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Association between arbuscular mycorrhizal fungi and *Pratylenchus brachyurus* in maize crop

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ABSTRACT

Maize (*Zea mays* L.) is one crop of major relevance in Brazil; however, nematodes are limiting factors in maize growth and productivity due to the difficulty to control them. Studies point out that arbuscular mycorrhizal fungi (AMF) increase plant tolerance to nematodes. Thus, the aim of this study was to assess the association between AMFs and *Pratylenchus brachyurus* in maize growth. Six AMF species (*Rhizophagus clarus*, *Claroideoglomus etunicatum*, *Gigaspora rosea*, *G. margarita*, *Scutellospora calospora*, *S. heterogama*), as well as a control treatment free of mycorrhizae, were assessed under two different nematode inoculum conditions (presence and absence). Plants were collected and analyzed 60 d after inoculation. The nematode population was higher in the treatments inoculated with AMFs. On the other hand, just the plants inoculated with *R. clarus* did not present improved development in comparison to the control. Nematodes increased AMF spore density, but despite the fact that AMFs have presented different root colonization capacity (ranging from 2.3% to 95.0%), such capacity was not affected by the nematodes.

Key words: Management, mycorrhizal, root-lesion nematode, *Zea mays*.

INTRODUCTION

Brazil is the third greatest maize (*Zea mays* L.) producer and the second exporter in the world (DEAGRO/FIESP, 2018). Maize is one of the crops of major economic relevance in the country, despite being one of the main alternatives for crop rotation and succession in soybean plantation areas (Inomoto, 2008). There is a whole variety of production systems, which go from high technological levels adapted to the national and international markets, to the total absence of technology in subsistence productions.

However, maize is severely attacked by nematodes, mainly by those belonging to genus *Pratylenchus* Filipjev, which is considered one of the most important for this crop. The main species causing the worse losses in maize crops are *Pratylenchus zeae* Graham and *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven (Goulart, 2008). The fact that maize is a crop used for soybean succession – soybean is also a good *P. brachyurus* host – in association with no-tillage, which keeps high soil humidity and enables root permanence in the field, promotes constant nematode population increase in the area and impairs a better control (Inomoto, 2008).

The main methods to manage this nematode are crop residue destruction, crop rotation, use of cultivars or resistant hybrids, and chemical and biological control. Biological control stands out as a feasible management alternative against

nematodes, because it minimizes environmental damages and may present good cost-benefit ratio in comparison to chemical treatments (Coimbra and Campos, 2005).

Different fungi and bacteria have been assessed for nematode biological control purposes (Sharma and Sharma, 2017). The arbuscular mycorrhizal fungi (AMFs) stand out among these species, they are distributed in different ecosystems, and associate themselves with 90% of the plant species (Smith and Read, 2008; Schouteden et al., 2015). They increase climatic stress tolerance, generate plant resistance against pathogens and reduce costs with fertilizers, since these fungi enlarge the nutrient-absorption area and allow the access to less-available nutrients or to nutrients presenting low mobility in the soil (Smith and Read, 2008; Schouteden et al., 2015).

Studies have been reporting that AMFs can increase plant tolerance to *Pratylenchus* species in some crops (Talavera et al., 2001; Elsen et al., 2008; Vos et al., 2012). However, the species *P. brachyurus* imposes research restrictions, because there are reports about nematode-tolerance increase in cotton crops colonized by AMF *Gigaspora margarita* Becker and Hall (Hussey and Roncadori, 1978).

Because of lack of information about the relation between AMF and *P. brachyurus*, the aim of the present study was to assess the association between AMFs and *P. brachyurus* in maize for nematode control purposes. Moreover, it aimed at studying the colonization capacity of mycorrhizal and plant growth.

MATERIALS AND METHODS

The experiment was conducted in greenhouse (23º47'28.46'' S, 53º15'23.46'' W; 430 m a.s.l.) The completely randomized design was adopted at factorial scheme 7 × 2, wherein seven is the number of treatments using AMFs (six AMFs + AMF free control) and two is the number of nematode inoculum conditions, namely: presence or absence of nematodes, with eight replicates for each treatment. The experiment was conducted between November 2014 and January 2015, when the mean minimum and maximum temperatures were 20.8 and 31.2 °C, respectively.

Firstly, the sandy soil (LVd19 – Dystrophic Red Latosol) was collected and doubly autoclaved at 120 °C for 2 h, with one day interval between autoclaving. Each pot was filled with 3 L soil; the autoclaved soil covered 3/5 of the pots. Subsequently, 1/5 of soil inoculum, containing approximately 250 spores of each AMF treatment, was added in the pots, followed by 1/5 of autoclaved soil. Nematode-free pots were used as control.

The AMF *Rhizophagus clarus* (Nicolson and Schenck) Walker and Schüssler, *Claroideoglomus etunicatum* (Becker and Gerd.) Walker and Schüssler, *Gigaspora rosea* Nicolson and Schenck, *Gigaspora margarita* Becker and Hall, *Scutellospora heterogama* (Nicolson and Gerd.) Walker and Sanders, and *Scutellospora calospora* (Nicolson and Gerd.) Walker and Sanders were selected from a well-established Glomales bank at UNIPAR (Paranaense University, Paraná State, Brazil) accessions number 10, 11, 12, 08, 06, and 04, respectively (Rosseto et al., 2012; Urcoviche et al., 2015).

All AMFs were previously multiplied in maize growing in pots containing 10 kg sterile soil. The species of AMF prior to inoculation were confirmed based on spore morphology, and the same when assessing spore production post-test. These six AMF species were selected due the diversity and importance in Brazilian soils and crops (Rosseto et al., 2012; Urcoviche et al., 2014); as reported by Veresoglou and Rillig (2012), AMF identity also is crucial for pathogen protection as nematodes.

Four maize seeds 'IPR 114' were sown in each pot, at the same day the soil was prepared. Thinning was performed 6 d after germination, and one seedling was kept in each pot. In half pots, the seedlings were inoculated with a population of 1000 *P. brachyurus* specimens in 4 mL solution, which was deposed in three holes, opened in the soil around the plant stem. The inoculum derived from a pure nematode population kept in maize, in greenhouse, during 2-mo. The specimens were extracted from roots through process performed in a blender, followed by centrifugation in sucrose solution (Coolen and D'Herde, 1972). The Peters' slide was used for nematode counting in optical microscope (100X).

Plants were collected 2-mo after inoculation and their shoots were separated from their root systems. The height and shoot fresh and dry weight were recorded. The dry weight was obtained after drying in forced air flow oven, at 65 ºC, until constant weight. The root system was carefully washed, and root fresh weight was subsequently recorded; 2 g root were separated from the median region to assess AMF root colonization. The rest of the root system was subjected to extraction and to *P. brachyurus* evaluation per root system, through extraction and counting, as aforementioned. The total number of nematodes was divided by the root weight in order to determine the number of nematodes g^{-1} root.

The collected root portion was cut in pieces of approximately 1 cm to set the root colonization by AMFs. The root pieces were inked through the method proposed by Phillips and Hayman (1970). Then, they were preserved in lacto-glycerol until the moment to prepare the slides. The analysis applied to the percentage of colonized segments was performed using microscopy slides. One hundred segments were analyzed per replicate, in each treatment (Giovannetti and Mosse, 1980), in stereoscopic microscope (100X) to identify the areas colonized by hypha, arbuscules and vesicles. The total root colonization by AMFs was transformed for data normalization through the equation: Total colonization $(Col_t) = (ArcSin\sqrt{Col(\%)/100})(180/\pi)$, where Col_t is the total root colonization, ArcSin is the inverse of sine, π is Pi.

The total of 100 cm^3 soil were removed from the rhizosphere region of each pot. The removed soil was subjected to nematode extraction process (Jenkins, 1964) and samples were assessed according to the previously mentioned procedure. Moreover, 10 g rhizosphere soil were also collected to AMF spore extraction, which was carried out according to the wet sieving method (Gerdemann and Nicolson, 1963). The samples were subjected to centrifugation in water (3000 rpm 3 min^{-1}) and in 50% sucrose (2000 rpm 2 min^{-1}) for counting; supernatant was once more wet sieved through 0.053 mm mesh. The extracted spores were preserved in 0.05% azide-sodium solution, until the time to perform the counting - when the samples were transferred to Petri dishes and counted in stereoscope microscope (40X).

The variance normality of data was assessed through Shapiro Wilk test, and through transformation by $\sqrt{(x+0.5)}$ - whenever necessary, except for colonization by AMFs. The collected data were subjected to ANOVA at 5% error probability. The mycorrhizal and nematode factors were compared to each other through the Scott-Knott and Bonferrini's *t* tests, respectively, at the same significance level, in the SisVar software (Ferreira, 2014).

RESULTS

All the assessed nematological variables presented larger numbers in plants inoculated with AMFs in comparison to non-inoculated plants. The highest values of *P. brachyurus* root system and total nematodes were recorded in plants inoculated with *S. heterogama*, *R. clarus* and *C. etunicatum* (Table 1).

The Mycorrhizal × Nematode interaction was significant ($p < 0.05$) in the variables shoot height and fresh weight. Mycorrhizal disruption for the nematode factor was significant in plants free from *P. brachyurus* in both variables (Table 2). Accordingly, the greatest development recorded for these variables (shoot dry weight, SDW) was observed in nematode-free plants when they were inoculated with *S. heterogama*, *C. etunicatum*, *S. calospora* and *G. rosea*. The presence of nematodes increased shoot height and fresh weight in the AMF-free control and in plants inoculated with *G. margarita*. The mycorrhizal factor in SDW was significant, but there was no interaction between factors. All other AMFs accept for *R. clarus*, which did not differ from the control, promoted SDW increase by 34% to 67% (Table 2).

Mycorrhizae and interaction between factors did not change root weight, but this variable was just affected by the presence or absence of nematodes. Thus, plants colonized by nematodes presented root weight (21.48 g) greater than nematode-free plants (18.02 g) (data not shown).

The presence of nematodes positively affected AMF spore density - general average 0.97 and 2.04 spores g^{-1} in plants with and without nematode, respectively. The interaction between factors was also significant ($p < 0.05$) for spore density. Greater spore density was recorded in maize inoculated with *R. clarus* when the mycorrhizal factor was assessed within the

Table 1. *Pratylenchus brachyurus* (Pb) per root system, per root gram, and total population in maize subjected to different treatments using mycorrhizae.

Treatments	Pb/root system	Pb g^{-1} root	Pb total
Scutellospora heterogama	21925a	1230a	22337a
Rhizophagus clarus	21345a	1341a	21795a
Claroideoglomus etunicatum	14150a	665a	14840a
S. calospora	10085b	557a	10127b
Gigaspora margarita	10012b	621a	11047b
G. rosea	9712b	710a	9910b
Control	2525c	123b	2847c
CV, %	24.53	31.76	23.73

Means followed by the same letter in the columns did not differ from each other, according to the Scott-Knott test. CV: Coefficient of variation.

Table 2. Shoot height and fresh weight in mycorrhized plants with and without nematodes and general mean shoot dry weight of maize subjected to different treatments using mycorrhizae.

Treatments	Height		Shoot fresh weight		Shoot dry weight
	Without nematode	With nematode	Without nematode	With nematode	General mean
	cm		g		g
Scutellospora heterogama	67.30aA	70.10aA	19.18aA	25.36aA	4.32a
Rhizophagus clarus	58.40bA	65.30aA	13.34bA	18.76aA	2.57b
Claroideoglomus etunicatum	69.30aA	76.30aA	22.09aA	31.11aA	4.60a
S. calospora	67.60aA	66.80aA	22.59aA	22.42aA	4.33a
Gigaspora margarita	58.40bB	71.10aA	14.11bB	24.84aA	3.68a
G. rosea	65.20aA	66.80aA	20.00aA	23.33aA	3.93a
Control	48.70bB	72.20aA	8.01bB	25.50aA	2.75b
CV, %	14.68		40.67		45.15

Means followed by the same lower-case letter in the columns and by the same uppercase letters on the lines did not differ from each other, according to the Scott-Knott and Bonferroni-*t* tests.
CV: Coefficient of variation.

Table 3. Spore density and root colonization by arbuscular mycorrhizal fungi in maize plants, with or without *Pratylenchus brachyurus*.

Treatments	With nematode	Without nematode	Colonization
	Number spores g^{-1} dry soil		General mean
			%
Scutellospora heterogama	1.45bA	0.47bA	11.70b
Rhizophagus clarus	7.09aA	3.10aB	95.20a
Claroideoglomus etunicatum	1.13bA	1.27bA	13.50b
S. calospora	1.88bA	0.90bA	12.90b
Gigaspora margarita	0.72bA	0.49bA	10.60b
G. rosea	1.64bA	0.60bA	2.30c
Control	0.30bA	0.00bA	3.50c
CV, %	82.33		39.44

Means followed by the same lower-case letter in the columns and by the same uppercase letters on the lines did not differ from each other, according to the Scott-Knott and Bonferroni-*t* tests.
CV: Coefficient of variation.

nematode factor, whereas nematode disruption within mycorrhizae showed that plants treated with *R. clarus* containing nematodes presented spore density higher than plants without nematode inoculation.

The root colonization, in its turn, was affected by mycorrhizae regardless of the presence or absence of the nematode. All other treatments presented greater colonization, mainly plants colonized by *R. clarus* (95.20%), except for *G. rosea*, which did not differ from the control (Table 3).

DISCUSSION

Regardless of the studied AMF species, *P. brachyurus* population increased in mycorrhized plants, and this result is different from reports in the literature (Vos et al., 2012; Veresoglou and Rillig, 2012; Baum et al., 2015; Schouteden et al., 2015). Studies have been pointing out that the best results for nematode management using mycorrhizae are recorded when AMFs are applied to the soil at least 30 d before sowing in comparison to the nematode. There are reports describing that colonization in some plants may happen within 2 to 4 wk (Alkan et al., 2006; Jansa et al., 2008). A well-succeeded colonization seems to be the requirement for effective nematode control (Pozo and Azcón-Aguilar, 2007).

However, this time advantage did not happen in agricultural soils in which nematodes and mycorrhizae are found and may also show the same competitive advantage (Schouteden et al., 2015). Moreover, results depend on a series of factors

including AMF isolates, host plant species, pathogens in association with the plant species, and soil and environmental conditions (Whipps, 2004; Veresoglou and Rillig, 2012; Baum et al., 2015).

Overall, plants trigger a basal defense response called PTI (PAMP-triggered immunity, in which PAMP is pathogen-associated molecular pattern) in the interaction location when they are infected by pathogens; however, AMFs are capable of suppressing this initial defense response in order to allow colonization to take place (Bhargava and Nair, 2016). Plants colonized by AMFs accumulate high jasmonic acid (JA) levels (Nair et al., 2015). This acid antagonizes the salicylic acid (SA) signaling, which, in turn, plays an important role in PTI expression. However, the defense signaling via SA is suppressed by high JA concentrations (López-Ráez et al., 2010; Thaler et al., 2012), fact that may have favored the initial nematode penetration and increased the number of nematodes in mycorrhized plants. Such result may explain the large number of *P. brachyurus* in plants inoculated with *S. heterogama*, *R. clarus* and *C. etunicatum*, which have recorded the greatest root colonization.

The effects from the interaction between nematode and AMF, which is expressed by the host's response, can be classified as neutral when there are no evident changes in AMFs, hosts or in the nematode. These effects are positive when plant damages caused by pathogens are compensated by the AMF, and when the development and number of nematodes are suppressed. The effects are negative if AMF sporulation and/or plant development are suppressed and nematode reproduction increases (Hussey and Roncadori, 1982).

Interaction results in the present study can be classified as negative, since there was nematode-number increase and the mycorrhizal presence in plants inoculated with nematodes did not influence their growth. Similar results were recorded in other pathosystems wherein the AMF/*Pratylenchus* interaction did not stimulate plant growth (Calvet et al., 1995; Pinochet et al., 1998). Such response can happen because colonization by AMFs may generate high energy cost for plants and, in some cases, such cost cannot be compensated by further mycorrhization (Vos et al., 2012). However, it is worth highlighting that there are reports about mycorrhizae promoting growth in cotton plants infected by *P. brachyurus* (Hussey and Roncadori, 1978) and in carrot plants infected by *P. penetrans* (Cobb) Filipjev and Schuurmans Stekhoven (Talavera et al., 2001).

Despite the results of plants inoculated with nematodes, the positive effect of mycorrhizae on maize crop can be observed in some interactions. Thus, except for *R. clarus*, which did not differ from the control, all other AMFs led to plant growth. The AMFs presented different growth promotion results in their study, and *G. margarita* and *R. clarus* had worse performance than *C. etunicatum*. Such result derived from the benefits provided by AMFs, which are correlated with the combination between plant-fungus and edaphoclimatic conditions (Smith et al., 2004; Smith and Read, 2008; Baum et al., 2015). Thus, although AMFs are usually beneficial to plants, they can have negative action over the hosts (Smith and Read, 2008), therefore, mycorrhizae performance is not positively correlated with plant development (Ba et al., 2000). It explains the results recorded for *R. clarus*, which presented lower growth response than the other treatments; however, it was the AMF recording the greatest spore density and root colonization.

The plants with nematodes presented more root weight than plants without them, and it is commonly observed in infected hosts. However, plants develop natural defense mechanisms and face different changes when they are infected by nematodes. It is possible highlighting the hormonal metabolism among these changes, because it acts in the concentrations of auxins, cytokinins and ethylene (Faria et al., 2003; Stangarlin et al., 2011). Auxins and cytokinins regulate root growth; in case of stress, ethylene can act in root horizontal growth (Taiz and Zeiger, 2013). Accordingly, it is possible hypothesizing that plants generate new roots when they are infected by nematodes to suppress damages caused by the pathogen.

With regard to the variable "mycorrhizae", plants with nematodes presented greater sporulation than nematode-free plants, and it allows assuming that the competition between nematode and AMF may have stimulated AMF sporulation. Such fact may have occurred when plants were under stress (Smith et al., 2004; Smith and Read, 2008; Baum et al., 2015; Schouteden et al., 2015), and it may even have led to greater *G. margarita* sporulation, as it was reported for tomato plants inoculated with *Meloidogyne javanica* (Treub) Chitwood (Cofcewicz et al., 2001), Sharma and Sharma (2017) also reported physiological and biochemical changes in tomato inoculated with AMF against root-knot nematode, observing that plants treated with inoculation of AMF showed significantly enhanced plant growth and reduced nematode infection.

On the other hand, the presence or absence of nematodes did not influence mycorrhizal colonization, and this result goes against that found by Grümberg et al. (2015). As the nematode inoculation was performed 6 d after mycorrhizal

inoculation, one can assume that the nematode did not influence the initial colonization by the fungus, since these organisms did not compete for infection and colonization sites in the first week. The other treatments presented greater root colonization among the studied AMFs, except for *G. rosea*, which did not differ from the control. *Rhizophagus clarus* presented the highest colonization average (95%) and the best spore density per soil gram. These data were similar to those recorded for *R. clarus* under nematode presence (Grümberg et al., 2015).

CONCLUSIONS

The mechanisms involved in the interactions among mycorrhizae, nematodes and plants remain non-elucidated, mainly because they concern high complexity relations. However, the current study allowed concluding that there was no *Pratylenchus brachyurus* suppression in maize plants inoculated with different arbuscular mycorrhizal fungi (AMFs). Moreover, the AMFs showed different colonization indices in maize and some species were efficient in promoting maize development.

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