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Pathogenicity of reniform nematode (*Rotylenchulus reniformis*) on the stinking passionflower (*Passiflora foetida*)

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Abstract: Stinking passionflower (*Passiflora foetida* L.) is a medicinal species that may be used as rootstock to sour passion fruit (*Passiflora edulis*) against wilting and collar rot caused by *Fusarium* spp. However, as it is a host of the reniform nematode (*Rotylenchulus reniformis*), the cultivation of this species may be constrained in crop fields infested by this nematode. The objective of this study was to evaluate the effect of *R. reniformis* on the growth of stinking passion flower. The length of plants inoculated with the highest dose of each trial (152,900 and 78,900 specimens per plant corresponding to 402.4 and 207.6 specimens / cm^3 of soil) was shorter than in plants not inoculated with the reniform nematode. Therefore, *R. reniformis* should be considered a pathogen of stinking passionflower and be properly managed.

Index terms: collar rot, Fusarium wilt, *Passiflora edulis*, pot trial.

Patogenicidade do nematoide-reniforme (*Rotylenchulus reniformis*) em maracujá-de-cheiro (*Passiflora foetida*)

Resumo: O maracujá-de-cheiro (*Passiflora foetida* L.) **é** uma espécie medicinal que pode ser utilizada como porta-enxerto para o maracujá-azedo (*Passiflora edulis*) contra a murcha e a podridão de colo e raiz causadas por *Fusarium* spp. No entanto, por ser uma planta hospedeira do nematoide-reniforme (*Rotylenchulus reniformis*), o cultivo desta espécie pode ser restringido em campos infestados por este nematoide. O objetivo deste estudo foi avaliar o efeito de *R. reniformis* no crescimento do maracujá-de-cheiro. O comprimento de plantas inoculadas com a dose mais alta de cada ensaio (152.900 e 78.900 espécimes por planta, correspondendo a 402,4 e 207,6 espécimes / cm^3 de solo) foi menor do que em plantas não inoculadas com o nematoide-reniforme. Portanto, *R. reniformis* deve ser considerado um patógeno de maracujá-de-cheiro e ser adequadamente manejado.

Termos para indexação: experimento em vasos, murcha de fusário, *Passiflora edulis*,podridão de colo e raiz.

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Stinking passionflower (*Passiflora foetida* L.) is a medicinal species that may be used as rootstock to sour passion fruit (*Passiflora edulis* Sim) in fields contaminated with *Fusarium oxysporum* f. sp. *passiflorae* and *Fusarium solani*. According to Silva et al. (2017), *P. foetida* showed resistance even when cultivated in areas with a history of fusariosis. However, Paes et al. (2022) showed that *P*. *foetida* is susceptible to the reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira). Therefore, this nematode may be a limiting factor for the use of stinking passion flower as main culture or rootstock, as it is widespread in sour passion fruit orchards. A survey in Fiji demonstrated that *R*. *reniformis* was found in 16 out of 19 orchards of *P*. *edulis* (KIRBY, 1978). In Brazil, the reniform nematode was present in 35% of samples collected in sour passion fruit orchards (SHARMA et al., 1999). Considering this scenario, two glasshouse trials were carried out in order to assess the effect of reniform nematode on the growth of *P*. *foetida* plants.

The *R. reniformis* isolate was obtained in Piracicaba (SP) from sour passion fruit plants (*P. edulis*) with symptomatic roots and kept on the same plant in a greenhouse (25 ± 10 ºC). The inoculum consisted of eggs and mobile stages (juveniles, males and immature females). The inoculum was obtained using a protocol adapted from Boneti and Ferraz (1981). The roots were chopped with a 1% sodium hypochlorite solution (NaClO) in a common kitchen blender (550 W) at low speed for 60 seconds. Subsequently, the resulting suspension was collected through a sieving sequence (60 – 200 – 500 mesh, corresponding to openings of 0.250 – 0.075 – 0.025 mm), removing the sodium hypochlorite with tap water. The material deposited on the 500-mesh sieve was collected in a beaker and the nematodes were counted using a Peters' slide in a compound light microscope (model CHS, Olympus Optical Co., Ltd., Tokyo, Japan) at 100x magnification.

Seeds of *P*. *foetida* were sowed in 500 cm^3 plastic pots (R=4,5cm / r=2,75cm / h=13,5cm) filled with autoclaved sand (121ºC/2h). When germinated, the seedlings were transplanted into 500 cm^3 pots containing 380 cm^3 of an autoclaved sandy-loam soil (83% sandy / 2% silt / 15% clay), keeping one plant per pot.

The experiment was conducted in two trials. Trial 1 was performed using the first germinated seeds, and trial 2 was using seeds germinated later. The plantlets formed were transferred to the pots and inoculated 12 days after transplanting (Trial 1), or 21 days after transplanting (Trial 2). Both trials were performed during the summer.

Three treatments were performed in each of the trials. For trial 1: a) control without nematodes; b) dose 1 (D1) = 30,600 specimens per pot (80.5 nematodes / cm^3 of soil) and dose 2 (D2) = 152,000 specimens per pot (402.4 / cm^3). For trial 2: a) control without nematodes; b) dose 1 (D1) = 16,900 specimens per pot (44.5 / cm^3) and dose 2 (D2) = 78,900 specimens per pot (207.6 / cm^3).

Nematode doses were based on inoculum availability and in the results of Kirby (1978), which registered over 36,000 specimens of *R*. *reniformis* per 200 cm^3 of soil (180 / cm^3) in a very infested orchard in Fiji. Inoculation was performed by pipetting an aqueous suspension containing the nematode specimens into two holes (2 cm deep) made in the soil, dividing half of the inoculated amount between the holes. Each treatment was composed by seven replicates, each replicate corresponding to one plantlet 10-12 cm high with 5-6 leaves (trial 1) or one plantlet 12-14 cm high with 7-8 leaves (trial 2). The plants were maintained in glasshouse (25 ± 10 ºC) until the evaluations that occurred on March 7, 2022 (44 days after the inoculation in trial 1) and May 19, 2022 (56 days after the inoculation in trial 2).

After this period of time, the soil was separated from the roots and the nematodes were recovered from it by the centrifugal flotation method proposed by Jenkins (1964) resulting in an aqueous suspension containing eggs, males and immature females. The nematodes from the roots (eggs, juveniles, immature females and males) were recovered by the adapted method of Boneti and Ferraz (1981) described previously for the production of inoculum. Finally, the nematodes were preserved alive at 10 ºC and counted twice on a Peters' counting slide with the aid of a light microscope at 100x magnification. The *R*. *reniformis* final population (Pf) was the sum of specimens from the soil and from the roots. Three plant growth variables were assessed: plant lenght (cm), root fresh weight (g) and aerial part dry weight (g). Both trials were set in a completely randomized design.

The statistical software R (R Core Team) was used for the Shapiro-Wilk test to ensure data normality and the means were compared using the Tukey honest test at the 5% significance level.

Plant length of *P*. *foetida* infected with the higher doses of *R*. *reniformis* was shorter than non-infected ones (Figure 1, Table 1). However, infected roots weighed similarly to non-infected ones. The plants inoculated with a high density of nematodes suffered damage, that triggered a greater emission of secondary roots, which could be responsible for the increase in the root volume. (Figure 1, Table 1).

Furthermore, the aerial part dry weight of infected plants did not differ from non-infected ones, which could be explained by the fact that the infected plants, being lower than the non-infected, maintained the older leaves (Figure 1, Table 1).

The present study was the first assessing the effect of the reniform nematode on the growth of stinking passionflower. Indeed, the literature only registered the effect of *R*. *reniformis* on sour passion fruit. Plants of sour passion fruit in a soil with high density of this nematode (22,750 specimens in 4,000 cm^3 soil) produced lighter fresh aerial parts than uninfected plants (30.3 *versus* 23.4 g), in a greenhouse trial. However, Kirby (1978) did not detect significant effects on aerial part length (129.9 *versus* 113.8 cm) and root fresh weight (4.9 *versus* 3.5 g). In another trial, the detrimental effect of *R*. *reniformis* was proved comparing the growth of *P*. *edulis* (aerial part length and weight; root weight) plants cultivated in a naturally infested soil (2,110 specimens in 1,000 cm^3 soil) with others planted in autoclaved soil (SUÁREZ; ROSALES, 2003).

Notwithstanding the large amount of egg masses observed in the roots (Figure 1), the nematode population decreased on *P*. *foetida* in both trials, probably due to the extremely high densities used, resulting in high competition for parasitism sites. This was observed in trial 1 in which the final nematode population in soil was significantly different between the nematode doses applied (Table 1).

Indeed, the susceptibility of *P*. *foetida* to *R*. *reniformis* was demonstrated previously by Paes et al. (2022) in a glasshouse trial, when the nematode density increased 16.3 times on *P. foetida* after 83 days of inoculation of 1,000 specimens.

In conclusion, *R*. *reniformis* should be considered pathogenic to stinking passionflower. Therefore, the cultivation of stinking passionflower as main culture or rootstock should be preceded by preventive control methods, ie. production of healthy seedlings and choice of fields free from the nematode.

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Table 1. Effect of *Rotylenchulus reniformis* on the growth of *Passiflora foetida* and nematode reproduction, in trial 1 (44 days after inoculation) and trial 2 (56 days after the inoculation).

	Treatments	Plant lenght (cm)	Root fresh weight (g)	Aerial part dry weight (g)	Final population in soil	Final population in root
Trial 1	Control	118 a	5.58 a	1.25 a	0.00 a	0.00 a
	80.5 / cm^3	108 ab	5.67 a	1.08 a	24,607 c	22,370 b
	402.4 / cm^3	78 b	4.87 a	1.02 a	12,970 b	29,910 b
Trial 2	Control	122 a	7.46 a	1.53 a	0.00 a	0.00 a
	44.5 / cm^3	100 ab	6.53 a	1.59 a	11,973 b	56,350 b
	207.6 / cm^3	80 b	6.14 a	1.39 a	19,230 b	44,270 b

*Each value is mean of seven replicates. Means followed by the same letter in column do not differ according to Tukey test at 5% significance.

Figure 1. Plants of *Passiflora foetida* 44 days after the inoculation with *Rotylenchulus reniformis* (trial 1). A. From left to right: non-inoculated plant, inoculated with 80.5 specimens / cm^3 soil (dose 1) and 402.4 specimens / cm^3 soil (dose 2). B. Roots of a plant inoculated with 402.4 specimens / cm^3 soil.

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