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Plant Protection

Reaction of passion fruit species to *Rotylenchulus reniformis* and *Meloidogyne incognita*

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Abstract -Passion fruit (*Passiflora* spp.) yield is limited by several factors, including soil pathogens, such as *Rotylenchulus reniformis* (the reniform nematode) and *Meloidogyne incognita* (the Southern root-knot nematode). Understanding the behavior of different cultivars, within different species of passion fruit could be important asset to the search for sources of resistance, and therefore for the effective management of phytonematodes. Three trials were carried out in greenhouse to evaluate the resistance/susceptibility of different passion fruit species to *R. reniformis* and *M. incognita*. For *R. reniformis*, cultivars from two species were tested: *Passiflora edulis* f. *flavicarpa* ('BRS Sol do Cerrado', 'BRS Rubi do Cerrado' and 'BRS Gigante Amarelo') and *Passiflora cincinnata* ('BRS Sertão Forte'). Regarding *M. incognita*, all the cultivars above were evaluated, with the addition of *Passiflora setacea* ('BRS Pérola do Cerrado'). The results showed that all tested cultivars were susceptible to the reniform nematode. Conversely, all tested cultivars were immune to root-knot nematode, although exhibiting root galling. Therefore, it is emphasizes the relevance of reniform nematode to passion fruit crops.

Index Terms: *Passiflora edulis*; *Passiflora cincinnata*; *Passiflora setacea*; reniform nematode; Southern root-knot nematode.

Reação de espécies de maracujazeiro a *Rotylenchulus reniformis* e *Meloidogyne incognita*

Resumo-A produção do maracujazeiro (*Passiflora* spp.) é limitada por vários fatores, incluindo patógenos de solo, como *Rotylenchulus reniformis* (nematoide-reniforme) e *Meloidogyne incognita* (nematoide-das-galhas). Entender o comportamento de diferentes cultivares, pertencentes às diferentes espécies de maracujazeiro, é de fundamental importância, especialmente quando se buscam fontes de resistência para o manejo efetivo de fitonematoides. Dois ensaios foram conduzidos em casa de vegetação para avaliar a resistência/suscetibilidade de diferentes espécies de maracujazeiro a *R. reniformis* e *M. incognita*. Para *R. reniformis*, foram testadas cultivares pertencentes a duas espécies: *Passiflora edulis* f. *flavicarpa* ('BRS Sol do Cerrado', 'BRS Rubi do Cerrado' e 'BRS Gigante Amarelo') e *P. cincinnata* ('BRS Sertão Forte'). Quanto a *M. incognita*, foram testadas todas as cultivares acima, além da espécie *P. setacea* ('BRS Pérola do Cerrado'). Os resultados mostraram que todas as cultivares de maracujazeiro testadas foram suscetíveis ao nematoide-reniforme. Por outro lado, todas as cultivares testadas foram imunes ao nematoide-das-galhas, apesar de apresentarem galhas nas raízes. Portanto, ressalta-se a relevância do nematoide reniforme para a cultura do maracujazeiro.

Termos para Indexação: *Passiflora edulis*; *Passiflora cincinnata*; *Passiflora setacea*; nematoide-reniforme; nematoide-das-galhas.

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The genus *Passiflora* L. is considered the most important and diverse within the Passifloraceae family, with approximately 400 described species. From these species, approximately 50-60 produce edible fruits and only a few are commercially important (RODRIGUEZ-AMAYA, 2003).

Among the species used in commercial passion fruit plantations for food purposes, *P. edulis* Sims f. *flavicarpa* Deg. (yellow or sour passion fruit) is the most common and important (VANDERPLANK, 2000). However, new species are being exploited in genetic breeding programs, either for their use as rootstock against biotic and abiotic stresses, or to diversify the production. For these purposes, Caatinga passion fruit (*Passiflora cincinnata* Mast.) and Sururuca passion fruit (*Passiflora setacea* D.C.) are the most used wild passion fruits (CERQUEIRA-SILVA et al., 2014; FALEIRO et al., 2011; MOURA et al., 2020).

Brazil is considered the world's largest producer (about 65% of global production) and largest consumer of passion fruit, with estimated planted area of 46,436 ha, concentrated in the Northeastern, Southeastern and Southern regions (ALTENDORF, 2018; IBGE, 2020).

Despite the economic numbers obtained by farmers, its yield is limited by several biotic and abiotic factors. Regarding biotic factors, soil pathogens stand out, for being able of reduce the life of plants. Phytonematodes are notably soilborne pathogens to passionfruit, posing difficult management (MANICOM et al., 2003; THOKCHOM; MANDAL, 2017).

Global losses caused by phytonematodes are estimated at US$ US$ 100 billion annually (BERNARD; EGNIN; BONSI, 2017). However, there are no estimates related to losses caused by its presence in passion fruit crops. Nevertheless, damage can be visible in the field, as these pathogens reduce the longevity of the orchard or may even make future crops unfeasible (MANICOM et al., 2003; FISCHER; REZENDE, 2008).

Although there are reports of different phytonematodes associated with passion fruits (BOESEWINKEL, 1977; LIBERATO; ZERBINI, 2020), only the reniform nematode (*Rotylenchulus reniformis*) and the root-knot nematodes (*Meloidogyne* spp.) appear to be able to cause economic losses (SHARMA; JUNQUEIRA; GOMES, 2000, 2001).

The symptoms of diseases induced by phytonematodes are often confused with nutritional deficiency; however, they cause alter and damage plant tissues, resulting in deep physiological changes, consequently hindering the absorption and transport of water and nutrients. Therefore, infected plants may show symptoms of dwarfism, leaf chlorosis, and reduced production and longevity may be observed (MANICOM et al., 2003; ORTIZ-PAZ; GUZMÁN-PIEDRAHITA; OCAMPO, 2012).

As passion fruit is a semi-perennial crop, some of these manage strategies to *R. reniformis* and *M. incognita* could not be easily applied. It is a crop with no resistant cultivar or commercial rootstock; therefore, the best strategy for managing this disease in passion fruit is the development and/or identification of these sources of resistance.

Passion fruit genotypes may show different degrees of resistance to the reniform nematode and root-knot nematode. As this is a crop with great genetic variability, studies evaluating the reaction of different commercial crop species against these nematode species are still incipient and divergent, especially with the root-knot nematode (CASTRO et al., 2010; EL-MOOR et al., 2006; ROCHA et al., 2013; SILVA et al., 2020).

In order to obtain yellow passion fruit genotypes with both high productivity and resistance to different biotic and abiotic factors, breeders have been developing and lauching new cultivars in Brazil, some to be used as rootstock, as the cultivars belonging the species *P. cincinnata* and *P. setacea*. However, the host status of most of these novel cultivars to both *R. reniformis* and *M. incognita* is still unknown. Therefore, the aim of this work was to evaluate the host status of *P. edulis* f. *flavicarpa* (Pe) 'BRS Sol do Cerrado' (BRS-SC1), Pe 'BRS Gigante Amarelo' (BRS-GA1), Pe 'BRS Rubi do Cerrado' (BRS-RC) and *P. cincinnata* 'BRS Sertão Forte' (BRS-SF) to *R. reniformis*; and all these cultivars, plus *P. setacea* 'BRS Pérola do Cerrado' (BRS-PC), were evaluated to *M. incognita*.

The trials were conducted between February 2020 and October 2020 in greenhouse conditions at the Laboratory of Nematology, located at "Luiz de Queiroz" College of Agriculture (ESALQ/USP) - (Piracicaba, Brazil, S22°42'14.4"; W47°38'00.7"). During the trials, the average temperature was 22.03ºC and the average humidity was 70.11%. Irrigation was carried out daily and fertilization fortnightly, by using 3 g of Osmocote® (N-P-K: 15-9-12) per plant.

Three trials were carried out in greenhouse to assess the susceptibility of commercial and wild cultivars to these nematodes. BRS-SC1, BRS-GA1, BRS-RC, BRS-SF and BRS-PC seeds were provided by Embrapa Cerrados. The sowing of these cultivars was carried out in trays containing autoclaved sandy loam soil (121ºC/2h), and after 25 days a single plant was transplanted into plastic cups (6.5cm in diameter x 13.6cm in height) previously filled with 450cm³ of autoclaved sandy loam soil (121ºC/2h).

In trial 1, the behavior of passion fruit cultivars mentioned above to the reniform nematode (*R. reniformis*) was addressed, except the BRS-PC, which seeds were not available during at the moment. The *R. reniformis* isolate was obtained from yellow passion fruit roots collected in the municipality of Piracicaba-SP and maintained on the same host in a greenhouse. The inoculum was extracted from infested yellow passion fruit roots by using a modified Baermann method for shallow containers (SOUTHEY, 1986). At the end of the process, an aqueous suspension containing immature juveniles, males and immature females was obtained. The inoculum was calibrated to 1,000 mobile forms/mL on a Peters' counting slide at 100x magnification by using light microscope (Olympus CH-2, Japan).

Two weeks after seedling transplantation, 1,000 *R. reniformis* specimens were inoculated, by pouring an aqueous suspension into two holes made in the soil close to plant stem. Passion fruit plants were kept in greenhouse and the evaluation was carried out 60 days after inoculation (DAI). Roots and soil were processed for nematode extraction, following methodologies proposed, respectively, by Coolen and D'Herde (1972) and Jenkins (1964). Nematodes were inactivated in low heat (60°C), fixed in formalin (2%) and counted as described above.

In trial 2 and 3, the host status of all cultivars of *P. edulis*, *P. cincinnata* and *P. setacea* to *M. incognita* race 3 was assessed. The *M. incognita* isolate was obtained from cotton (*Gossypium hirsutum* L.) roots collected in the municipality of Campo Verde-MT, and maintained on cotton, in a greenhouse. For inoculum extraction, roots of cotton 'TMG 44 B2RF' previously colonized for 60 days by the nematode were processed to obtain eggs and second-stage juveniles (J_2) by the method of Coolen and D'Herde (1972). The suspension was calibrated on Peters' counting slide at 100x magnification using light microscope (Olympus CH-2) for 1,000 eggs and J2/mL.

Two weeks after seedling transplantation, 1,000 *M. incognita* individuals/mL were inoculated by pouring the suspension into two holes made in the soil close to plant stem. To prove the inoculum infectivity, cotton plants cv. 'TMG 44 B2RF' were inoculated with 1,000 *M. incognita* eggs or J2/mL and placed together with passion fruit plants. These were maintained in greenhouse and the evaluation was carried out in two different periods: 77 DAI and 167 DAI, respectively, trial 2 and 3. Root processing was carried out by the method described by Coolen and D'Herde (1972).

In the end of the evaluation, the final population (Pf), reproduction factor (R) and number of nematodes per gram of root (Nema/g of root) were determined. The R value corresponds to the relationship between the final nematode population in roots and soil (Pf) and initial population (Pi) introduced in the inoculation. The cultivars were classified as resistant when $R < 1$, and susceptible when $R \geq 1$.

The trials were conducted in a completely randomized design (CRD), with four treatments (cultivars) and 11-14 replicates (trial 1 – with *R. reniformis*) / five treatments (cultivars) and 5-8 replicates (trial 2 and 3 – *M. incognita*). For trial 1, the data obtained (R, Pf and Nema / g of root) were transformed into $\sqrt[2]{(x+1)}$ using the R package (r-project.org) and the mean values were compared by the Tukey test at the 5% significance level.

The reniform nematode reproduced at high rates in all cultivars evaluated (Table 1). Caatinga passion fruit (*P. cincinnata* cv. BRS-SF) presented the lowest R value, in accordance to Inomoto and Fonseca (2020). The susceptibility of BRS-GA1 to *R. reniformis* was demonstrated previously (INOMOTO; FONSECA, 2020), but, to our knowledge, the host status of BRS-SC1 and BRS-RC were not assessed yet. Our data reassure the susceptibility of yellow passion fruit to *R. reniformis* (Fig. 1a; b), which is well documented in literature, suggesting the absence of source of resistance in *P. edulis* and other cultivated species of *Passiflora*, namely, *P. alata* Curtis, *P. cincinnata* and *P. setacea* (KIRBY, 1978; SHARMA; JUNQUEIRA; GOMES, 2000; SHARMA et al., 2003; SUÁREZ-H.; ROSALES, 2003; ORTIZ-PAZ; GUZMÁN-PIEDRAHITA; OCAMPO, 2012; INOMOTO; FONSECA, 2020)

Table 1. Number of passion fruit plants (*Passiflora edulis* f. *flavicarpa* - Pe; *Passiflora cincinnata* - Pc) and cotton evaluated (N), final population density of *Rotylenchulus reniformis* recovered from the roots (Pf root), final population density of *R. reniformis* recovered from the soil (Pf soil) e reproduction factor (R).

Trial 1 (60 dai)				
Species / cultivars	**N**	**Pf Root[1]**	**Pf Soil[1]**	**R[1]**
Pe / BRS GA1	14	7,539 a	12,473 a	20.01 a
Pe / BRS SC1	12	3,416 a	7,474 ab	10.89 ab
Pe / BRS RC	11	6,496 a	8,040 ab	14.54 ab
Pc / BRS SF	12	2,842 a	3,667 b	6.51 b
CV	-	10,63%	8,83%	25,95%

Within a column, averages sharing a letter are not significantly different at P ≤0.05 according to Tukey's test. [1]Data were transformed using $\sqrt[g]{(x+1)}$ before performing the statistical analysis.

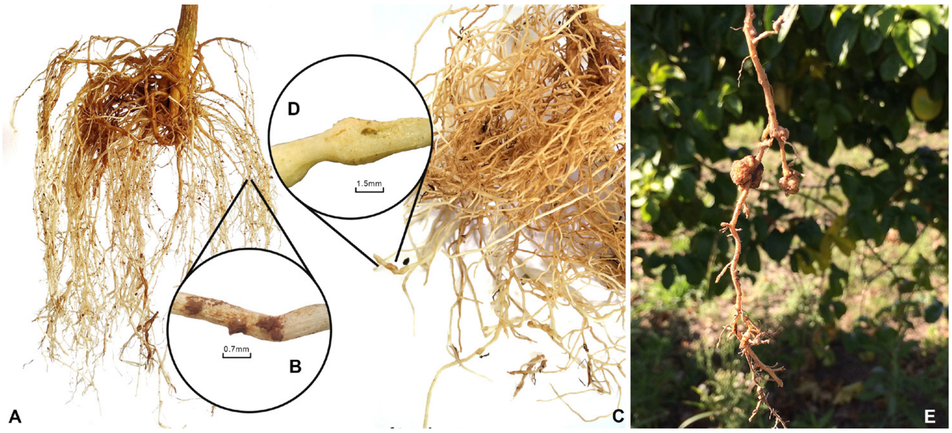

Figure 1. 1a) Yellow passion fruit cv. BRS 'Gigante Amarelo' (*P. edulis f. flavicarpa*) roots infested with reniform nematode (*R. reniformis*). 1b) The gelatinous matrix of reniform nematode. 1c) Yellow passion fruit cv. BRS 'Gigante Amarelo' (*P. edulis f. flavicarpa*) roots showing few galls due to *M. incognita* infestation. 1d) Small galls due to *M. incognita* infestation. 1e) Galled roots were observed in visit to a passion fruit area in Laranjal Paulista-SP.

Contrary to *R. reniformis*, there no consensus about host status of yellow passion fruit to *M. incognita*. Indeed, the results available in the literature are strongly divergent. According to results of trial 2 and 3, all tested cultivars were immune to *M. incognita* (R = 0). Instead, *M. incognita* densities on cotton increased 4.34-fold at 77 DAI and 5.84-fold at 167 DAI, confirming the inoculum infectiveness.

Caatinga passion fruit (*P. cincinnata* cv. BRS-SF) and Sururuca passion fruit (*P. setacea* cv. BRS-PC) were also rated as immune to *M. incognita* (R = 0), in partial accordance to the previous reports of Paula (2006) for the accession CPAC MJ 12-1 of *P. setacea* (R = 0.19) and of Rocha et al. (2013) for an indetermined genotype of *P. cincinnata* (R = 0.3). However, *M. incognita* reproduced on an indetermined genotype of Sururuca passion fruit (R = 1.9) in Rocha et al. (2013), and indetermined genotypes of yellow and Caatinga passion fruits (R = 12,0 and 8.3) in Zucareli et al., 2020. To our knowledge, the host status of BRS-SF and BRS-PC for *M. incognita* was not assessed yet.

Interestingly, purple passion fruit allowed strong reproduction of *M. incognita* (one cultivar tested: R = 57.3) and yellow passion fruit was rated as almost immune (six cultivars tested: R = 0.0-0.4) in Costa et al. (2017).

Although *M. incognita* failed to reproduce on the cultivars assessed in trial 2 and 3, small galls were easily visible on the roots (Fig. 1c; d), in accordance to Kirby (1978), which reports root galling in yellow passion fruit inoculated with *M. arenaria*, *M. incognita* and *M. javanica*, in spite of absence of nematode reproduction. Furthermore, in July 2020, we personally sample yellow passion fruit with conspicuous galls (Fig. 1e), but without nematode females, in an orchard located in Laranjal Paulista-SP. In the same orchard, we collected roots of white pitahaya [*Hylocereus undatus* (Haw.) Britton and Rose] infected with *M. incognita* (SOUZA et al., 2022). Conversely, Nascimento et al. (2016) reported that 19 out the 20 yellow passion fruit genotypes were susceptible to *M. incognita*, with R value ranging from 1.1 to 6.3; however, root galling was not observed.

In conclusion, passion fruits (*P. edulis*, *P. alata*, *P. cincinnata* and *P. setacea*) cultivation is not recommended in areas infested with the reniform nematode, as resistant cultivars of passion fruits were not yet available. Conversely, the host status of passion fruits to *M. incognita* was not completely understood. Most of the researches indicate that *P. edulis*, *P. cincinnata* and *P. setacea* are resistant to *M. incognita*, but some demonstrated reproduction of this nematode on *P. edulis* and *P. setacea*, suggesting variability in *M. incognita* fitness to *Passiflora* spp.. Therefore, the implanting of passion fruits, mainly *P. edulis* and *P. setacea*, in areas infested with *M. incognita* presents reasonable risk.

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