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Biology (Plant-Microorganism Interaction)

Suppression of *Pratylenchus brachyurus* and soybean growth inoculated with arbuscular mycorrhizal fungus

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ABSTRACT

Arbuscular mycorrhizal fungi perform a variety of plant-beneficial processes including increased resistance to disease. The objective of this work was to study arbuscular mycorrhizal fungus *RhizoGlomus clarum* effect on phytonematode *Pratylenchus brachyurus* suppression and on soybean plants growth. Two experiments were performed under greenhouse conditions. First soybean plants growth was evaluated in mycorrhizal fungi presence and absence. In the second experiment phytonematode damage in soybean cultivated in mycorrhizal fungi presence and absence was evaluated. During soybean flowering was evaluated mycorrhizal colonization, dry matter, nodulation, chlorophyll and nutrient content in plant tissue, nematodes number in soil and root penetration, and nematode reproduction factor was obtained, *R. clarum* mycorrhizal colonization reduced by 64% the number of nematodes penetrated in roots and increased soybean plants nodulation, nutrient absorption and dry matter accumulation. The stimulation to mycorrhization is a strategy to reduce damage caused by *Pratylenchus brachyurus* to soybean plants.

Keywords: *Glycine max; Rhizoglomus clarum*; Inoculant; Mycorrhization; Phytonematode

1 INTRODUCTION

Soil microorganisms are very important for agricultural systems and are directly related to soil quality and soybean yield (*Glycine max* L. Merril) (MONDANI *et al*., 2019). Mutualistic association between arbuscular mycorrhizal fungi (AMF) and plants provides an increase soil volume explored by the root system (MATSUO *et al*., 2012; SCHNEIDER *et al*., 2016; RASMUSSEN *et al*., 2019), increasing water and nutrients absorption (SMITH e READ, 2008; HASHEM *et al*.,

2018). In addition to the direct benefits for soybean growth, AMF presence also has a positive influence on soil physical and chemical properties (PEREIRA *et al*., 2013; SALGADO *et al*., 2016; EL MUJTAR *et al*., 2019), biological activity near roots (WANG *et al*., 2011) and acts against biotic and abiotic stress (SALGADO *et al*., 2016).

Phytonematoids are among the most important biotic factors in soybean cultivation and have become a serious problem for many crops in recent years (BRIDA *et al*., 2017). Several nematodes species harm soybean, but the *Pratylenchus brachyurus* have been highlighted by the severity of the damage caused to the plants and by the rapid increase of the infested area (FREITAS *et al*., 2017). This nematode is responsible for lesions formation in plants roots due to its feeding habits, movement, and toxins and enzymes injection into the root cortex (SANTANA-GOMES *et al*., 2014). Thus, plants water and nutrient absorption processes are affected, as well as infection by secondary pathogens is facilitated (LIMA *et al*., 2015; FREITAS *et al*., 2017).

Alternatives for phytonematode control have been studied, among them, suppression by AMF (TCHABI *et al*., 2016; RASMUSSEN *et al*., 2019). These fungi are essential for productive systems sustainability, but their populations have been reduced due to inadequate management practices (CASTILLO *et al*., 2016). In Brazil, soybean is usually cultivated with low quality crop rotation and use of large amounts of fertilizers and agrochemicals (BELO *et al*., 2012) which impairs AMFs and favors phytonematodes (PASARIBU *et al*., 2013).

Several studies have used these fungi as antagonists to parasitic nematodes from other agricultural crops, but not to soybean (ELSEN *et al*., 2008; VOS *et al*., 2012). Recently in Brazil, Rootella BR™ mycorrhizal inoculant was registered in Livestock and Food Supply Ministry (Ministério da Agricultura, Pecuária e Abastecimento - MAPA) for use in soybean and corn crops (AGROLINK, 2019). However, studies involving *P. brachyurus* suppression by mycorrhizal fungi in soybean crop are very scarce or nonexistent. Considering this gap of

information and *P. brachyurus* importance for soybean cultivation in Brazil, and in the world, the objective of this work was to study the effect of arbuscular mycorrhizal fungus *RhizoGlomus clarum* on *Pratylenchus brachyurus* suppression and soybean plants growth.

2 MATERIAL AND METHODS

2.1 Soil

A Typic Hapludalf was collected in the 0-20 cm depth layer excluding the surface vegetal residues in a natural pasture area with no crop history. This soil was sterilized by autoclaving for 4 hours at 121°C, dried at room temperature sieved and separated into pots containing 3.5kg of soil. Subsequently, autochthonous microorganisms reinoculation was performed, by the addition of solution extracted from the same non-sterile soil, passing through 500 mesh sieves to exclude presence of nematodes and AMF spores (HAYMANN e MOSSE, 1971). To raise soil pH, calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) were added in 3:1 Ca and Mg molar ratio and the soil was then incubated for 40 days at a humidity equivalent to 70% of field capacity.

Soil fertilization was carried out according to Soil Chemistry and Fertility Commission - CQFS-RS/SC (2016), adding 50% of P recommended dose so that there was no mycorrhizal colonization inhibition of plants according to Ferreira *et al*. (2015) that used both same soil and fungus. Before sowing the soybean 22.9 mg kg^{-1} P and 28 mg kg^{-1} K were added to soil as a KH_2PO_4 solution. Fertilized soil presented the following characteristics: clay (densimeter) 170 g kg^{-1}; pH in water (1:1) 4.9; P (Mehlich^{-1}) 35.7 mg dm^{-3}; K (Mehlich^{-1}) 196 mg dm^{-3}; organic matter (Walkley-Black) 26 g kg^{-1}; exchangeable Al (KCl 1 mol L^{-1}) 1.1 cmolc dm^{-3}; Ca (Mehlich^{-1}) 3.6 $cmol_c$ dm^{-3}; Mg (Mehlich^{-1}) 1.8 $cmol_c$ dm^{-3}; Base Saturation

40.7%; Saturation by Al 15.5%; Zn(Mehlich^{-1}) 1.4 mg dm^{-3}; Cu (Mehlich^{-1}) 0.2 mg dm^{-3}; S available [$Ca(H_2 PO_4)_2$] 31 mg dm^{-3}; and B (hot water) 0.7 mg dm^{-3}.

2.2 Biological Material

Nematode inoculum was composed of a pure population of *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev, Schuurmans Stekhoven 1941 multiplied in sorghum plants (*Sorghum bicolor* (L.) Moench) cultivar BRS 506, in a greenhouse. Inoculum preparation was carried out by roots grinding, according to technique modified by Bonetti e Ferraz (1981).

Initial inoculum of AMF *RhizoGlomus clarum* (TH Nicolson; NC Schenck) C. Walker e A. Schüßler (formerly *Rhizophagus clarus* and *Glomus clarum*) was obtained from International Glomeromycota Culture Collection (CICG) of Regional University of Blumenau, Santa Catarina State, Brazil and multiplied in culture trap of *Brachiaria decumbens*. Soil spore extraction was performed according to the methodology described in Ferreira *et al*. (2015). Spores were counted using a stereomicroscope microscope and separated in 100 units aliquots in microtubes.

2.3 Experiment

Two experiments were conducted in a greenhouse, arranged in a completely randomized design, with eight replications per treatment using the same soil and following the same installation, conduction and evaluation procedures. In the first experiment, soybean growth was evaluated in two treatments: mycorrhizal fungus *RhizoGlomus clarum* presence and absence in soil. In the second experiment, AMF effect on *Pratylenchus brachyurus* nematode suppression in soybean plants was evaluated, with all pots inoculated with the nematode and soybean cultivated in *R. clarum* presence or absence.

The sowing was carried out with soybean seeds, cultivar Nidera 5909. which were inoculated with *Bradyrhizobium* spp., according to technical recommendations. Under the seeds, approximately 100 *R. clarum* viable spores were added per culture pot. In the soybean V3 stage nematoid inoculation was performed. Three approximately 5 cm deep orifices were opened around seedlings, in which 3 mL of a suspension containing 2000 *P. brachyurus* eggs and juveniles, diluted in 25 mL of water, were added. During the experiment, pots irrigation was performed daily with distilled water, aiming to maintain the soil with approximately 70% of the field capacity.

2.4 Analysis

The evaluations were carried out on soybean flowering. Plants relative chlorophyll content was determined on a portable chlorophyllometer (SPAD-502 Minolta Japan). Readings were taken on central trifolium of last fully developed leaf, at 5 points of leaf of each plant, Shoot, roots and nodules dry matter was determined in a greenhouse with forced air circulation, at 65 ºC, until constant mass. Mycorrhizal colonization was evaluated by Giovannetti e Mosse (1980) methodology, by roots discoloration with 10% KOH and subsequent Trypan blue 0.05% staining. Mycorrhizal colonization percentage of the roots system was evaluated by the presence of hyphae or vesicles presence in 20 segments of the 0.5 cm in length, segments arranged between slides and coverslips, and visualized with a microscope at 40x of magnification. The P, K, Ca, Mg, Fe, Mn, Cu, Zn and S concentrations of the shoot were determined after nitric-perchloric digestion in an atomic absorption spectrophotometer (932 AA, GBC, Australia) (EMBRAPA, 1997). N content was determined by the Kjeldahl^{-1} method after sulfur digestion (BREMNER e MULVANEY, 1982).

Nematodes number in soil was determined according to Noronha *et al*. (2017), by the centrifugal flotation method in sucrose solution. Evaluation of

nematode penetration in the root system followed the root coloring methodology of Byrd *et al*. (1983). Roots were arranged between two glass slides under a microscope with a 40x magnification for counting *P. brachyurus* penetrated number. Nematode reproduction factor (FR = FP / IP) was calculated by dividing the final nematode population in pot (soil + roots) (FP) and the initial inoculated population by pot (IP).

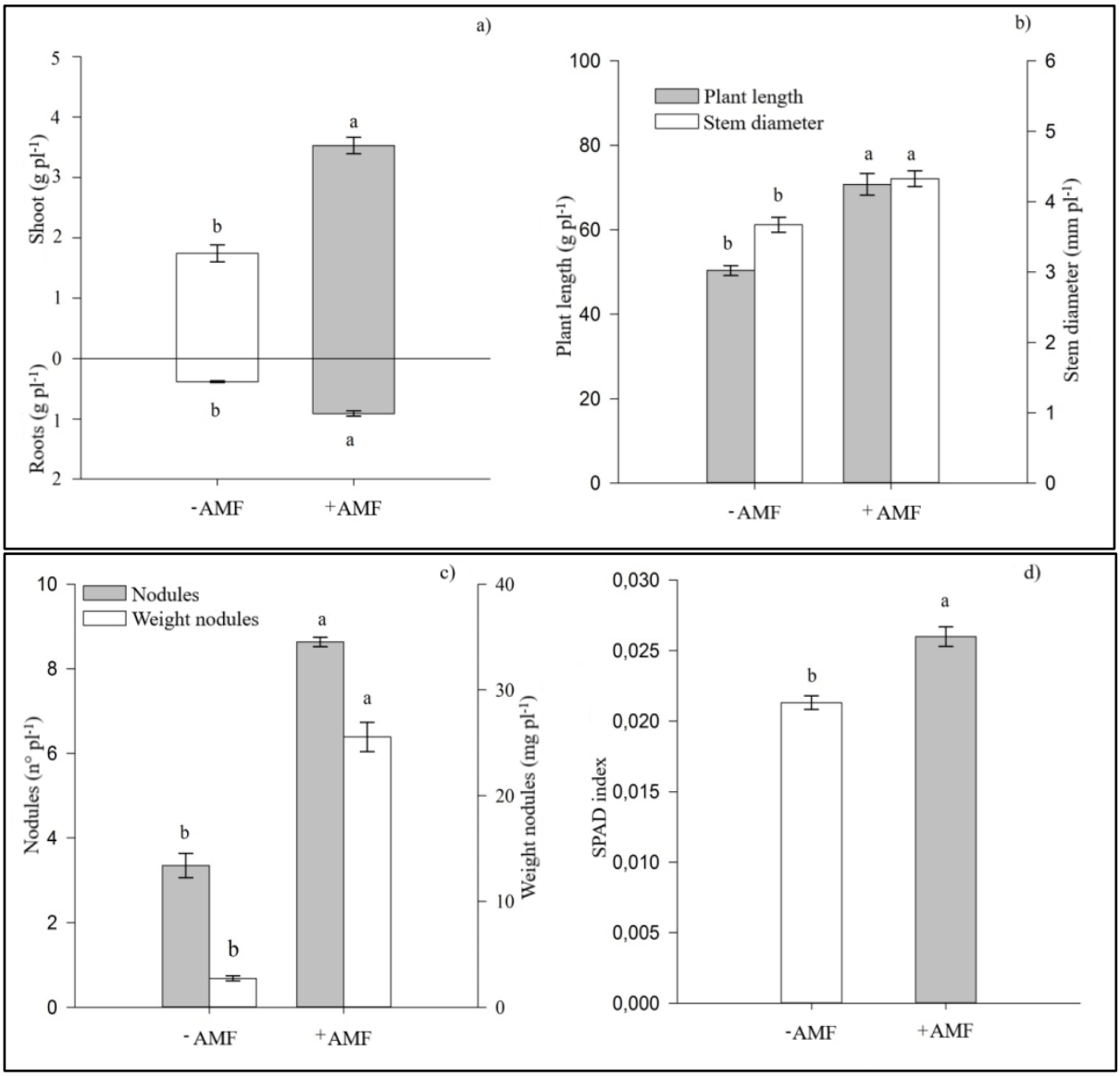
The data were submitted to analysis of variance (ANOVA) and means were compared by t test (LSD) at 5% error probability, with SISVAR software (FERREIRA, 2014).

3 RESULTS AND DISCUSSION

3.1 Experiment I: Soybean Growth

Soybean mycorrhization in fungus *RhizoGlomus clarum* presence was efficient, with approximately 100% of root segments with fungal hyphae and 60% of vesicle segments (data not shown). In high P levels soils root mycorrhizal colonization can be inhibited (SMITH e REED, 2008). In the present study, although the P level of 35.7 mg dm^{-3} is considered as very high by Chemistry and Soil Fertility Commission - CQFS-RS/SC (2016) there was no mycorrhizal colonization inhibition. This fact is in agreement with Ferreira *et al*. (2015), which used same soil and mycorrhizal fungal specie. No mycorrhizal colonization was observed in plants cultivated in *R. clarum* absence. The presence of *R. clarus* in the soil stimulated the growth of soybean plants (Figure 1). The dry matter values of shoot and root system, plant height and stem diameter were higher in plants inoculated with mycorrhizal fungus. A similar result was found by Pereira *et al*. (2013), which observed that the presence of AMF *Gigaspora margarita* and *Glomus clarum* exerts a positive influence on the growth and development of soybean plants, quantified through the dry matter of the shoot and the root system.

Figure 1 – Shoot and root dry mass (a), plant height and stem diameter (b), nodules number and dry mass (c) and chlorophyll relative index (SPAD) (d) at soybean flowering, grown in arbuscular mycorrhizal fungus *RhizoGlomus clarum* presence (+AMF) and absence (-AMF). Data presented are averages of eight replicates. Means followed by same letter were not significantly different according to t test (LSD) at 5% error probability.



Mycorrhizal colonization resulted in a nodulation stimulus (Figure 1c), with a 40% increase in number and 87% in nodules mass. The mycorrhization favors biological activity in the rhizosphere and consequently, nitrogen-fixing bacteria (ANZANELLO, SOUZA, CASAMALI, 2011). Relative chlorophyll index was also 20% higher in plants inoculated with

mycorrhizal fungus (Figure 1d), which may be related to higher biological N fixation and higher nutrient intake due to AMF presence.

The plants without mycorrhizae had higher shoot nutrients concentrations as N, P, Ca, Mg, Mn and Zn (Table 1). This result can be explained by the concentration effect due to lower growth. When accumulated value was calculated, all nutrients were absorbed in greater quantity by plants inoculated with mycorrhizal fungus. Bressan *et al*. (2001) evaluating AMFs *Gigaspora margarita, Glomus etunicatum* and *Glomus clarum* inoculation effect on sorghum and soybean plants, observed that AMF presence increased N, P, K, Zn and Cu concentration in both plants and similarly Ca, Mg and Mn concentrations were higher in the treatment without inoculation, due to nutrient dilution in plant tissue. Results observed in the present work demonstrate that mycorrhization stimulated soybean plants growth and was not affected by high P levels in soil.

Table 1 – Nutrients concentration and accumulation in soybean shoot in presence and absence of arbuscular mycorrhizal fungus (AMF) *Rhizoglomus clarum*.

Nutrient	AMF	Concentration		Accumulation	
		(g kg^{-1})	CV%	(g plant^{-1})	CV%
N	-	45.19 a	2.39	85.41 b	3.31
	+	30.87 b		101.16 a	
P	-	2.17 a	3.98	4.03 b	1.95
	+	1.74 b		5.72 a	
K	-	10.39 b	1.67	20.12 b	1.53
	+	13.52 a		44.42 a	
Ca	-	6.44 a	4.19	12.34 b	4.47
	+	4.42 b		14.55 a	
Mg	-	3.83 a	4.50	7.45 b	5.45
	+	3.45 b		12.92 a	

Continuation...

Table 1 – Conclusion...

Nutrient	AMF	Concentration		Accumulation	
		(g kg^{-1})	CV%	(g plant^{-1})	CV%
S	-	1.87 b	3.15	3.53 b	0.38
	+	2.36 a		7.59 a	
Cu	-	8.79 b	3.03	17.00 b	0.53
	+	11.17 a		35.95 a	
Fe	-	86.23 a	3.64	165.10 b	3.52
	+	93.08 a		308.20 a	
Mn	-	238.14 a	1.89	455.55 b	1.25
	+	222.17 b		737.49 a	
Zn	-	42.92 a	3.95	80.22 b	0.25
	+	36.73 b		118.44 a	

Data presented as averages of eight replicates. Means followed by the same letters did not present statistical differences between treatments with and without AMF inoculation for each nutrient by t test (LSD) at 5% probability of error.

3.2 Experiment II: Phytonematode suppression

An efficient mycorrhizal colonization in plants is a prerequisite for a possible nematode suppression effect (ELSEN *et al*., 2008; SCHOUTEDEN *et al*., 2015). In the present study, *R. clarum* efficiently colonized soybean root system even in *Pratylenchus brachyurus* presence, resulting in 100% colonized roots by hyphae and 35% by vesicles (data not shown). Again, no mycorrhizal colonization was observed in plants not inoculated with *R. clarum.*

Mycorrhization stimulated soybean growth even when soil was infested with phytonematodes. Dry mass increase was of 18% in shoot and 20% in roots (Figure 2a). No statistically significant differences were observed in plant height. However, values tended to be higher in treatment with AMF presence (Figure 2b). Regarding stem diameter, once again plants with mycorrhizae presented a significantly better performance. Talavera *et al*. (2001) observed that *G. mosseae* presence increased carrot plants mass and reduced in 49% nematodes *Pratylenchus* penetrans density in soil. Relative chlorophyll index was higher in the treatment with *R. clarum* inoculation (Figure

2d). This can be a consequence of better nutritional status of plants inoculated with mycorrhizal fungus, also a result of higher nodulation observed in root system (Figure 2c). This result evidences mycorrhization beneficial effects on phytonematode damage attenuation. According to Asmus e Ferraz (2002) there was a reduction in soybean leaves chlorophyll content in nematode *Heterodera glycines* presence.

Figure 2 – Shoot and root dry mass (a), plant height and stem diameter (b), nodules number and dry mass (c) and relative chlorophyll index (d) at soybean plants flowering cultivated in soil infested by *Pratylenchus brachyurus* nematode, in arbuscular mycorrhizal fungus *RhizoGlomus clarum* presence (+AMF) and absence (-AMF). Data presented as averages of eight replicates. Means followed by same letter were not significantly different according to t test (LSD) at 5% probability of error.

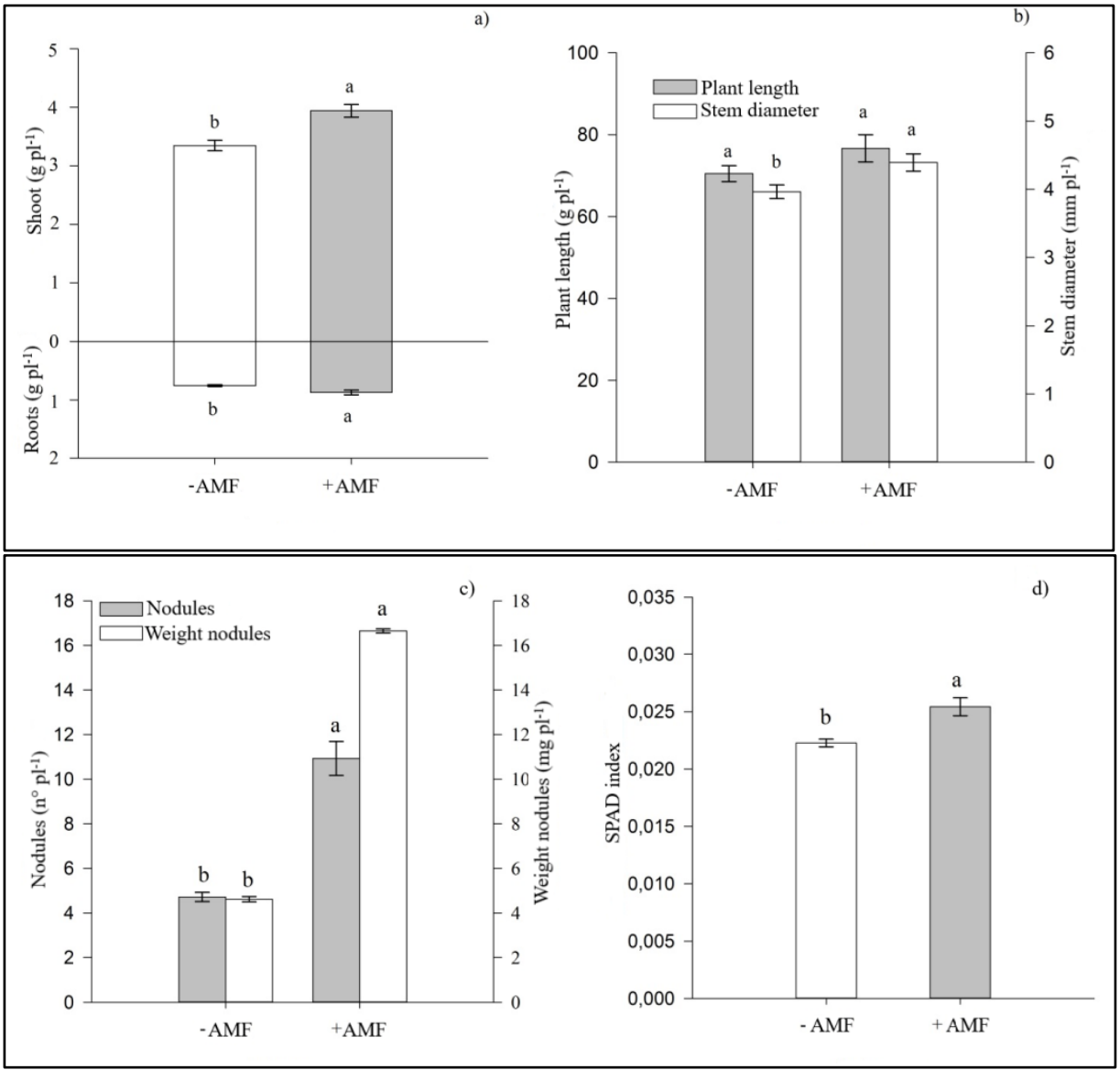


Table 2 – Nematodes number in roots and soil, and reproduction factor (RF) in soybean cultivated in soil infested by *Pratylenchus brachyurus* phytonematode and inoculated or not with arbuscular mycorrhizal fungus *Rhizoglomus clarum*

Treatments	Nematodes in 50 g roots	Nematodes in 100g soil	Reproduction Factor
P. brachyurus	19.75 a	24.99 b	1.43 b
P. brachyurus + AMF	7.11 b	60.51 a	4.22 a
CV%	10.65	15.47	13.64

Data presented as averages of eight replicates. Means followed by same letters in the column did not present statistical differences between treatments with and without *R. clarum* inoculation, by t test (LSD) at 5% of error probability

The AMF presence, besides contributing to plant nutrition, induces the activation of plant defense mechanisms against nematode damages (PEREIRA *et al*., 2013; SALGADO *et al*., 2016; WANG *et al*., 2011). These authors differentiated possible AMF modes of action against nematodes: increased competition for space and food in root tissue; growth stimulus, nutrition and morphological changes in plants, such as increased tissue lignification and suberization, and increased water and nutrients absorption by the root system; biochemical changes related to defense mechanisms and induced resistance of plants, such as modification in root exudates composition, phenolic compounds, hormones and phytoalexins production, which act as repellents to nematodes; and development of an antagonistic microbiota.

Total *P. brachyurus* number in soil was 59% higher when plants were inoculated with *R. clarum* (Table 2). This result can be explained by phytonematodes lower penetration in roots due to AMF presence, resulting in a greater number in soil. Due to the greater nematodes amount present in soil. Reproduction Factor was higher in mycorrhizal fungus presence. Since it is superior to 1, we can infer that the cultivar is susceptible to *P. brachyurus*.

Mycorrhization also improved nutritional status from plants infested by the nematodes (Table 3). Increases in N, P, Ca, Cu, Fe, Mn and Zn in soybean shoot were observed. Only K and Mg were absorbed in greater amounts in the treatment without

mycorrhizal fungus. This result can be attributed to plants metabolic disturbances caused by nematode presence. Plants reaction to nematodes is highly variable and dependent on a number of factors, such as nematode species, host plant and both soil and climatic conditions (ELSEN *et al*., 2008; CAMERON *et al*., 2013; SCHOUTEDEN *et al*., 2015).

Table 3 – Nutrients concentration and accumulation evaluated at flowering in soybean shoot cultivated in soil infested by nematode *Pratylenchus brachyurus* and inoculated or not with arbuscular mycorrhizal fungus *Rhizoglomus clarum*

Nutrient	AMF	Concentration (g kg^{-1})	CV%	Accumulation (g $plant^{-1}$)	CV%
N	-	37.52 a	2.20	122.68 b	0.10
	+	35.46 b		132.39 a	
P	-	1.93 b	2.39	6.27 b	2.91
	+	2.09 a		8.25 a	
K	-	13.57 a	2.50	44.66 a	2.30
	+	10.02 b		37.61 b	
Ca	-	5.45 a	6.73	18.64 b	2.82
	+	5.32 a		20.21 a	
Mg	-	4.30 a	4.93	14.35 a	1.87
	+	3.58 b		12.92 b	
S	-	2.28 a	9.30	7.73 a	2.82
	+	2.19 a		8.07 a	
Cu	-	8.64 b	4.58	27.31 b	0.81
	+	10.38 a		41.48 a	
Fe	-	89.61 b	1.34	296.60 b	2.56
	+	98.02 a		365.98 a	
Mn	-	222.94 a	2.07	729.33 b	3.24
	+	219.82 a		812.37 a	
Zn	-	37.61 a	3.12	124.41 b	0.76
	+	36.47 a		136.14 a	

Data presented as averages of eight replicates. Means followed by the same letters did not present statistical differences between with and without AMF inoculation treatments for each nutrient. by the t test (LSD) at 5% probability of error

Mycorrhizal colonization reduced phytonematode penetration. increased nodulation and nutrient uptake by soybeans. which is directly related to increased tolerance to nematode attack and higher plant growth (LIMA *et al*., 2015; SALGADO *et al*., 2016). Thus, maintenance of high AMF populations in soil may represent a strategy to stimulate plant growth and reduce nematode damage in soybean. For this, adoption of agricultural practices that stimulate soil biological activity is recommended, as crop rotation, permanent soil cover, reduction of soil compaction, erosion, pesticides and synthetic fertilizers, addition of large amounts of organic residues to soil, among others.

4 CONCLUSIONS

Mycorrhizal colonization by *RhizoGlomus clarum* can contribute to reduces *Pratylenchus brachyurus* penetration in soybean roots.

RhizoGlomus clarum mycorrhization increases nodulation, nutrient uptake and dry matter accumulation of soybean plants, in presence and absence of *Pratylenchus brachyurus* in soil.

Promoting mycorrhization is a strategy to reduce *Pratylenchus brachyurus* damage to soybean plants.

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The authors confirm that there are no conflicts of interest associated with this article.

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