternative mechanisms. Nevertheless, it is believed that the benefits of mycorrhizal fungi on plane trees are outweighed by the negative consequences of raised appeal to *Z. pulchra*.

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