

Article



Is the Arbuscular Mycorrhizal Fungus *Funneliformis mosseae* a Suitable Agent to Control Criconematid Populations?

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Abstract: Several studies have shown the potential of using mycorrhizal fungi in increasing the plant yield by simultaneously reducing damages caused by pathogens. Plant parasitic nematodes (PPNs) are among the most feared pathogens for crops. This study aimed to evaluate the effects of Funneliformis mosseae as a mycorrhizal fungus on the population abundance of three world widespread species of nematodes from the family Criconematidae: Mesocriconema xenoplax, Mesocriconema antipolitanum, and Criconemides informis. Pure and highly abundant populations of each species were collected from Urmia city in Northwestern Iran, after the identification morphological and morphometric characteristics. The experiments were carried out in greenhouse conditions on three different rhizospheres of alfalfa, sugar beet, and wheat. After five months, the final population of nematodes and fungus, and the root surface on host plants inoculated and noninoculated with the fungus F. mosseae, were evaluated. The results showed that the population of nematodes was increased in the presence of the fungus. It could be assumed that the extension of the host surface level of roots by the fungus resulted in more feeding sites for nematode activity and, consequently, higher population densities. In this study, the fungus did not seem to play a suitable role in controlling ectoparasitic nematode growth. However, since there are still many open questions about mycorrhizal fungi's role in agriculture, more research should be conducted.

Keywords: Criconematidae; alfalfa; sugar beet; wheat; mycorrhiza; plant root; biological agents

1. Introduction

Agricultural products facing plant pathogens are subject to resulting damages, which can have high economic and strategic impacts. Nematodes represent one of the primary plant pests, causing significant direct and indirect damages to crops, which, in turn, could lead to qualitative damages to host plants [1,2]. Indeed, the total damage caused by pathogens in the world is estimated to be around 118 billion dollars, of which 12% are caused by nematodes [3]. Plant parasitic nematodes (PPNs) show a wide range of lifestyles, but all of them are classified into different groups based on their feeding strategies, including ecto- and endo- parasitism [4].

Yield losses caused by PPNs are expected to rise soon because of climate change and the intensification of cropping systems [1]. Considering the significant overuse of synthetic chemical products and increasing ecosystem alterations, scientists are trying to exploit bio-control agents, including diverse microorganisms, to achieve a possible PPN control and, at the same time, fit Agenda 2030 goals (i.e., reducing climate and environmental footprint, increasing biodiversity, guaranteeing food security, strengthening sustainable food systems, and developing alternatives to contentious

Citation: Manteghi, A.; Rezaee Danesh, Y.; Hesar, A.M.; Demir, S.; Boyno, G.; Catani, L.; Semprucci, F. Is the Arbuscular Mycorrhizal Fungus *Funneliformis mosseae* a Suitable Agent to Control Criconematid Populations? *Diversity* 2022, *14*, 898. https://doi.org/10.3390/ d14110898

Academic Editor: Luc Legal

Received: 31 August 2022 Accepted: 22 October 2022 Published: 24 October 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/license s/by/4.0/). inputs and other plant protection products) [5–7]. Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts that can protect their host plant against many stress factors, improving plant growth and increasing the absorption of minerals, especially immobile nutrients, from the soil by the host [5] (see Figure 1). They are used as biological agents to increase plant tolerance to different living and non-living environmental stressors, as well as plant productivity, in sustainable agricultural systems [5,8–12]. The effects of the AMF symbiosis on plant-parasitic nematodes can be variable, and the mechanisms driving such variability remain unknown. However, AMFs seem to directly or indirectly affect PPN populations becoming a possible alternative to nematicide products [9,10,13–15]. So far, they have been used to reduce damage of endo-parasites nematodes such as Meloidogyne spp. [16–23]; Pratylenchus spp. [24–28]; Xiphinema index [29]; Ditylenchus dipsaci [30]; Radopholus similis [31]; Globodera pallida [32]; Nacobbus aberrans [33]; Tylenchulus semipenetrans; and Rotylenchulus reniformis [34]. However, there is a lack of data on the effects of arbuscular mycorrhizal fungi on criconematids, as well as on migratory ectoparasite nematodes. The family Criconematidae includes more than 480 species of ectoplant parasitic soil nematodes [35,36]. Among them, Mesocriconema xenoplax, Mesocriconema antipolitanum, and Criconemides informis are the most common cosmopolitan roots damaging ectoparasites, with a wide host span ranging from herbaceous plants to woody trees [36–41].

Thus, in order to find new and more sustainable tools to control PPNs, a glasshouse experiment was conducted to assess the influence of arbuscular mycorrhizal fungus, i.e., *Funneliformis mosseae*, on the population abundance of *M. xenoplax*, *M. antipolitanum*, and *C. informis*. The treatments were tested on different host plants that serve a great role in Iranian cultivation and worldwide, i.e., alfalfa (*Medicago sativa* belonging to Fabaceae family), sugar beet (*Beta vulgaris*, Amaranthaceae), and wheat (*Triticum aestivum*, Poaceae). The experiment was carried out by using *F. mosseae* with different treatments in the three host plants: i) pure *F. mosseae* (without ecto-plant parasites); and ii) *F. mosseae* along with criconematids to test the following hypotheses: (1) H₀, host plants did not affect the number of spore populations; (2) H₀, nematodes did not affect *F. mosseae* sporulation, and (3) H₀, *F. mosseae* did not affect the number of criconematids in the plant hosts.



Figure 1. Schematic representation of the interaction between arbuscular mycorrhizal fungi (AMF) with the host plant and their functions (Figure by Boyno, G.).

2. Materials and Methods

2.1. Protocol of Nematode Species Selection

The *M. xenoplax* population was extracted from the rhizosphere soil of an apricot tree from the Dalampar valley (37°11.103′N, 44°54.147′E; 1740 m), the *M. antipolitanum* population was derived from the rhizosphere of a walnut tree from the Balanoj region (37°32.554′N, 45°06.354′E; 1331 m), and the *C. informis* population was obtained from the rhizosphere soil of an apple tree from the Heidarlo region (37°28.699′N, 45°02.807′E; 1551 m). Criconematid specimens were isolated using the Whitehead tray method [42] and prepared according to Jagdale et al. (2013) [43].

For morphological and morphometric characterization and taxonomical identification, Criconematidae were extracted from the soil using sieve and centrifuge methods [44]. The specimens were killed and fixed by hot FPG (4:1:1, formaldehyde: propionic acid: glycerol), then processed in anhydrous glycerol [45] and mounted on permanent slides using paraffin wax. For taxonomic identification, the specimens were observed under a Nikon Y300 light microscope equipped with Dino-eye eyepiece camera and its relative image capture software (Dino-Lite v. 2.0). Identification of the three nematode populations was further confirmed by molecular analyses using COI gene

haplo-typing [46]. After ensuring that each population of criconematids was pure with only one species in the soil, the number of specimens was counted in 100 g of soil (76 specimens for *M. xenoplax*, 87 for *M. antipolitanum*, and 48 specimens for *C. informis*). Then,

2.2. Preparation of Arbuscular Mycorrhizal Fungus Inoculum

To prepare the inoculum of *F. mosseae*, wheat seeds were disinfected with 20% sodium hypochlorite solution and cultured in wet sterile Petri dishes. Two days after germination, the seeds were transferred to pots containing inoculum of pure mycorrhizal fungus provided from the fungal culture collection at the department of Plant Protection, Faculty of Agriculture, Urmia University, Iran; they were kept for 6 months at 25°C in greenhouse conditions. During this time, non-phosphorous supplemented solutions (Hoagland) were used to feed the plants.

the primary inoculum for all three nematode species was set up to 50 specimens.

The dietary supplement included only calcium nitrate solution, potassium nitrate, magnesium sulfate, Fe-EDTA, and microelements. This solution was used by irrigating each pot twice a week (25–30 mL). After 6 months, to create stress for the stimulation of mycorrhizal fungi sporulation, the aerial green parts of the plants were discarded, and the pots were stored for a month in dry conditions without irrigation [47]. The determination of the fungal population number was carried out by isolating spores using standard wet sieving and centrifugation in 50% sucrose solution [44,48].

2.3. Cultivation in the Greenhouse

Cultivation was carried out under greenhouse conditions in plastic pots (30 cm in diameter and 20 cm height). For cultivation, a thin layer of sterilized soil (free of any microorganisms) was poured into the bottom of the plastic pots. Then, depending on the treatments, a soil layer containing only nematodes (50 specimens for each species) or nematodes (50 specimens for each species) + mycorrhizal fungus (20% v:v; containing 195 spores/g soil inoculum) was added. The plant seeds were cultivated in this soil layer and followed by adding, again, a thin layer of sterilized soil. In control treatments, seed cultivation was carried out only in sterilized soil. The pots were kept in greenhouse conditions and irrigated with water and Hoagland's nutrient solution. After five months, all of the treatments were checked for the quantity of nematodes and mycorrhizal fungus spores. The plants' root lengths were further measured in all the treatments.

2.4. Image Processing of the Plant Roots

The image acquisition of the roots was conducted using a digital camera (Power Shot G7, Canon, Tokyo, Japan) with 1200 × 1600 pixels resolution. A lens (ANB847, Panasonic, Osaka, Japan) with 50 mm focal length was mounted on the camera using an adapter ring (ANB848, Panasonic, Osaka, Japan). The camera was fixed on a stand, which provided easy vertical movement and stable support. Clear focused images of the paddy seed were obtained at a distance of 140 mm between the lens and the sample platform. A whiteboard was then used as background for the root photos. Digital images were stored in uncompressed JPEG format. MATLAB (version 2012, Math Works, Natick, MA, USA) software was then used to develop the required algorithms for image segmentation and texture feature extraction.

2.5. Statistical Analyses

Analysis of variance (ANOVA) was used to test for significant differences between treatments. A factorial experiment, including two factors (AMF and nematode inoculation) in a randomized complete block design with three replications, was used. Tukey's multiple-comparison tests were then used when significant differences (p < 0.05) were detected. The data set was analyzed using the SPSS, v 21, software.

3. Results

3.1. Morphological and Morphometric Data on Criconematid Populations

All morphometric data calculated for nematode species are presented in Table 1. Furthermore, the main characters of each nematode species are briefly described as follows:

Mesocriconema antipolitanum (de Guiran, 1963) Loof and De Grisse, 1989 (Figure 2) Female: The body annuli retrorse; about 4.5 μm thick at the anterior end and 10 μm thick in the middle of the body. Annuli smooth and rounded; generally, 0–3 anastomoses in the post-vulval body part. Head truncated; labial disc flattened, sub-median lobes well developed, anteriorly flattened; lateral labial plates present; first annulus retrorse. Stylet short and stout with rounded knobs. Vulva with anterior lip bi-lobed. Tail terminus rounded.

Male: not found.

Mesocriconema xenoplax (Raski, 1952) Loof and De Grisse, 1989 (Figure 3)

Female: Cuticular body annuli retrorse, no lateral differentiation of anastomoses in the studied population. The two lip annuli were not retrorse, smaller and narrower than the subsequent body annuli, but not set off, presenting a bluntly rounded outline; first annulus directed laterally, anteriorly indented. Labial disc elevated, sub-median lobes relatively large, projecting outward and forward, labial plates notched. Stylet strong and robust, knobs 9–11 μ m across. Vagina was always sinusoidal, parallel to the body axis just anterior to vulva and continued inward and upward. Anterior vulval lip variable usually with two evident projections. Post-vulval body part conical to rounded. Tail terminus with 2–4 lobes.

Male: not found.

Criconemoides informis (Micoletzky, 1922) Taylor, 1936 (Figure 4)

Females: Cuticular annuli retrorse with rounded edges, no lateral differentiation, margins smooth or slightly irregular; anastomoses rare in the studied population. Head rounded; most anterior annuli variable, usually directed sideways; sub-median lobes distinct, relatively large, and angular; labial disc slightly elevated above lobes. Stylet very strong with thick knobs and anteriorly concave, with marginal processes directed anteriorly. Vulval lips bulging but not projecting above body contour. Tail plump with rounded tip; terminal annulus simple or bilobed.

Male: not found.

Both morphometric and morphological characteristics of the studied populations did not reveal significant differences from populations reported from other geographical regions.

| Demenseter | Species | | | | |
|------------------------------|---------------------------|---------------------------|-----------------------------|--|--|
| Parameter | M. xenoplax | M. antipolitanum | C. informis | | |
| Ν | 47 | 54 | 45 | | |
| L (μm) | 556 ± 36.1 (509–600) | 525 ± 41 (462–625) | 528.3 ± 78.1 (357–600) | | |
| Α | 11.5 ± 1.1 (10.8–13.5) | 10.8 ± 1.1 (9.7–13.3) | $7.8 \pm 0.9 \ (6.5 - 8.9)$ | | |
| В | $4.1 \pm 0.3 (3.7 - 4.7)$ | $3.7 \pm 0.3 (3.4 - 4.4)$ | 4 ± 4.1 (3.2–4.5) | | |
| С | 34.1 ± 7.9 (27.8–47.5) | 24.5 ± 3.5 (19.5–32.3) | 16.5 ± 3.6 (11.6–22.5) | | |
| \mathbf{V} | 94.2 ± 0.5 (92.5–95.2) | 94.5 ± 0.8 (93.1–96.2) | 91 ± 1.6 (87.5–93) | | |
| Stylet | 76.5 ± 2.1 (74–79) | 72.5 ± 3.5 (69–79) | 81.2 ± 4.6 (76–88) | | |
| Oes. | 132.3 ± 4.6 (125–141) | 135 ± 6.4 (126–145) | 130 ± 10.5 (109–141) | | |
| Tail | 17.6 ± 3.6 (13–21) | 21.3 ± 3.2 (17–25) | 31 ± 6.5 (22.5–43) | | |
| BW 47.6 ± 6.1 (37–56) | | 48.7 ± 4.6 (41–56) | 65 ± 7.1 (53–74) | | |
| R | 102 ± 2.5 (99–107) | 81.6 ± 2.6 (78–88) | 60.1 ± 2.7 (57–65) | | |

Table 1. Morphometric characteristics of *M. xenoplax, M. antipolitanum,* and *C. informis* used in this study. All measurements are in μ m given as mean ± SE (standard error), and range of minimum and maximum measurements.

| Rst | 16 ± 08 (15–17) | 14.5 ± 0.9 (13–18) | 11.1 ± 1.1 (10–14) |
|-------|---------------------------|-----------------------------|---------------------------|
| Roes | 24 ± 1.6 (23–28) | 22.8 ± 1.4 (21–25) | 16.4 ± 1.8 (14–21) |
| RV | 6.1 ± 0.5 (6–8) | 6.7 ± 0.4 (6–9) | $6.2 \pm 0.5 (5-7)$ |
| Ran | $3.5 \pm 0.6 (3-5)$ | 5.4 ± 0.4 (5–6) | $3.6 \pm 1 (2-5)$ |
| Rvan | $2.7 \pm 0.6 (2-3)$ | $0.3 \pm 0.4 (0-1)$ | 2.4 ± 0.4 (2–3) |
| VL/VB | $1.1 \pm 0.1 (0.9 - 1.2)$ | $0.8 \pm 0.1 \ (0.6 - 1.1)$ | $1.2 \pm 0.3 (0.8 - 1.4)$ |

N: number of specimens measured; L: Body length, A: Body length/maximum body width, B: Body length/oesophageal length, C: Body length/tail length, V: distance from head end to vulva/body length ×100, Stylet: stylet length, Oes.: Oesophageal length, Tail: tail length, BW: maximum body width, R: total number of body annules, Rst: number of annules in stylet region, Roes: number of annules in oesophageal region, RV: number of annules between posterior end of body and vulva, Ran: number of annules on tail, Rvan: number of annules between vulva and anus, VL/VB: distance between vulva and posterior end of body/body width at vulva.



Figure 2. Diagnostic characters in the females of *Mesocriconema antipolitanum*. (**A**): Total body; (**B**) and (**C**): detail of the anterior end stylet and pharyngeal region; (**D**,**H**): head with big sub-median lobes; (**E**,**F**,**I**,**H**): posterior end, vulva, and tail region; (**G**): surface margins; and (**K**): pharyngal region. (Scale bars: (**A**–**C**,**E**,**F**) = 100 µm; (**D**,**G**,**K**) = 20 µm).



Figure 3. Diagnostic characters in the females of *Mesocriconema xenoplax*. (**A**): Total body; (**B**,**C**): details of the anterior end stylet and pharyngeal region; (**D**,**G**): head with small sub-median lobes; (**H**–**J**): posterior end, vulva, and tail region; (**F**): surface margins; and (**K**): pharyngal region. (Scale bars: (**A**–**C**,**E**) = 100 μm; (**D**,**F**–**K**) = 20 μm).



Figure 4. Diagnostic characters in the females of *Criconemoides informis*. (A): Total body; (B,C): detail of the anterior end stylet and pharyngeal region; (D,H): head with sub-median lobes; (E,F,I,J): posterior end, vulva, and tail region; (G): surface margins; and (K): pharyngal region. (Scale bars: (A–C,E,F) = 100 μ m; (D,G,K) = 20 μ m).

3.2. Fungal Spore Analysis

ANOVA showed that the main and mutual effects of mycorrhiza and nematode inoculation had a significant effect on the number of mycorrhizal fungal spores in all three host plants (Table 2). When the combined effects of criconematids and AMF on the spore population was evaluated, differential responses were observed in the three host plants: the highest number of fungal spores was found in the nematode-free treatment of alfalfa, in the inoculation with AMF and *C. informis* in sugar beet, and in inoculation with AMF and *M. antipolitanum* in wheat (Figure 5 A–C). The lowest values were found in the treatment with AMF and *C. informis* in alfalfa, with AMF and *M. antipolitanum* in sugar beet, and with AMF and *M. xenoplax* in wheat (Figure 5 A–C). Therefore, alfalfa was the only host plant that showed a progressive decrease in the spore number from the nematodefree treatment to the *Mesocriconema* species (i.e., *M. antipolitanum* and *M. xenoplax*) and *C. informis* treatments (Figure 5A). Sugar beet displayed an increase in spore population only in relation to *C. informis* treatment, while the other three treatment combinations did not reveal significant differences (Figure 5B). In wheat, greater variability was recognizable, even if the resulting spores were higher in relation to *M. antipolitanum*, while free-nematodes and *M. xenoplax* treatments were similar (Figure 5C).

| II. at Diant | Treatments | Degree of freedom | F values and Significance Levels | | |
|--------------|-----------------|-----------------------|---|--|---|
| Host Plant | | | Root Length (cm) | Nematode Population | Spore Population (per 1 g Soil) |
| Alfalfa | Fungus | 1 | 232.68 ** | 119.21 ** | 1412.08 ** |
| | Nematode | 3 | 26.85 ** | 314.76 ** | 4.29 * |
| | Fungus×Nematode | 3 | 7.03 ** | 21.76 ** | 4.29 * |
| | Tukey test | Pair-wise comparisons | (-) Nemas > than in all the other treat- | (+) Mesocriconema antipolitanum > than (+) | (-) Nemas > than (+) <i>Criconemoides</i> |
| | | | ments ** | Mesocriconema xenoplax and (+) Criconemoides | informis * |
| | | | | informis ** | |
| Sugar beet | Fungus | 1 | 623.91 ** | 76.23 ** | 4471.12 ** |
| | Nematode | 3 | 19.54 ** | 521.34 ** | 5.80 ** |
| | Fungus×Nematode | 3 | 5.20 * | 9.34 ** | 5.80 ** |
| | Tukey test | Pair-wise comparisons | (-) Nemas > than (+) <i>Mesocriconema</i> | (+) Criconemoides informis > than (+) | (+) Criconemoides informis > than (-) |
| | | | antipolitanum and (+) Criconemoides | Mesocriconema xenoplax and (+) Mesocriconema | Nemas and (+) Mesocriconema |
| | | | informis * | antipolitanum ** | antipolitanum * |
| Wheat | Fungus | 1 | 1096.40 ** | 103.64 ** | 3068.46 ** |
| | Nematode | 3 | 35.62 ** | 129.97 ** | 15.33 ** |
| | Fungus×Nematode | 3 | 8.93 * | 11.86 ** | 15.33 ** |
| | Tukey test | Pair-wise comparisons | (-) Nemas > than (+) <i>Mesocriconema</i> | (+) Mesocriconema antipolitanum and (+) Cricone- | (+) <i>Mesocriconema antipolitanum</i> > than |
| | | | antipolitanum and (+) Criconemoides | moides informis > than (+) Mesocriconema xeno- | all the other treatments * |
| | | | informis ** | plax * | |

Table 2. Analysis of variance (ANOVA) on the effects of fungus and nematode treatments on the root length of alfalfa, sugar beet, and wheat; spore and nematode populations ((-) = without inoculation; (+): with inoculation; * p < 0.05; ** p < 0.001).



Figure 5. Main effects of arbuscular mycorrhizal fungi (AMF) and criconematids treatments on the number of fungal spores, nematodes, and root length of the three host plants. The treatments are indicated with numbers as follow: 1: without AMF and nematodes inoculation; 2: with AMF inoculation but without nematodes; 3: without AMF but with *M. antipolitanum* inoculation; 4: with both AMF and *M. antipolitanum* inoculation; 5: without AMF but with *M. xenoplax* inoculation; 6: with both AMF and *M. xenoplax* inoculation; 7: without AMF but with *C. informis* inoculation; and 8: with both AMF and *C. informis* inoculation).

3.3. Nematode Population Analysis

As reported in Table 2, ANOVA showed a significant increase in the three criconematid species in all three host plants when they were compared with the treatments without AMF inoculation (Figure 5 D–F). To describe in detail, *M. antipolitanum* appeared to be more advantaged by AMF inoculation in alfalfa, and *C. informis* more in sugar beet, while both *M. antipolitanum* and *C. informis* species were positively influenced by AMF in wheat (Figure 5 D–F).

3.4. Plant Root-Length Analysis

The ANOVA results showed that the single factors (i.e. AM fungus or nematode species), as well as their interactions, had significant effects on the root length in all three host plants (Table 2). To discuss in more detail, AMF treatment appeared to affect root length; indeed, AMF alone (i.e., nematode-free treatments) corresponded to the highest root length in all three host plants (see Figure 5 G–I) (mean values of 3.39, 2.02, and 4.76 cm for alfalfa, sugar beet, and wheat, respectively); meanwhile, in all the treatments without mycorrhiza inoculation, average root lengths were lower (mean values of 1.68, 0.93, and 1.64 cm for alfalfa, sugar beet, and wheat, respectively) (Figure 5 G–I). Despite this, the controls did not reveal significant differences with the treatments when based only on the inoculation of one of the three criconematid species (i.e., *M. antipolitanum, M. xenoplax,* and *C. informis*); the interaction between AMF and the three ectoparasites revealed significantly higher values of the root length, especially when compared to the treatments without mycorrhiza (Figure 5 G–I).

4. Discussion

This is the first study of the effect of the arbuscular mycorrhiza fungus *F. mosseae* on the population densities of the three criconematid species *M. antipolitanum*, *M. xenoplax*, and *C. informis*. Overall, in alfalfa, the highest number of fungal spores was obtained from the nematode-free treatment; however, in sugar beet and wheat, *C. informis* and *M. antipolitanum* treatments had the highest number of spores. The trends observed seem to support the idea that both of the different host plants alone (e.g., alfalfa seems to be the most suitable host plant for the symbiosis with *F. mosseae*) and the interaction host plants × AMF × ectoparasite species (i.e., AMF + *C. informis* and *M. antipolitanum* in sugar beet and wheat) could affect the spore population. Based on the number of individuals of each criconematid species produced in each host plant's rhizosphere, it could be concluded that alfalfa and wheat are the primary host plants for *M. antipolitanum*, while sugar beet would be more suitable for *C. informis*. However, in wheat, overall, the total number of all three criconematid species was rather low.

Since nematode damage is directly related to their population density, their increasing abundance led to a higher impact on the host plants.

Therefore, the observation of a higher abundance of the criconematid species in the treatments with AMF inoculation, as compared to those without AMF, made it possible to conclude that the mycorrhizal fungus *F. mosseae* not only failed to control the ecto-parasites under scrutiny but even increased their populations with a highly significant impact on the plants under scrutiny (see Figure 6). These findings are in contrast to the majority of the studies, which show the effects of AM fungi on decreasing the damages and populations of some endoparasite nematodes such as *Meloidogyne hapla*, *M. incognita*, *M. javanica*, and *Pratylenchus penetrans* [5,17,21,24,25,48]. However, similar to our study, Bell et al. 2022 [32] have recently documented the increased abundance and fitness of the endoparasite *Globodera pallida* on potato plants colonized by AMF (i.e., *Rhizophagus irregularis*), compared with non-colonized plants. AMF-acquired nutrients may not solely benefit the host plant but may also enhance the fitness of *G. pallida* while simultaneously lending tolerance to the host plant against an increasing parasite burden [32]. The mechanisms by

which AMF can exert bio-control against PPNs are numerous (e.g., increasing host-plant nutrient uptakes, the alteration of root morphology that facilitates direct nutrient uptake, direct competition for nutrients and space, induced systemic resistance, and altered rhizosphere interactions [5]) and cannot be considered as completely independent from each other. In our study, the increasing root length (due to the increase in root branching) exerted by the AMF in all the three host plants might be related both to a different nutrient supply, AMF, and nematode effects. However, since soil and nutrition conditions were the same for all treatments, it can be concluded that the increase in the root length was only positively influenced by mycorrhizal fungus.



Figure 6. Schematic representation of the results obtained from the arbuscular mycorrhizal fungi (AMF) and nematode species inoculation treatments on the root length of three host plants (Ma = *M. antipolitanum*; Mx = *M. xenoplax*; and Ci = *C. informis*) (Figure by Boyno, G.).

5. Conclusions

Despite the promising results on endoparasite nematodes, this research revealed an increase in the population of *M. antipolitanum*, *M. xenoplax*, and *C. informis* when the plant host was treated with *F. mosseae*. This could be related to the feeding strategy of Criconematidae as migratory ecto-parasites. Criconematidae can be regarded as k-strategists in feeding because of their high competition for nutrition space around roots. Compared to other ectoparasitic nematodes, these nematodes move very slowly, and for this reason, they perform better in occupying the feeding places around the roots. Thus, it can be argued that the mycorrhizal fungus may increase the host root surface, resulting in a higher root surface available for criconematidae activity and nutrition and a consequent increase in their population densities. Therefore, *F. mosseae* did not appear to be a biological agent suitable for the control of Criconematidae in agriculture; while other AMF species should be investigated, the action mechanism with which AMF acts cannot limit the occurrence of these nematode species. Studying the changes of different elements in plant tissues in the presence of mycorrhizae and these nematodes can help one to better understand the relationship between these factors.

Author Contributions: Conceptualization, Y.R.D. and A.M.H; methodology, A.M. and G.B; validation, Y.R.D., A.M.H. and S.D.; formal analysis, Y.R.D., A.M.H., L.C. and F.S.; investigation, A.M. and G.B.; resources, Y.R.D.; data curation, S.D.; writing—original draft preparation, Y.R.D., F.S. and L.C.; writing—review and editing, Y.R.D. and F. S.; supervision, Y.R.D.; and funding acquisition, Y.R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Institutional Review Board Statement: Not applicable

Conflicts of Interest: The authors declare no conflict of interest.

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