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ORIGINAL ARTICLE

Behavior of peach and mume rootstocks to the nematode *Meloidogyne enterolobii*

Comportamento de porta-enxertos de pessegueiro e umezeiro ao nematóide Meloidogyne enterolobii

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ABSTRACT: The purpose of this study was to evaluate the response of one mume rootstock and five peach rootstocks (Capdeboscq, Aldrighi, Tsukuba, Flordaguard, Okinawa) to the nematode *Meloidogyne enterolobii*. Plants averaging 12 cm in height were transferred to a greenhouse. Genotypes were individually inoculated with a suspension of 10^4 eggs of *M. enterolobii*. The study was carried out in a completely randomized design with six treatments (rootstocks) and six replicates. The degree of infectivity of rootstocks (number of galls and number of eggs per root system) and the foliar content of macro- and micronutrients were assessed. Based on the reproduction factor of eggs/root (RF) and infectivity, rootstock hospitability was characterized as highly resistant (mume), resistant (Okinawa, Capdeboscq, Aldrigui, Tsukuba), and moderately resistant (Flordaguard). All *Prunus* species, except mume, exhibited galls and egg masses when inoculated with *M. enterolobii*. Foliar contents followed descending orders of macro- and micro-nutrients for rootstocks 'Capdeboscq', 'Tsukuba' and 'Aldrighi' (N >Ca> K > Mg > P > S and Mn> Fe > B > Zn > Cu), for mume (N >Ca> K > Mg > S > P and Mn> Fe > B > Zn > Cu), and for 'Flordaguard' (N >Ca> K > S > Mg > P and Mn> Fe > B > Zn > Cu).

RESUMO: *Foi avaliada a resposta de seis porta-enxertos, sendo um com umezeiro e os demais com pessegueiros (Capdeboscq, Aldrighi, Tsukuba, Flordaguard, Okinawa), para frutas de caroço ao nematóide M. enterolobii. Plantas com média de 12 cm de altura foram transferidas para casa de vegetação. Os genótipos foram inoculados individualmente com suspensão de* 10^4 *ovos de M. enterolobii. O delineamento foi inteiramente casualizado com seis porta-enxertos e seis repetições. Foram avaliados o grau de infectividade dos porta-enxertos (número de galhas e número de ovos por sistema radicular) e o teor foliar de macro e micronutrientes. Baseado no fator de reprodução de ovos/raiz (FR) e na infectividade hospedabilidade dos porta-enxertos, estes foram caracterizados como altamente resistente (umezeiro), resistente (Okinawa, Capdeboscq, Aldrigui, Tsukuba) e mediamente resistente (Flordaguard). Todos os Prunus, à exceção do umezeiro, apresentaram galhas e massas de ovos quando inoculados com M.enterolobii. Os teores foliares seguiram uma ordem decrescente para os porta-enxertos Capdeboscq, Tsukuba e Aldrighi: N>Ca>K>Mg>P>S e Mn>Fe>B>Zn>Cu; para o umezeiro: N>Ca>K>Mg>S>P e Mn>Fe>B>Zn>Cu, e para o Flordaguard: N>Ca>K>S>Mg>P e Mn>Fe>B>Zn>Cu.*

KEYWORDS
Stone fruits
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1 Introduction

Modern fruticulture demands technologies that enable large, regular, high-quality yields with the lowest possible investment (FINARDI, 2003). In this sense, studies aiming to identify sources of resistance to pests and diseases constitute important contributions, not only to increase the productivity and final quality of fruits, but also from the economic and ecological standpoint of production (MAYER; SANTOS; PEREIRA, 2005). Plant parasitic nematodes are among the pests that cause damage and affect the productivity of temperate fruit trees. In surveys conducted in peach orchards, Carneiro et al. (2006) verified that the species of root-knot nematodes most frequently found in the root system of plants were *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne* spp. In the Brazilian context, the entire production of stone fruits uses rootstocks from seeds. In southern Minas Gerais state, a booming region in the production of stone fruits, 'Okinawa' is the most commonly used rootstock. It is considered a genotype resistant to root-knot nematodes (genus *Meloidogyne*), compatible with almost all crown cultivars, and little demanding in cold weather. Currently, this rootstock is the most commonly used by nursery owners and producers (CARNEIRO et al., 2007).

For the past 10 years, *M. enterolobii*, which is a species of root-knot nematode highly aggressive to guava, has drastically reduced the acreage of this crop in the Brazilian northeast region. It is currently spread over several regions of the country where there are orchards of guava, including southern Brazil (PEREIRA et al., 2009).

M. enterolobii species should be evaluated for additional information on the resistance of temperate fruit trees to this nematode, so that a complete resistance and/or susceptibility profile can be established. This information is important to assist the definition of which genotypes would be most adequate for the genetic improvement and commercial use of peach tree rootstocks. Phytoparasitic nematodes are harmful to plants because of their negative effect on the root system, affecting the absorption and translocation of nutrients, altering the physiology and possibly predisposing plants to diseases and environmental stresses, or acting as transmitters of other pathogens (GOMES; CAMPOS, 2003). Mineral nutrition, although often relegated to a secondary level, sometimes functions as an important primary component of disease control (ZAMBOLIM; COSTA; VALE, 2001), because well-nourished plants are more tolerant to the presence of pathogens. On the other hand, the imbalance of nutrients can predispose plants to attack by pathogens and contribute to greater severity of some diseases (SILVA, 2009). Should these genotypes be recommended for cultivation or use in plant breeding programs, the first step is to confirm whether main agronomic traits, such as resistance to nematodes, as well as nutritional changes in plants attacked by nematodes are observed.

The objective of this study was to assess the behavior (resistance/susceptibility) of one mume rootstock and five peach rootstocks (Capdeboscq, Aldrighi, Tsukuba, Flordaguard, Okinawa) to the nematode *Meloidogyne enterolobii* and also measure the levels of macronutrients and micronutrients in the rootstocks analyzed regarding the nematode infection process.

2 Materials and Methods

The experiment was carried out in two phases: first, in a greenhouse at the Hydroponics Sector of the Soil Science Department and, subsequently, in a greenhouse at the Nematology Laboratory of the Plant Pathology Department at the Federal University of Lavras (UFLA), located in the municipality of Lavras, Minas Gerais state, Brazil, (21° 13' 55" S; 44° 57' 43" W), 925 m above sea level. The climate in the study area is Cw according to Köppen classification (mesothermal, with mild summers and dry winters).

We used seeds from rootstocks of peach tree *Prunus persica* (L.), genotypes Capdeboscq, Aldrighi, Tsukuba, Flordaguard, Okinawa, and of mume tree (*Prunus mume* sieb & Zucc) from the Federal University of Pelotas, Rio Grande do Sul state. The seeds were sown in plastic vials, 5 cm in diameter and 20 cm in height, containing vermiculite as substrate. They were then placed in appropriate supports and transferred to leveled shallow boxes, here called pools, which received the nutrient solution proposed by Faquin and Chalfun (2009).

The irrigation and nutrition of plants were conducted by capillarity of the vermiculite. When the plants reached the average height of 12 cm, they were transferred to a greenhouse in pots containing five liters of Plantimax® as substrate, where the genotypes were inoculated with *M. enterolobii* as in the following procedure: the inoculum provided by Embrapa Semi-árido CPATSA, Petrolina, Pernambuco state, was inoculated into tomato roots (*Lycopersicon esculentum* Mill), strain 684, which is considered to be resistant to *M. incognita* and *M. javanica.* Two months after inoculation, the roots of tomato plants were carefully removed from the substrate, washed, and cut into small segments (1-2 cm); the eggs were then collected according to the technique described by Hussey and Barker (1973).

The rootstocks of peach and mume were inoculated with 10^4 eggs/plant –1 10^4 eggs/plant *M. enterolobii*. The inoculum was soil deposited in four 5 cm-deep holes, spaced by 2 cm around the stem of each plant, using an automated pipette (Macroset). Tomato plants, strain 684, were inoculated to attest the efficiency of the inoculum. The plants were scattered in the greenhouse; they were fortnightly irrigated with the nutrient solution proposed by Faquin and Chalfun (2009) and the other cultural treatments for a trial period of four months. The average maximum and minimum temperatures inside the greenhouse (120 μm coverage) were 18 and 38 °C, respectively. The study was carried out in a completely randomized design, with six treatments: five peach rootstocks (*Prunus* genotypes) and one mume rootstock, with six replications, one plant per plot, with two plants (not inoculated with the nematode) of each genotype added as control.

Approximately four months (120 days) after inoculation, the roots of each plant were separated from the aerial part, rinsed for soil removal, and evaluated for gall index (GI) according to the methodology described by Taylor and Sasser (1978). The eggs were extracted from the roots according to Hussey and Barker (1973). Quantification and determination of the reproduction factor (RF) were performed considering RF as the quotient of the final population by the initial population (OOSTENBRINK, 1966). The reaction of plants was estimated by the *M.enterolobii* RF: plants presenting RF=0.00 were considered immune; those showing RF<1.00 were regarded as resistant; and those presenting RF>1.00 were accounted as susceptible (OOSTENBRINK, 1966).

To obtain the dry matter content, the leaves were collected and dried to constant weight in a forced air circulation oven at 60-65 °C; they were then ground against a 20 mesh in a Willey type mill. The levels of macronutrients and micronutrients were chemically analyzed according to Malavolta, Vitti and Oliveira (1997).

Statistical analysis was conducted using the Sisvar software package (FERREIRA, 2011), analysis of variance (ANOVA), and the Scott-Knott test at 5% probability for comparison of means. For these analyses, data from galls/root were transformed into $(x100)^{0.5}$.

3 Results and Discussion

All rootstocks were immune to *M. enterolobii* and high rates of galls on the roots and RF=42.81 were detected in the tomato plant, confirming that the inoculum was perfectly viable (Table 1).

Susceptibility and resistance is measured by reproduction, not by galls (TAYLOR; SASSER, 1978). However, difference in the hospitality of the nematode *M.enterolobii* was observed, and they were classified as highly resistant or immune (mume), resistant (Okinawa, Capdeboscq, Aldrigui, Tsukuba), and moderately resistant (Flordaguard), based on the number of galls in the root system (Table 1). Neither galls nor *M. enterolobii* eggs were observed in the mume rootstock and the plant was therefore considered immune. Galls and eggs were found in the other peach rootstocks, but they were still classified as resistant because they presented reproduction factors smaller than 1.00 (Table 1).

The 'Okinawa' peach rootstock showed the lowest reproduction factor for *M. enterolobii* among the species assessed in this study. Rossi, Ferraz and Montaldi (2002) reported that the 'Okinawa' genotype may be a reference of immunity, because when these authors inoculated *M. javanica*, although they found few galls and no eggs in the roots, they verified that juvenile nematodes in stage two (J2s) penetrated the root system and showed initial development, but eventually died due to a resistance mechanism of the plant. This immunity had also been observed by Scherb, Campos and Chalfun (1994) for *M. incognita* in the 'Okinawa' genotype. In 'Okinawa' peach rootstocks, Mayer, Santos and Pereira (2005) found an average of 0.33 galls in the root system, but no eggs or J2s. A similar result was found in the present study, and the rootstock was considered immune. The 'Flordaguard' genotype immunity to *M. incógnita* detected in our study corroborates the information by Sherman, Lyrene and Sharpe (1991), Ferguson and Chaparro (2013), and Paula et al. (2011), who reported immunity to root-knot nematode as one of the characteristics of this rootstock.

In a research on the reaction to *M. enterolobii* parasitism in six peach rootstocks in Florida, USA, Nyczepir, Brito and Dickson (2008) verified that 'Lovell' and 'Halford' rootstocks behaved as susceptible and 'Flordaguard', 'Okinawa', 'Nemaguard' and 'Guardian' rootstocks were considered resistant. In the works carried out by Mayer, Santos and Pereira (2005) and Rossi, Ferraz and Montaldi (2002), the 'Okinawa' cultivar was considered immune to *M. incognita*. Considering that 'Flordaguard' has the 'Okinawa' genotype, which is a significant source of resistance to *Meloidogyne* spp., as its ancestor, these are potential rootstocks for use in genetic improvement programs and an alternative for use in the implantation of orchards in infested areas. It was verified that *Meloidogyne* spp. is extremely important in peach tree cultivation (ROSSI; FERRAZ; MONTALDI, 2002); therefore, evaluating the resistance reaction only by the gall index is not sufficient to characterize whether the genotype is resistant; it is also necessary to assess nematode reproduction in the plant.

Resistance to nematodes, for both genera and species of *Prunus*, is specified according to the observations of Ledbetter (2013), this difference in resistance to various species of *Meloidogyne* spp. was verified by Esmenjaud et al. (1997) and Rossi, Ferraz and Montaldi (2002). Thus, rootstocks should be tested with each nematode species to determine resistance or susceptibility. According to Gomes and Campos (2003), phytoparasitic nematodes are harmful to plants because of their negative effect on the root system, affecting the absorption and translocation of nutrients, altering the physiology and possibly predisposing plants to diseases and environmental stresses, or acting as transmitters of other pathogens.

Table 1. Number of galls and reproduction factor (RF) in roots of peach and mume rootstocks inoculated with *Meloidogyne enterolobii.*

Genotypes	RF	Reaction to susceptibility/ resistance	Galls/ RSR	Reaction	Hospitability
Tomato plant	42.81	S	8034.00	S	Susceptible
Mume	0.00 c	R	00.00 c	R	Resistant
Okinawa	0.12 c	R	66.50 b	S	Resistant
Capdeboscq	0.07 b	R	85.67 b	S	Resistant
Aldrighi	0.09 b	R	100.3 b	S	Resistant
Tsukuba	0.22 a	R	105.0 b	S	Moderately resistant
Flordaguard	0.23 a	R	210.1 a	S	Resistant
CV (%)	2.19		14.91		

Means followed by the same letters in the column do not differ by the Scott-Knott test at 5% probability. RF= reproduction factor; RSR = Number of galls/ root system of the rootstock; CV (%) = coefficient of variation.

No differences were observed regarding the foliar contents of N, K and Mg (macronutrients) in peach and mume rootstocks inoculated with *M. enterolobii.* Significant differences between rootstocks were observed only for the contents of P, Ca and S, with 'Flordaguard' showing a smaller content of P (Table 2) and higher contents of Ca and S. The other rootstocks presented similar levels of P and Ca in their leaves. The contents of S presented greater variation among rootstocks, with intermediate values for the mume and high values for 'Capdebosq', 'Tsukuba' and 'Aldrighi' rootstocks.

With respect to micronutrients, no significant differences were observed for Mn and Zn foliar concentrations in the rootstocks tested, but the contents of B were smaller for 'Flordaguard'; Cu contents were higher for 'Capdebosq'; Mn and Zn concentrations were higher for cultivars 'Capdebosq', 'Tsukuba' and mume; and Fe values were higher for mume and 'Flordaguard' (Table 3).

Based on the data presented in Tables 2 and 3, we established a descending order of level for macro- and micro-nutrients (Table 4), which may be used as benchmarks for nutritional requirement for peach and mume rootstocks.

According to Fachinello et al. (2005), the optimum leaf contents of a healthy peach seedling present the following descending order for macronutrients: N>K>Ca>Mg> P > S; and for micronutrients: Fe>Mn> B > Zn> Cu. Inoculation with *M. enterolobii* caused an unbalanced nutrition in the leaf contents of macronutrients of the 'Flordaguard' rootstock compared with those without inoculation (Table 4).

Table 2. Macronutrient contents of peach and mume rootstocks, control without inoculation and at 120 days after inoculation with *Meloidogyne enterolobii.*

Genotypes	N		P		K		Ca		Mg		S	
	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.
	g kg^{-1}											
Capdebosq	23.09	22.50 a	3.39	3.09 a	8.92	8.68 a	12.01	11.21 b	3.73	3.33 a	2.82	2.62 c
Mume	24.03	23.83 a	3.38	3.08 a	8.98	8.85 a	12.99	12.07 b	3.64	3.50 a	3.27	3.27 b
Tsukuba	24.00	23.00 a	2.96	2.86 a	8.19	8.42 a	12.15	12.02 b	3.34	3.46 a	2.52	2.46 c
Aldrighi	24.37	23.33 a	2.99	2.94 a	8.97	8.26 a	12.21	11.20 b	3.77	3.54 a	2.61	2.41 c
Flordaguard	25.13	24.16 a	3.67	2.19 b	9.02	8.57 a	14.97	14.16 a	3.8	3.55 a	3.55	4.07 a
Okinawa	25.12	23.28 a	3.99	2.98 a	8.97	8.64 a	13.11	12.15 b	3.71	3.48 a	3.65	2.45 c

Means followed by the same letters in the column do not differ by the Scott-Knott test at 5% probability. [1]Control (without inoculation). [2]Inoculated (with inoculation).

Table 3. Micronutrient contents of peach and mume rootstocks 120 days after inoculation with *Meloidogyne enterolobii.*

Genotypes	B		Cu		Fe		Mn		Zn	
	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.	[1]Cont.	[2]Inoc.
	mg kg^{-1}									
Capdebosq	81.05	78.95 a	8.17	8.05 a	131.28	128.98 b	279.2	288.20 a	29.34	29.56 a
Mume	80.12	79.57 a	4.67	4.65 b	195.66	194.62 a	313.3	311.28 a	33.45	31.74 a
Tsukuba	81.95	80.55 a	5.16	4.16 b	161.23	150.32 b	300.0	295.03 a	32.79	31.79 a
Aldrighi	82.11	78.10 a	6.15	5.21 b	138.52	134.56 b	284.6	284.60 a	32.67	32.75 a
Flordaguard	78.67	56.67 b	4.71	3.73 b	162.49	194.49 a	292	262.00 a	31.97	32.86 a
Okinawa	80.13	78.23 a	5.12	5.12 b	169.85	149.75 b	300.2	297.12 a	31.23	31.23 a

Means followed by the same letters in the column do not differ by the Scott-Knott test at 5% probability. [1]Control (without inoculation); [2]Inoculated (with inoculation).

Table 4. Leaf contents of macronutrients and micronutrients of peach and mume rootstocks in descending order, without inoculation and 120 days after inoculation with *Meloidogyneenterolobii.*

Genotypes	Macronutrients		Micronutrients	
	Control	Inoculated	Control	Inoculated
	g kg^{-1}		mg kg^{-1}	
Capdebosq	N > Ca > K >Mg> P > S	N > Ca > K >Mg> P > S	Mn > Fe > B > Zn > Cu	Mn > Fe > B > Zn > Cu
Tsukuba	N > Ca > K >Mg> P > S	N > Ca > K >Mg> P >S	Mn > Fe > B > Zn > Cu	Mn > Fe > B > Zn > Cu
Aldrighi	N > Ca > K >Mg> P > S	N > Ca > K >Mg>P > S	Mn > Fe > B > Zn > Cu	Mn > Fe > B > Zn > Cu
Flordaguard	N > Ca > K >Mg>P >S	N > Ca > K > S >Mg> P	Mn > Fe> B > Zn > Cu	Mn > Fe> B > Zn > Cu
Mume	N > Ca > K >Mg> S > P	N > Ca > K >Mg> S > P	Mn > Fe> B > Zn > Cu	Mn > Fe > B > Zn > Cu
Okinawa	N > Ca > K >Mg> P > S	N > Ca > K >Mg> P > S	Mn > Fe > B > Zn > Cu	Mn > Fe > B > Zn > Cu

Regarding the micronutrients, the rootstocks of mume and peach did not present unbalanced nutrition in the leaf contents, either with or without inoculation with *M. enterolobii*.

The mineral composition of plants parasitized by nematodes usually differs from the composition of non-parasitized plants. These changes in mineral composition do not follow a strict pattern. There may be an accumulation or a decrease in these levels, and in some cases, the contents of certain nutrients may remain unchanged (GOMES et al., 2008).

The literature presents no studies on leaf contents for the species of the genus *Prunus* infected by root-knot nematodes, which hinders the development of other researches. Thus, the intensification of studies on foliar concentrations of species of this genus infected by nematode *M. enterolobii* can provide valuable scientific contribution.

4 Conclusions

The mume rootstock showed resistance to the nematode *M.enterolobii*, reinforcing the need for studies on this species for use as rootstock in commercial peach cultivars. Leaf contents of macro- and micro-nutrients in plants inoculated with the nematode *M. enterolobii* were very similar to those observed in plants without this parasite, indicating that root infestation did not affect their nutritional capacity.

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