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Mycorrhization and grafting improve growth in the tomato and reduce the population of *Nacobbus aberrans*[1]

Micorrização e enxertia melhoram o crescimento de tomateiro e diminuem a população de *Nacobbus aberrans*

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ABSTRACT - Inoculation with mycorrhizal fungi and the use of rootstock that is tolerant or resistant to nematodes are two strategies that allow the nematode to be controlled, and plants to coexist with the pathogen. However, the two techniques have always been tested in isolation, when their positive effects are less compared to when they are able to act together. In the present work, two rootstocks combined with the mycorrhizal fungus *Rhizophagus intraradices* were compared, with the aim of evaluating their behaviour on the growth of tomato plants in soils infested with *N. aberrans*, the false root-knot nematode. The experiment was set up in a completely randomised design with ten replications, in a 3 x 2 x 2 factorial scheme. The treatments consisted of a combination of three factors: a) plant composition: two rootstocks (*Solanum lycopersicum* var. cerasiform 'Carolina' and *S. lycopersicum* 'Maxifort'), onto which the 'Santa Clara' tomato (*S. lycopersicum)* was grafted, and a non-grafted plant, considered the control, which was the same cultivar as the graft; b) mycorrhization: mycorrhizal or non-mycorrhizal roots; c) a substrate infested or not infested with *N. aberrans*. The plants grafted onto 'Maxifort' showed significantly greater growth for shoot dry weight, root fresh weight and stem diameter. The rootstock under test had a lower pathogen reproductive factor than did the ungrafted plant. Mycorrhization contributed to a reduction in the number of days until flowering, and a reduction in the final population of *N. aberrans* for the three plant compositions under test.

Key words: False root-knot nematode. *Rhizophagus intraradices*. Reproductive factor.

RESUMO - A inoculação com fungos micorrízicos e o uso de porta-enxertos tolerantes ou resistentes aos nematoides são duas estratégias que permitem o controle e a convivência das plantas com esse patógeno. Contudo, ambas as técnicas sempre foram testadas de forma isolada, sendo seus efeitos positivos menores, se comparados com a possibilidade de suas somatizações. No presente trabalho compararam-se dois porta-enxertos em combinação com o fungo micorrízico *Rhizophagus intraradices* com o objetivo de avaliar seu comportamento no crescimento de plantas de tomate em solos infestados com *N. aberrans*, o falso nematoide das galhas. O experimento foi instalado em delineamento interiramente casualizado, com dez repetições, sob um esquema fatorial 3 x 2 x 2. Os tratamentos consistiram na combinação de três fatores: a) as composições de plantas, dois porta-enxertos (*Solanum lycopersicum* var. cerasiforme cv. 'Carolina' e *S. lycopersicum* cv. 'Maxifort'), nos quais foram enxertadas *S. lycopersicum* tomate 'Santa Clara'; e, a planta pé-franco, considerada controle, que foi a mesma cultivar utilizada como enxerto; b) micorrização: raízes micorrizadas ou não micorrizadas; c) substrato infestado ou não infestado com *N. aberrans.* Para a massa seca da parte aérea, a massa fresca radicular e o diâmetro do caule as plantas enxertadas em 'Maxifort' tiveram um crescimento significativamente maior. Os porta-enxertos testados tiveram fatores de reprodução do patógeno inferiores ao do Pé franco. A micorrização contribuiu para uma diminuição dos dias ate florescimento e uma redução da população final do *N. aberrans* para as três composições de plantas testadas.

Palavras-chave: Falso nematoide das galhas. *Rhizophagus intraradices*. Fator de reprodução.

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INTRODUCTION

Current knowledge of integrated pest and disease management shows that there is more than one efficient and sustainable tool for controlling such problems. On the contrary, the greater the number of effective tools available, the greater the likelihood of successful crops. For *Nacobbus aberrans* (THORNE, 1935) Thorne & Allen, 1944., also known as the false root-knot nematode, this principle can perfectly well be applied, requiring the study of control methods, both individually and together, which would make production possible in infested areas.

N. aberrans is a sedentary endoparasitic nematode that causes significant production losses in Peru, Bolivia, Chile, Argentina, Ecuador, Mexico, and the United States (EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION, 2018). In Brazil, it is considered a major quarantine pest, classified as A1, as it can potentially cause severe economic damage. *N. aberrans* can parasitise a wide range of hosts, from commercial crops to weeds. The infestation has been identified in 18 botanical families and 84 species. Once the pathogen settles in the root, it produces anatomical changes that cause a water deficit of around 40%. This was verified by a reduction in the stomatal conductance and transpiration of infested plants. As a result, the absorption of macro- and micronutrients becomes deficient, there is a reduction in apical growth, chlorosis and decreased productivity (LORENZO *et al*., 2001). The fruit is smaller in size, less firm, and deteriorates faster compared to fruit from non-parasitised plants (CRISTÓBAL, 2001).

Within this context, grafting and symbiosis with arbuscular mycorrhizal fungi are important as effective tools for controlling soil nematodes, having the advantage of low economic cost and not causing a negative impact on the environment or human health (SCHOUTEDEN *et al*., 2015).

Despite the high number of compatible and efficient rootstocks for tomato (*Solanum lycopersicon* L.) production under various unfavourable conditions, there is no indication of any that might be resistant to *N. aberrans* (CORTADA *et al*., 2010). Some studies which used rootstock carrying the Mi gene, which confers resistance to *Meloidogyne incognita* (KOFOID; WHITE, 1919), *M. javanica* (TREUB, 1885) and *M. arenaria* (NEAL, 1889) in soils infested with *N. aberrans*, presented high reproductive factors for the pathogen (GARBI *et al*., 2013), and as such cannot be recommended as rootstock for the false root-knot nematode.

Mycorrhizae are a symbiotic association formed by phylo Glomeromycota fungi and the roots of most plants (SARABIA OCHOA *et al*., 2010). In the soil, the fungi in the symbiosis present an extensive network of hyphae that favour the absorption of water and nutrients (BONFANTE; GENRE, 2010) allowing a larger volume of soil to be exploited. According to Brundrett and Tedersoo (2018), between 70% and 80% of plants are potentially mycorrhizal. Several researchers have shown the negative effect of mycorrhizal fungi of the genus *Glomus* on populations of the root-knot nematode of the genus *Meloidogyne* (PINHEIRO; SOUZA; COIMBRA, 2014; SOUSA *et al*., 2010), however, there is no consensus on the mechanisms that operate in the relationship.

Based on the above, the aim of this study was to evaluate the effect of two rootstocks and one strain of arbuscular mycorrhizae on tomato plants grown in a substrate, both infested and not infested with *N. aberrans*.

MATERIAL AND METHODS

The experiment was conducted in the experimental area of the Institute of Plant Physiology - INFIVE, of the National University of La Plata and the National Council of Science and Technology, located in the city of La Plata, Argentina, at 34°55' S and 57°57' W.

Seedlings of the 'Santa Clara' tomato were grafted onto two types of rootstock: the 'Carolina' cherry tomato (*Solanum lycopersicon* var. Cerasiform) and the 'Maxifort' tomato (rootstock carrying the Mi gene). As the control for comparing the rootstock, ungrafted 'Santa Clara' tomato plants were submitted to the same treatments. Immediately after grafting, the plants were placed in a humidity chamber under controlled conditions (temperature: 25 °C, luminosity: 12,000 Lux, humidity: 99%, photoperiod: 12 hrs). One gram of mycorrhizal inoculum per cell was placed in half of the trays used.

The inoculum comprised a mixture of soil, fragments of mycorrhizal roots, spores (50 spores.g^{-1} inoculum) and *Rhizophagus intraradices* mycelium. To generate the same conditions of substrate mixture in the treatments with no mycorrhizae, inoculum, autoclaved at 120 °C at a pressure of 1 atmosphere for 90 minutes, was added.

Ten days after grafting, the seedlings were transplanted into 10 L pots, spaced 0.5 m between plants and 1.0 m between rows, and exposed to field conditions under controlled irrigation. A mixture of 75% tyndallized soil and 25% sand was used to fill the pots. The soil used to prepare the mixture was a Vertic Argiudoll, with 5.5 pH, 10 mg.kg^{-1} total P, 3.5% organic matter, 2.0% total C, and 0.24% total N. During the experimental period, the mean minimum temperature in the region was 17 °C, the mean

maximum temperature was 30 °C and the mean relative humidity was 68%.

One day after transplanting the grafted and ungrafted seedlings (control), each pot was inoculated with 5000 eggs *of N. aberrans*. The inoculum was extracted from tomato plants infested with *N. aberrans* only, as per Coolen (1979). For the inoculation, three holes, approximately two centimetres deep, were opened next to the seedlings with the aid of a glass rod. After opening the holes, five millilitres of the solution were deposited on each plant using an automatic pipette. The holes were then closed with substrate taken from the pot.

Starting from when the plants were transplanted, the phenological stages were monitored; 60 days after inoculation, the plants were removed from the pots and the following parameters quantified: a) shoot dry weight (g); b) root fresh weight (g); c) stem diameter, 10 cm below the first bunch; d) final nematode population; e) nematode reproductive factor, as per the methodology of Oostenbrink (1966); f) degree of root colonisation by the mycorrhizal fungus (PHILLIPS; HAYMAN, 1970) and g) viability of the fungal structures, using the succinate dehydrogenase method (SMITH; GIANINAZZI-PEARSON, 1990).

The study was carried out in a completely randomised design, in a 3 x 2 x 2 factorial scheme, of three rootstock cultivars (ungrafted 'Santa Clara' - control, 'Santa Clara' grafted onto 'Maxifort', and 'Santa Clara 'grafted onto 'Carolina'), two mycorrhizal conditions (mycorrhizal plants with *Rhizophagus intraradices* and non-mycorrhizal plants) and two soil conditions (substrate with and without the *N. aberrans* nematode).

In total, 10 replications were carried out per treatment, each replication comprising one plant. The results were submitted to analysis of variance (ANOVA), and the mean values compared by LSD at a significance level of 0.05.

RESULTS AND DISCUSSION

The analysis of variance of the growth parameters under evaluation showed that there are significant differences between the treatments (Table 1).

There were significant differences in the accumulation of shoot dry weight between the rootstock or between the mycorrhizal and non-mycorrhizal plants. 'Maxifort' promoted the greatest accumulation of dry weight and 'Carolina' less accumulation compared to the ungrafted plants (Table 2a). The mycorrhizal plants had greater shoot dry weight than did the non-mycorrhizal plants (Table 2b).

Both rootstocks showed greater root fresh weight than did the ungrafted plants, with 'Maxifort' having the greatest growth under all the experimental conditions (Table 3a and b). The roots of 'Carolina' and Maxifort had significantly greater growth when mycorrhizal, but this response was not seen in the ungrafted plants (Table 3a). Infestation by *N. aberrans* contributed to a reduction in root fresh weight in the ungrafted plants and those grafted onto 'Carolina'. Root weight in 'Maxifort' was not affected by the presence of the nematode (Table 3b).

The plants grafted onto 'Maxifort' had larger-diameter stems, larger than those produced by the ungrafted plants, with the 'Carolina' rootstock showing significantly smaller diameters than did the ungrafted plants.

Table 1 - Analysis of variance of the growth parameters of plants of the 'Santa Clara' tomato (*Solanum lycopersicon* L.), ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, mycorrhizal and non-mycorrhizal, and in soil infested and not infested with *Nacobbus aberrans*

Sv	----------Fc----------		
	Shoot dry weight	Root fresh weight	Stem diameter
Rootstock (R)	282.27**	201.39**	158.5**
Mycorrhizal (M)	42.29**	24.29**	3.56
Nematode (N)	2.59	73.77**	3.72
R x M	1.57	5.73**	0.157
R x N	0.29	16.17**	0.918
M x N	0.04	0.58	0.213
R x M x N	2.34	0.91	1.482
CV	14.73%	13.98%	10.01%

Sv: Source of variation. Fc: F calculated. CV: Cofficient of variation. * and ** indicate significant differences at a level of 0.05 e 0.01 respectively

Table 2 - Comparison test of the mean values for shoot dry weight. a) Comparison of the rootstock. b) Comparison of the mycorrhizal and non-mycorrhizal plants

----------a----------		----------b----------	
Rootstock	Mean value	Mycorrhization	Mean value
'Carolina'	8.37 A	No mycorrhizae	13.58 A
Ungrafted	14.61 B	With mycorrhizae	16.78 B
'Maxifort'	22.56 C		

*Mean values followed by the same letter do not differ statistically by Fisher's LSD test at 5% probability

Table 3 - Comparison test of the mean values for root fresh weight in plants of the 'Santa Clara' tomato (*Solanum lycopersicon* L.), ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato. a) Mycorrhizal and non-mycorrhizal plants. b) In infested soil and not infested with *Nacobbus aberrans*

----------a----------			----------b----------		
non-mycorrhizal		Mycorrhizal	No nematodes		With nematodes
Ungrafted	31.92 g Aa	36.53 g Aa	Ungrafted	41.53 g Ba	27.28 g Aa
'Carolina	40.10 g Ab	53.88 g Bb	'Carolina	58.90 g Bb	35.08 g Ab
'Maxifort'	68.54 g Ac	75.61 g Bc	'Maxifort'	73.14 g Ac	71.01 g Ac

Mean values followed by the same uppercase letter on a row and lowercase letter in a column do not differ by Fisher's LSD test at 5% probability

The plants grafted onto 'Maxifort' also showed vigorous growth and had larger roots, which was reflected in greater shoot growth, unlike the plants grafted onto 'Carolina', which were less vigorous, with shoot and root growth that was more reduced than in the ungrafted plants. Due to the vigour of 'Maxifort', root growth was unaffected by the parasitism of the nematodes; however, this did occur with the roots of the ungrafted plants and 'Carolina'. This vigorous growth is one of the qualities sought in rootstock, where it can make up for the damage caused by biotic or abiotic stress.

The mycorrhizal plants had greater root and shoot growth, explained by an improvement in the nutritional status of the plant. The mycorrhizal association resulted in greater exploitation of the soil by the mycelium which expand beyond the roots. In addition, the hyphae are also more efficient in absorbing low-mobility mineral elements than are the roots (SCHNITZER *et al*., 2011).

For date of flowering, 21 days after transplanting, the ungrafted mycorrhizal plants presented racemes; however, none of the plants of the other treatments showed flowering by this date. At 28 days, flowering developed in

Table 4 - Analysis of variance and comparison test of the mean values for flowering date in plants of the 'Santa Clara' tomato (*Solanum lycopersicon* L.), ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, mycorrhizal and non-mycorrhizal, and in soil infested and not infested with *Nacobbus aberrans*

Sv	Fc
	Days to flowering
Rootstock (R)	579.84**
Mycorrhizal (M)	300.65**
Nematode (N)	0.76
R x M	26.90**
R x N	3.894*
M x N	0.653
R x M x N	-1.633
CV	4.13 %

	Non-mycorrhizal	Mycorrhizal
Ungrafted	27.07 B a	21.02 A a
'Carolina	35.28 A b	34.52 A c
'Maxifort'	35.35 B b	28.71 A b

	No nematodes	With nematodes
Ungrafted	23.28 A a	24.57 B a
'Carolina	34.92 A b	34.85 A c
'Maxifort'	32.22 A b	31.68 A b

Sv: Source of variation. Fc: F calculated. CV: Cofficient of variation. * and ** indicate significant differences at a level of 0.05 e 0.01 respectively. Mean values followed by the same uppercase letter on a row and lowercase letter in a column do not differ by Fisher's LSD test at 5% probability

the ungrafted non-mycorrhizal plants and the mycorrhizal 'Maxifort' plants. Finally, at 35 days, the 'Carolina' and non-mycorrhizal 'Maxifort 'plants began to flower. In relation to the nematodes, flowering was delayed in the ungrafted plants when infested with *N. aberrans*.

One of the negative consequences of grafting is a delay in the flowering process, which appears to be aggravated when *N. aberrans* is parasitising the plants. The results show that mycorrhization with *R. intraradices* had a clear influence on early flowering in the ungrafted and 'Maxifort' plants, and is therefore a good alternative to partially compensate for the delay caused by grafting and parasitism.

As regards the level of mycorrhizal colonisation, it can be seen in Figure 1 that the presence of the nematode in the soil caused a reduction in percentage mycorrhization in the ungrafted plants and in the 'Carolina' rootstock. Conversely, in the 'Maxifort' rootstock, the greatest percentage mycorrhization occurred in plants infested with the nematode.

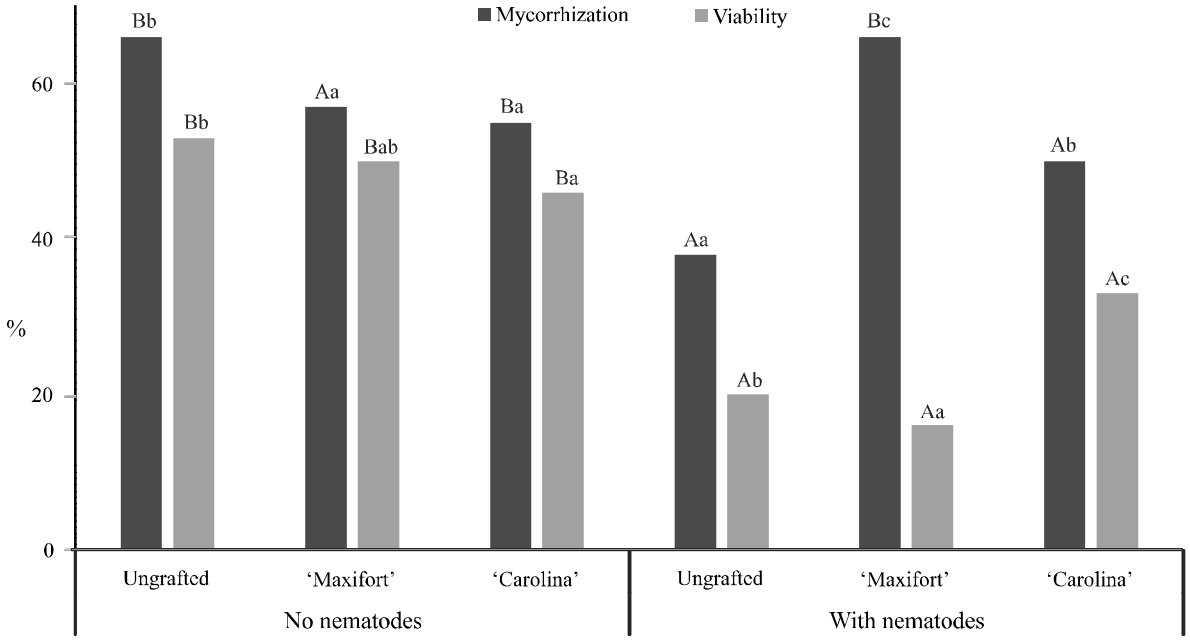
The establishment of mycorrhizal symbiosis depends mainly on the Host-Environment-Fungus combination. There are cases in which nematode infection can produce an increase or reduction, or have no effect, on the percentage colonisation of mycorrhizae-forming fungi (MFF) (MARRO *et al*., 2014; SOUSA *et al*., 2010).

In treatments where the pathogen was present, irrespective of the plant composition used, the viability of the fungal structures, evaluated by the activity of the enzyme succinate dehydrogenase, was lower than in treatments where the nematode was absent (Figure 1). Cofcewicz *et al*. (2001) suggest that the nematode has no direct influence on the MFF. Interference may occur indirectly by a change in the flow of nutrients between the symbionts, which may cause a reduction in the development and efficiency of fungal colonisation.

As regards analysis of the *N. aberrans* population, the nematodes were able to reproduce under all the soil treatments being evaluated (Fr>1). The analysis of variance indicated a significant interaction between plant type and mycorrhization. The symbiosis with *R. intraradices* reduced the population of *N. aberrans* by 51.7%, 35.4% and 6.05% in the ungrafted plants, and in the 'Maxifort' and 'Carolina' rootstock respectively (Figure 2a).

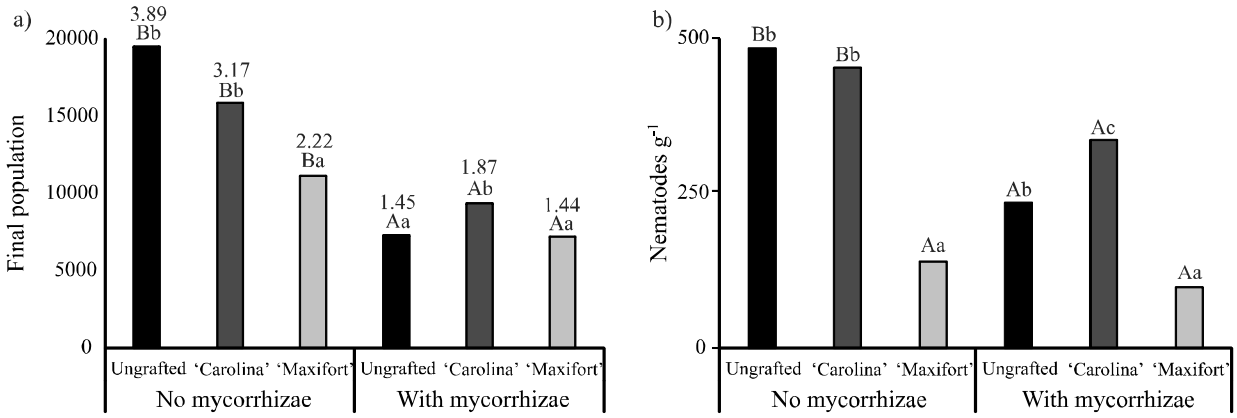
Calculating the reproductive index (IR%), and comparing the values obtained for each composition of plants with the classification established by Hadisoeganda and Sasser (1982), it can be seen that each plant presented a higher category, i.e. less susceptible when mycorrhizal (Table 5).

Figure 1 - Level of mycorrhizal colonisation and viability of fungal structures in plants of the 'Santa Clara' tomato (*Solanum lycopersicon* L.), ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, mycorrhizal and non-mycorrhizal, and in soil infested and not infested with *Nacobbus aberrans*



Uppercase letters compare the same cultivar under the two conditions of infestation. Lowercase letters compare cultivars for the same condition of infestation. Mean values followed by the same letter do not differ by Fisher's LSD test at 5% probability

Figure 2 - Nematological analysis of plants of the 'Santa Clara' tomato, ungrafted and grafted onto the 'Carolina' or 'Maxifort' tomato, mycorrhizal and non-mycorrhizal, and in soil infested with *Nacobbus aberrans*. a) Final population and reproductive factor b) number of nematodes per gram of root



Uppercase letters compare the same cultivar under the two conditions of mycorrhization. Lowercase letters compare cultivars for the same condition of mycorrhization. Mean values followed by the same letter do not differ by Scott-Knott test at 5% probability

Table 5 - Reproductive index of *Nacobus aberrans* according to Hadisoeganda and Sasser (1982) in plants of the 'Santa Clara' tomato, ungrafted or grown on two tomato rootstocks ('Carolina' and 'Maxifort'), mycorrhizal and non-mycorrhizal

	No mycorrhizae	With mycorrhizae
Ungrafted	100%	36.58%
	Standard susceptibility	Slightly resistant
'Carolina'	55.95%	34.74%
	Susceptible	Slightly resistant
'Maxifort'	25.83%	15.39%
	Slightly resistant	Moderately resistant

IR: Quotient between nematodes g^{-1} of root in the cultivar to be evaluated and standard of cultivar susceptibility

Through use of the 'Carolina' and 'Maxifort' rootstock, it was possible to obtain lower reproductive factors than seen in the ungrafted plants, with mycorrhization of the rootstock further reducing the reproductive factors.

A few of the actions that have been described for mycorrhizal symbiosis might explain the reduction in nematodes in mycorrhizal hosts: a) the development of antagonistic bacteria around the extraradical hyphae (BAREA; AZCÓN; AZCÓN-AGUILAR, 2002.); b) the release of root exudates which interfere with pathogen-host signalling, resulting in less penetration of J2 juveniles (SIKORA; FERNÁNDEZ, 2005); c) the better nutritional status of the plants that increases tolerance and compensates for the damage caused by the nematodes (GIANINAZZI *et al*., 2010); and d) the synthesis of compounds that participate in plant defence (VOS *et al*., 2012).

CONCLUSIONS

1. The 'Maxifort' rootstock has an invigorating effect, and the 'Carolina' rootstock a reducing effect, on growth in grafted 'Santa Clara';
2. Mutual symbiosis with *Rhizophagus intraradices* has a stimulating effect on growth in grafted tomato plants and reduces the delay in flowering caused by grafting and the *N. aberrans* parasite;
3. Mycorrhization of tomato plants with the fungus *Rhizophagus intraradices* reduces the multiplication of *N. aberrans*.

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