

## Susceptibility of four passion fruit species to the reniform nematode

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**Abstract**– Reniform nematode (*Rotylenchulus reniformis*) is a major pest of sour-passion fruit (*Passiflora edulis*) in Brazil, and few management methods are currently available, mainly due to the lack of host-plant resistance. Other species of passion fruit species are also commercially available in Brazil, which could be a source of resistance. Cultivars of three species (*P. setacea* ‘BRS-Pérola-do-Cerrado’, *P. cincinnata* ‘BRS-Sertão-Forte’, and *P. alata* ‘BRS-Mel-do-Cerrado’), and one susceptible cultivar (*P. edulis* BRS-Gigante-Amarelo) were evaluated for reniform nematode susceptibility in two greenhouse trials. Results showed that the cultivars were susceptible. Therefore, reniform nematode management in passion fruit must be rely on a combination of nematode-free seedlings and nematode-free fields.

**Index-terms:** *Passiflora alata*, *Passiflora cincinnata*, *Passiflora edulis*, *Passiflora setacea*, *Rotylenchulus reniformis*.

## Reação de quatro espécies de maracujazeiro ao nematoide reniforme

**Resumo** – O nematoide reniforme (*Rotylenchulus reniformis*) é uma das principais pragas do maracujá-azedo (*Passiflora edulis*), e há poucos métodos de manejo, pois não há fontes de resistência. Outras espécies de maracujá estão disponíveis comercialmente no Brasil e poderiam ser fontes de resistência. Cultivares de três espécies (*P. setacea* ‘BRS-Pérola-do-Cerrado’, *P. cincinnata* ‘BRS-Sertão-Forte’ e *P. alata* ‘BRS-Mel-do-Cerrado’) e uma cultivar suscetível (*P. edulis* ‘BRS-Gigante-Amarelo’) foram avaliadas quanto à suscetibilidade ao nematoide reniforme, em dois experimentos conduzidos em casa de vegetação. Os resultados mostraram que as cultivares de maracujá testadas são suscetíveis a *R. reniformis*; portanto, a base para o manejo do nematoide reniforme em maracujá deve ser a combinação do uso de mudas sadias e campos livres dos nematoides.

**Termos para indexação:** *Passiflora alata*, *Passiflora cincinnata*, *Passiflora edulis*, *Passiflora setacea*, *Rotylenchulus reniformis*.

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In Brazil, reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira, 1940) was reported for the first-time attacking passion fruit (*Passiflora* sp.) roots in the municipality of Votuporanga, São Paulo state (CURI and BONA, 1972). Now, *R. reniformis* is widespread in Brazilian sour passion fruit (*Passiflora edulis*) orchards. According to a 1990s survey, *R. reniformis* was present in 35% of passion fruit samples from Brazilian Federal District, Minas Gerais state and Goiás state (SHARMA et al., 1999). In 2007-2009, a survey of 14 yellow passion fruit (*P. edulis* Sims f. *flavicarpa* Deg) orchards in the Federal District of Brazil showed its occurrence in nine out of 20 samples (CASTRO et al., 2012).

The effects of the reniform nematode on sour passion fruit has been fairly documented. A survey of yellow passion fruit orchards in Fiji showed the occurrence of reniform nematode in 16 out of 19 sampled orchards. Additionally, a glasshouse trial was carried out in order to evaluate the effect of reniform nematode on the growth of passion fruit seedling. After 81 days in 4,000 cm<sup>3</sup> pots containing non-infested soil or with two densities of *R. reniformis* (2,275 and 22,750 specimens), the nematode did not affect the root fresh weight, but the higher nematode density resulted in a 23% reduction in fresh weight of passion fruit vines (KIRBY, 1978). As the densities exceeded 1,000 individuals of *R. reniformis* per 200 cm<sup>3</sup> soil in 11 orchards of the survey, and the maximum density reached 36,000 specimens, the reniform nematode should be considered a major pest of passion fruit in Fiji.

A study conducted in Venezuela over a 7-week period showed a significant reduction in plant height within two weeks after inoculation (9%), and intensified along the trial progress, reaching 22%. Additionally, fresh and dry weight of passion fruit vines decreased 51 and 60% respectively; fresh and dry weights of roots decreased 22 and 50% respectively. Other effects of reniform nematode on passion fruit plants were the foliar yellowing and stem thinning (SUÁREZ-H. and ROSALES, 2003).

Few management methods were available for the sour passion fruit plants / *R. reniformis* pathosystem, because of the lack of host-plant resistance. However, 70 or more species of *Passiflora* that have a potential economic value were not rated yet for their host suitability to *R. reniformis* in a way to be used as a reniform nematode management strategy.

Some cultivars of wild passion fruit were launched recently in Brazil: 'BRS-Pérola-do-Cerrado' / BRS-PC (*Passiflora setacea* DC) (RANGEL-JUNIOR et al., 2018), 'BRS-Sertão-Forte' / BRS-SF (*P. cincinnata* Mast) (ARAÚJO et al., 2016), and 'BRS-Mel-do-Cerrado' / BRS-MC (*P. alata* Curtis) (FALEIRO et al., 2017). Due to the importance of *R. reniformis* to the passion fruit crop, two greenhouse trials were carried out in order to evaluate the susceptibility of these cultivars to the reniform

nematode. The intraspecific hybrid 'BRS-Gigante-Amarelo' / BRS-GA1 of yellow passion fruit was used as susceptible standard host.

The isolate of *R. reniformis* was obtained from yellow passion fruit roots collected in Piracicaba (SP). Seeds of yellow passion fruit (indeterminate cultivar) were sown in an autoclaved (121 °C/2h) sandy-loam soil, and plants were infected with egg masses of *R. reniformis*. The infected plants were maintained in 1,000-cm<sup>3</sup> clay pots in a greenhouse. The inocula for the trials were obtained from potted soil by a modified Baermann method for flat recipient (SOUTHEY, 1986), resulting in an aqueous suspension containing juveniles, males and young females.

One single seed of each of the four passion fruit species was sown in a 450-cm<sup>3</sup>-plastic cup (6.5cm diameter x 13.6cm height) filled with an autoclaved (121 °C/2h) sandy-loam soil. The germination was very poor and irregular, but seven seedlings of BRS-GA1, eight of BRS-MC, four of BRS-PC, and eight of BRS-SF were obtained three weeks after the sowing. Forty-five days after sowing, each seedling was inoculated with 2,000 specimens of *R. reniformis* by pouring an aqueous suspension containing the nematodes in a hole (7 mm diameter / 20 mm depth) made in the soil, at 1 cm distant from plant stem. The passion fruit plants were maintained in a glasshouse until the evaluation, 59 days after inoculation (DAI). Soil and roots were separated in 4 L of tap water in a 10 L bucket. For evaluations, the soil nematodes were recovered by centrifugal-flotation method (JENKINS, 1964), resulting in a 20 mL aqueous suspension containing adults (females and males), juveniles and eggs of *R. reniformis*. The nematodes were heat-inactivated (60 °C), fixed by adding formalin until reach 0.25% concentration, maintained at 10 °C, and counted twice in a Peters' counting slide (1 mL aliquot) at 100x magnification using a light microscope (Olympus CH2).

Roots were examined at 13x magnification under a stereomicroscope (Leica EZ4) in order to counting *R. reniformis* egg masses. Thereafter, the root nematodes were recovered by blender-centrifugal-flotation method (COOLEN and D'HERDE, 1972), using 0.5% NaOCl solution and a kitchen blender at low speed. The nematodes from the roots were inactivated, fixed and counted as previously performed for the soil nematodes. The *R. reniformis* final population (Pf) was the sum of the population recovered from the soil (Pf Soil) plus the population from the roots (Pf Roots); thus, the reproductive factor (RF=Pf/Pi) was calculated. To compare the susceptibility of the passion fruit species to *R. reniformis*, the following variables were considered: 1-number of egg masses per plant, 2-Pf Soil, 3-Pf Roots, and 4-RF value.

The trial was repeated once using another ten seeds of each *Passiflora* species, which were sown in a 450-cm<sup>3</sup>-plastic cup filled with an autoclaved (121 °C/2h)

sandy-loam soil. One single seedling was transplanted to other 450-cm<sup>3</sup>-plastic cup with autoclaved sandy-loam soil 36 days after sowing. Seven seedlings of BRS-GA1, six of BRS-MC, eight of BRS-PC were obtained; however, seeds of BRS-SF did not germinate at all. Eight days after transplanting (44 days after sowing), the inoculation was performed as the trial 1. The evaluation was similar to of the trial 1, but 129 DAI.

Both experiments were set up in a completely randomized design, with four treatments and 4-8 replicate (trial 1) / three treatments and 5-8 replicates (trial 2). Data obtained (egg masses, Pf Soil, Pf Roots, and RF) were analyzed without transformation using R-package (r-project.org), and the mean values were compared by Tukey's honestly significant difference test ( $P = 0.05$ ).

The results of both trials are presented in the Table 1. Reniform nematode reproduced at high rates in all *Passiflora* species. The three species were not different from the susceptible control for egg masses, Pf Soil and Pf Roots. The *P. cincinnata* showed a lower RF, but this cultivar failed to germinate in the second trial to confirm this result. The population increase obtained in trial 1 ( $R=1.61-6.19 / 59$  DAI) was similar to the values previously obtained for ten cultivars and hybrids of *P. edulis* ( $RF=2.4-6.4 / 51$  DAI) (SHARMA et al., 2001). The soil populations (Pf Soil) were markedly higher in trial 2, probably due to the longer experimental period (129 DAI vs. 59 DAI), resulting in higher values of R. Therefore, the population densities of *R. reniformis* in soil might attain very high values during the economic production period of passion fruit, which ranges from 18 months to 4 years for *P. edulis* (KIRBY, 1978).

**Table 1.** Number of passion fruit plants evaluated (N), number of egg masses per plant, *Rotylenchulus reniformis* recovered from the roots and soil, *R. reniformis* reproductive factor (FR) on *Passiflora alata* BRS-Mel-do-Cerrado (Pa / BRS-MC), *P. cincinnata* BRS-Sertão-Forte (Pc / BRS-SF), *P. edulis* BRS-Gigante-Amarelo-1 (Pe / BRS-GA1) and *P. setacea* BRS-Pérola-do-Cerrado (Ps / BRS-PC).

Species / cultivars	Trial 1 (59 dai)					Trial 2 (129dai)				
	N	Egg Masses	Pf Roots	Pf Soil	R	N	Egg Masses	Pf Roots	Pf Soil	R
Pe / BRS-GA1	7	476 a	4,089 a	8,291 a	6.19 a	5	418 a	6,199 a	30,367 a	18.13 a
Pa / BRS-MC	8	351 a	6,219 a	5,667 a	5.94 a	6	347 a	7,114 a	45,803 a	26.38 a
Ps / BRS-PC	4	172 a	1,350 a	5,045 a	3.20 ab	8	538 a	4,191 a	16,210 a	10.43 a
Pc / BRS-SF	8	167 a	755 a	2,473 a	1.61 b	-	-	-	-	-

Means followed by the same letter in column do not differ according to Tukey test (0.05).

The absence of a remarkable resistance of BRS-MC, BRS-PC, and BRS-SF, which might be ranked as susceptible hosts to the reniform nematode, comparable to BRS-GA1, shows that other techniques should be considered for *R. reniformis* management. However, as mentioned above, Brazilian nurseries have not adopted any specific measure to prevent nematode contamination in passion fruit seedlings, as nearly no legislation exists to prevent the commercialization of nematode-infected passion fruit seedlings in Brazil. Some nurseries applies the use of artificial substrates to produce passion fruit seedlings, which are supposed to be free of phytonematodes. In addition, they could use water from artesian aquifer to irrigate the seedlings; however in Brazil these procedures were not compulsory in passion fruit nurseries (BRAGA and JUNQUEIRA, 2003; SILVA, 2006). Therefore, the passion fruit producers must select carefully the nurseries and the fields where the orchard will be implanted.

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