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The Southern root-knot nematode (*Meloidogyne incognita*) is an important constraint for stinking passion flower (*Passiflora foetida* L.)

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Abstract: In Brazil, stinking passion flower (*Passiflora foetida* L.) is exploited as a rootstock for the commercial planting of passion fruit (*Passiflora edulis* Sims) and also planted for medicinal purposes. Its use as a rootstock is justified by its resistance to *Fusarium solani* and *F. oxysporum* f. sp. *passiflorae*. However, is susceptible to the reniform nematode (*Rotylenchulus reniformis*) and possible to the Southern root-knot nematode (*Meloidogyne incognita*). This deserves attention, as plant resistance to diseases caused by soil fungi is often compromised when the roots of these plants are infected by nematodes. The objective of this research was to evaluate the effect of *M. incognita* on *P. foetida*. Two trials were carried out in a glasshouse. The first trial comprised three treatments: T1: non-inoculated control; T2: 1,600 *M. incognita* specimens per plant; T3: 8,000 specimens. For the second trial T2: 5,500; T3: 22,500. Both trials were evaluated 56 days after inoculation. The results showed difference between the inoculated plants and control for the following variables: root weight, vine length, nematodes per gram of roots and reproduction rate. It was concluded that *P. foetida* is susceptible to *M. incognita* and infested crop fields should be managed before planting *P. foetida*.

Index terms: Wild passion fruit, phytonematode, rootstock, field management.

Meloidogyne incognita é um importante fator limitante para maracujá-de-cheiro (*Passiflora foetida* L.)

Resumo: No Brasil, o maracujá-de-cheiro (*Passiflora foetida* L.) é explorado como porta-enxerto para o plantio comercial de maracujá (*Passiflora edulis* Sims), além de cultivado para fins medicinais. Seu uso como porta-enxerto é justificado pela sua resistência à *Fusarium solani* and *F. oxysporum* f. sp. *passiflorae*. No entanto, é suscetível ao nematoide-reniforme (*Rotylenchulus reniformis*) e, possivelmente, ao

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nematoide-das-galhas (*Meloidogyne incognita*). Este fato merece atenção, uma vez que a resistência das plantas a doenças causadas por fungos do solo é, frequentemente, comprometida quando as raízes destas plantas são infectadas por fitonematoides. Além disso, em solos infestados, *P. foetida* pode sofrer danos diretos. O objetivo deste trabalho foi avaliar o efeito de *M. incognita* sobre *P. foetida*. Dois ensaios foram realizados em casa de vegetação. O primeiro ensaio compreendeu três tratamentos: T1: testemunha não inoculada; T2: 1.600 espécimes por planta; T3: 8.000 espécimes. Para o segundo ensaio: T2: 5.500 espécimes; T3: 22.500 espécimes. Ambos os ensaios foram avaliados 56 dias após a inoculação. Os resultados mostraram uma diferença entre as plantas inoculadas, quando comparadas com o controle, para as seguintes variáveis: massa das raízes, altura da rama, nematoides por grama de raízes e fator de reprodução. Concluiu-se que *P. foetida* é suscetível a *M. incógnita*, e os campos de cultivo infestados devem ser manejados antes do plantio de *P. foetida*.

Termos para indexação: Maracujá silvestre, fitonematoide, porta-enxerto, manejo de campo.

The genus *Passiflora* L. comprises about 500 species of tendril-bearing vines, shrubs or trees and sour passion fruit (*Passiflora edulis* Sims) stands out for its economic importance (DHAWAN et al., 2004). The stinking passion flower (*Passiflora foetida* L.) is a valuable species due to two characteristics: it is a plant with several medicinal properties, a fact that makes it exploited and produced on a large scale (CAVICHIOLI et al., 2020); it is tolerant to salinity and fusariosis [*Fusarium oxysporum* f. sp. *passiflorae* W.L. Gordon 1954 (FOP) and *F. solani* (Mart.) Sacc. 1881], therefore can be used as a rootstock for *P. edulis* in unbalanced soils (DARIVA et al., 2015; SOUZA et al., 2023).

However, stinking passion flower is susceptible to the reniform nematode (*Rotylenchulus reniformis*) and possibly to the Southern root-knot nematode (*Meloidogyne incognita*) (PAES et al., 2022; SAUER; ALEXANDER, 1979). This is concerning, as plant resistance to diseases caused by soil fungi is often compromised when the roots of these plants are infected by nematodes (TOFOLI et al., 2019). In addition, phytonematodes may compromise the cultivation of stinking passion flower as a medicinal plant. The host status of *P. foetida* to three nematodes was briefly reported by Sauer and Alexander (1979). The authors stated that *P. foetida* is resistant to the Javanese and Northern root-knot nematodes (*Meloidogyne javanica* and *M. hapla*), but probably susceptible to the Southern root-knot nematode (*M. incognita*), as the infected roots contained nematode females and eggs. The resistance of *P. foetida* to *M. javanica* was later confirmed by Paes et al., (2022).

Considering that *M. incognita* is widespread in tropical and subtropical countries (EISENBACK, 2020), this nematode may restrict the cultivation of *P. foetida* in many growing areas. Furthermore, the aim of this study was to confirm the susceptibility of *P. foetida* to *M. incognita* and to evaluate the damage caused by this nematode.

The isolate of *M. incognita* was collected from cotton (*Gossypium hirsutum* L.) roots in 2004 in Campo Verde (MT) and has been maintained in glasshouse, alternating cotton, bell pepper, common bean, corn and tomato as hosts in order to preserve its infectiveness. Once a year, species identification was confirmed based on the perineal configuration of mature females (JEPSON, 1987; KLEYNHANS, 1986). Just before obtaining the inoculum, the electrophoretic profile of the esterase isoenzyme was conducted (ALFENAS; BRUNE, 2006).

The *M. incognita* inoculum was obtained by homogenizing infected roots in a blender and the resultant suspension was poured through three stacked sieves (60-200-500 Mesh, corresponding to 0.250-0.074-

0.025mm aperture), resulting in an aqueous suspension containing eggs and second-stage juveniles (nearly 70% eggs and 30% J2) of *M. incognita* (BONETI; FERRAZ, 1981). The nematodes were counted under a compound light microscope at 100x magnification with the aid of a Peters' counting slide. This slide consists in a glass plate with an area of 1 cm² divided into smaller squares. Under a microscope, nematodes are counted within a series of these smaller squares, enabling the measurement of the number of specimens per mL, which is then multiplied by the volume of the suspension.

Seeds of *Passiflora foetida* cultivar UFERSA BRSRM 153 was provided by Dr. Fabio G. Faleiro (Embrapa Cerrados, Planaltina DF - Brazil) and sowed in 500-cm³ plastic pots (R=4,25cm / r=2,7 /h=13) filled with autoclaved (121°C/2h) sandy soil (83% sandy / 2% silt / 15% clay). Cotton cultivar 1370 GLT was included to check the infectiveness of nematode, and sown one month later, in order to better match the phenological stage with the period of plant evaluation.

Seventy-two days after sowing, the *P. foetida* seedlings were transplanted to 500-cm³ plastic pots, at the rate of one seedling per pot. Seventy days after the transplant, the trial was performed with 3 treatments, namely: T1 - control without nematodes; T2 - with 1,600 specimens of *M. incognita* per pot; T3 - with 8,000 specimens per pot. Cotton plants were inoculated with 1,600 specimens. Each treatment was composed by six replicates, each replicate corresponding to one 10-11 cm high plantlet with 6-7 leaves.

The inoculum was poured into a 2-cm hole made in the soil. After the inoculation the plants were maintained in a glasshouse (max 37°C; min 24°C) until the assessment, 56 days after the inoculation (DAI). The root nematodes (eggs and J2) were recovered as described above to obtain the inoculum. Nematodes were preserved alive at 10°C and counted twice with the aid of a Peters' counting slide (1mL aliquot) at 100x magnification using a light microscope (Olympus CH2, Japan). The final population (eggs and J2) of *M. incognita* (Pf), fresh root weight (FRW), reproduction rate (R=Pf/Pi) and nematodes per gram of root (Nem./g) were evaluated (Table 1). Unfortunately, the plant vines were inadvertently discarded. Therefore, some data were missing and are not available (weight and length of vines).

Furthermore, a similar trial was carried out using more developed plants (130 days after transplanting 70-day seedlings) with higher *M. incognita* population densities (T2 - 5,500 specimens and T3 - 22,500). Therefore, the stinking passion flower plants were 15-16 cm high with 10-11 leaves at the time of inoculation. The plants were kept in a glasshouse (max 33.5°C; min 22°C) until evaluation (56 DAI). The variables evaluated were fresh root weight (FRW), reproduction rate (R = Pf/Pi), nematodes per gram of root, dry weight of vine in gram (DWV) and vine length in cm (VL) (Table 2).

Both experiments were conducted in a completely randomized design. Normality of the data was assessed using Shapiro-Wilk test, and data were transformed as necessary using log10 (x+1). For the analysis, it was used the R package (R Core Team) and the mean values were compared by the Tukey test at the 5% significance level.

Inocula infectiveness were confirmed on cotton (R = 24.92 in trial 1 and 8.41 in trial 2). Plants inoculated with the nematode showed very characteristic root galls in both trials (Figure 1), especially in the main root closest to the stem. A yellowing of the leaves was also observed at the end of the trial, as well as reduction in vine length (Figure 1).

In trial 1, there was a significant difference in FRW in T3 compared to control and T2, but T2 did not differ from to control. For the variable R, T2 showed a much higher value than T3, however there was not statistical difference between T2 and T3 in the variables Pf (T2: 23,154; T3: 22,886) and Nem/g (T2: 5,652; T3: 7,420).

Table 1- Reproduction of *M. incognita* on *P. foetida* (Pf = final population in the roots; Nem./g = number of nematodes per gram of roots; R = Pf/Pi) and fresh root weight (FRW) at 56 DAI.

Treatment	FRW (g)	Pf	Nem./g	R
T1: Control	5.13 a	-	-	-
T2: Pi -1,600	4.35 a	23,154 ns	5,652 ns	13.98 a
T3: Pi- 8,000	3.17 b	22,886	7,420	2.76 b

Means followed by the same letter in column do not differ according to Tukey test at 5% significance. Data were transformed using log10 (x+1) before performing the statistical analysis. *Pi: population inoculated. *ns: not significant.

In trial 2, the Southern root-knot nematode reduced vine length at both population densities. As in trial 1, yellowing of the leaves was observed (Figure 1–A); additionally, root galls were very conspicuous (Figure 1-B). However, FRW and DWV were not affected, perhaps because the nematode reduced the internode distance, but not the number of leaves (n=16 in control; 16 in Pi 5,500; 14 in Pi 22,500) and the infected roots had the weight enhanced by due to galls formation. There was also a statistical difference in Nem./g between the treatments T2 and T3. Different from trial 1, the reproduction rate did not differ between T2 and T3, probably because the plants of this trial were more developed, consequently provided more roots for nematode colonization (Table 2). Finally, both Pi 5,500 and Pi 22,500 had an R value greater than 1 showing that *P. foetida* was susceptible to *M. incognita*.

Table 2- Reproduction of *M. incognita* on *P. foetida* (Pf = final population density in the roots; Nem./g = number of nematodes per gram of roots; R = Pf/Pi), fresh root weight (FRW), vine length (VL) and dry weight vine (DWV) at 56 DAI.

Treatment	VL (cm)	FRW (g)	DWV (g)	Pf	Nem./g	R
T1: Control	66.83 a	9.17 ns	2.14 ns	-	-	-
T2: Pi 5,500	47.50 b	8.19	1.97	8,448 b	1,031 b	1.53 ns
T3: Pi 22,500	43.34 b	9.60	1.87	29,745 a	3,198 a	1.46

Means followed by the same letter in column do not differ according to Tukey test at 5% significance. Data were transformed using log10 (x+1) before performing the statistical analysis. *Pi: population inoculated. *ns: not significant.

Figure 1. Plants of *Passiflora foetida* 56 days after the inoculation with *Meloidogyne incognita* (trial 2). A. From left to right: non-inoculated plant, inoculated with 5,500 specimens (T2) and 22,500 specimens (T3). B. Roots of a plant inoculated with 5,500 specimens.

Sauer and Alexander (1979) inoculated egg masses of *M. incognita*, *M. javanica* and *M. hapla*, corresponding to 2000-2500 eggs per plant. Three months after inoculation with *M. incognita*, *P. foetida* roots exhibited galls containing females and eggs, but the final population was not estimated. In addition, stinking passion flower plants inoculated with *M. incognita* exhibited several galls and two out of eight plants died. Conversely, roots inoculated with *M. javanica* and *M. hapla* were free of galls, suggesting that *P. foetida* is resistant or imune to both root-knot nematodes. Therefore, the current results partially confirmed Sauer and Alexander (1979) findings.

Indeed, stinking passion flower proved to be a susceptible host for *M. incognita* at different initial population of the nematode, ranging from the lowest concentrations to the highest (Pi 1,600 – R = 13.98; Pi 5,500 – R = 1.53; Pi 8,000 – R = 2.76; Pi 22,500 – R = 1.46). It is noteworthy that the nematode reproduction rate is reduced at high nematode population densities, as nematodes begin to compete with each other for feeding sites on the host plant (AFSHAR et al., 2014; OOSTENBRINK, 1966).

In conclusion, stinking passion flower is susceptible to *M. incognita*. As infected plants are weakier than uninfected ones, crop fields infested by the Southern root-knot nematode should be properly managed to reduce nematode density before planting *P. foetida*, either as medicinal plant or as rootstock for sour passion fruit.

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