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# Host status of stinking passion flower and sour passion fruit to *Rotylenchulus reniformis*, *Meloidogyne javanica* and *Pratylenchus brachyurus*

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**Abstract:** Stinking passion flower (*Passiflora foetida* L.) is an herbaceous vine used due its medicinal properties. It could be an option to be used as a rootstock for sour passion fruit (*P. edulis*). This interest was especially motivated by its resistance to *Fusarium oxysporum* f. sp. *passiflorae* and *F. solani*, two soilborne pathogens that severely limit the sour passion fruit cultivation in Brazil. Phytonematodes are other important soil pathogens to most crops, including *Passiflora* species, and they often interact synergistically with pathogenic *Fusarium* spp. strains. However, the host status of stinking passion flower to phytonematodes was not assessed yet. Therefore, three pot experiments were carried out in order to assess the reproduction of *Rotylenchulus reniformis*, *Meloidogyne javanica* and *Pratylenchus brachyurus* on stinking passion flower and sour passion fruit. Both *P. foetida* and *P. edulis* were susceptible to *R. reniformis*, but immune (or highly resistant) to both *M. javanica* and *P. brachyurus*. Based on previous and the present work, *R. reniformis* stands out and could be pointed as the most important species to species of *Passiflora*.

**Index terms:** lesion nematode, *Passiflora edulis*, *Passiflora foetida*, reniform nematode, root-knot nematode.

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# Reação de maracujazeiro-de-cheiro e maracujazeiro-azedo a *Rotylenchulus reniformis*, *Meloidogyne javanica* e *Pratylenchus brachyurus*

**Resumo:** O maracujá-de-cheiro (*Passiflora foetida* L.) é uma trepadeira herbácea com propriedades medicinais. Pode ser uma opção a ser utilizada como porta-enxerto para o maracujazeiro-azedo (*P. edulis* Sims). O interesse foi especialmente motivado pela sua resistência a *Fusarium oxysporum* f. sp. *passiflorae* e *F. solani*, dois fitopatógenos de solo que limitam severamente o cultivo do maracujazeiro-azedo no Brasil. Os fitonematoides são outros importantes patógenos de solo para a maioria das culturas, incluindo espécies de *Passiflora*, e muitas vezes interagem sinergicamente com isolados patogênicos de *Fusarium* spp.. Entretanto, a reação de *P. foetida* aos nematoides fitoparasitas ainda não foi avaliada. Dessa forma, três experimentos em vasos foram realizados em casa de vegetação, com o objetivo de avaliar a reprodução *de Rotylenchulus reniformis*, *Meloidogyne javanica* e *Pratylenchus brachyurus* em maracujazeiro-de-cheiro e maracujazeiro-azedo. Tanto *P. foetida* quanto *P. edulis* foram suscetíveis a *R. reniformis*, mas imunes (ou altamente resistentes) a *M. javanica* e *P. brachyurus*. Com base em trabalhos anteriores e no presente, *R. reniformis* se destaca e pode ser apontada como a espécie mais importante para as espécies de *Passiflora*.

**Termos para indexação:** fusariose, nematoide-das-lesões-radiculares, *Passiflora edulis, Passiflora foetida*, nematoide-reniforme, nematoide-das-galhas.

## Introduction

Passion fruits plants belong to the genus *Passiflora* L., which groups about 500 species mostly distributed in tropical and subtropical regions. Brazil has about 150 native species with some of them exploited in agriculture. *Passiflora edulis* Sims is the main commercial species in Brazil, especially the sour passion fruit (*Passiflora edulis*). It is widely appreciated due to its flavor, aroma and calming properties (BERNACCI et al., 2015; MUNHOZ et al., 2018). Sour passion fruit is very susceptible to soilborne pathogens, mainly *Fusarium oxysporum* f. sp. *passsiflorae* (FOP), causing fusarium wilt, and *Fusarium solani* (FS), causing collar rot (FISCHER; REZENDE, 2016).

To manage these root diseases, the safest strategy would be avoided infested areas, but it is not possible in many situations. Thereafter, the most efficient option in infested areas would be the use of resistant cultivars or rootstocks (DARIVA et al., 2015). The studies carried out by Santos et al. (2016) confirmed that grafting using resistant rootstocks is an excellent alternative to reduce the harmful effects of *Fusarium* in sour passion fruit vines. The most valuable rootstocks are wild *Passiflora* species, such as *P. foetida* (CARVALHO et al., 2021; CHAVES et al., 2004; SILVA et al., 2017).

Stinking passion flower (*P. foetida* L.) is widely used due its medicinal properties and could also be used as rootstock to sour passion fruit in areas infested with FOP and FS.

This wild *Passiflora* species showed resistance to FOP even when cultivated in areas with previous reports of fusarium wilt (SILVA et al., 2017). According to Preisigke et al. (2017), *P. foetida*, *P. nitida*, *P. morifolia* and *P. mucronata* were the most resistant to FOP among 14 wild species of *Passiflora*, which were maintained in Hoagland's nutrient solution for 40 days. However, *P. foetida* is susceptible to *Meloidogyne incognita* (Kofoid and White) Chitwood (SAUER; ALEXANDER, 1979), a polyphagous phytonematode widespread in tropical and subtropical countries (EISENBECK, 2020). Additionally, *Meloidogyne* spp. (including *M. incognita)* are known to increase the incidence of fusarium wilt (HILLOCKS, 1985). Therefore, the presence of root-knot nematode is a considerable risk for implantation of vine of *P. foetida*, or even vines of *P. edulis* grafted on *P. foetida*.

Due this context, the host status of *P. foetida* to other species of phytonematodes is an important step for risk assessment in infested areas. The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) are the most important nematodes of *Passiflora* species (INOMOTO; FONSECA, 2020). The root-lesion nematode *Pratylenchus brachyurus* (Filipjev) is prevalent in soybean and common bean crop fields of Brazil (BONFIM JUNIOR et al., 2021; DEBIASI et al., 2016; MÁRQUEZ et al., 2021), but it is widespread in the country, and the host status of species of *Passiflora* to this nematode was not evaluated yet. According to Sauer and Alexander (1979), *M. javanica* (Treub) caused small galls in *P. foetida* roots, but did not reproduce. Therefore, the present work aims to assess the host status of stinking passion flower and sour passion fruit to *R. reniformis*, *M. javanica* and *P. brachyurus*, which are three of the most widespread plant parasitic nematodes in Brazilian crop fields.

# Material and methods

## Nematode isolates

The isolate of *R. reniformis* was obtained from sour passion fruit roots collected in Piracicaba (SP - Brazil) and maintained on the same plant in a glasshouse. The isolate of *M. javanica* was obtained from soybean roots [*Glycine max* (L.) Merr.] collected in Londrina (PR - Brazil) and maintained on spring onion (*Allium fistulosum* L.). The isolate of *P. brachyurus* was obtained from cotton roots (*Gossypium hirsutum* L.) in Sapezal (MT – Brazil) and maintained on cotton. The inocula of the three phytonematodes were obtained by homogenizing infected roots in a blender and the resultant suspension was poured through three sieves stacked (60-200-500 Mesh, corresponding to 0.250-0.074-0.25mm aperture), resulting in an aqueous suspension containing: eggs and motile forms (eggs, juveniles, males and immature females) of *R. reniformis;* eggs and second stage juveniles of *M. javanica*; eggs and a mix mobile life stages (juveniles and females) of *P. brachyurus*.

## Plant material

Seeds of *P. foetida* and *P. edulis* 'BRS-Gigante-Amarelo' ('BRS-GA1') were provided by Dr. Fabio G. Faleiro (Embrapa Cerrados, Planaltina DF - Brazil), and sowed in a 500-cm$^3$ plastic pots (R=4,5cm / r=2,75cm / h=13,5cm) filled with autoclaved sandy soil (121ºC/2h) in Apr 5, 2021 (*P. foetida*) and Jun 17, 2021 (*P. edulis*). The *Passiflora* spp. seedlings were transplanted in Sep 8, 2021 into pots containing 450 cm$^3$ of an autoclaved sandy-loam soil (83% sandy / 2% silt / 15%

clay), keeping one plant per pot. Cotton 'Deltapine-1730' was included to check the infectiveness of *R. reniformis* and *P. brachyurus*, and muskmelon 'Asturia' (*Cucumis melo* L.) to check *M. javanica*.

### Reniform nematode (experiment 1)

Each seedling was inoculated with an initial population density (Pi) of 1,000 specimens of *R. reniformis* by pouring the inoculum in a 2-cm-hole made in the soil (Sep 9, 2021 / 1 day after transplanting). The plants were maintained in a greenhouse until the evaluation (Dec 1, 2021 / 83 days after inoculation - DAI). Using a 10-liter bucket containing 4 liters of tap water, the soil and roots were separated. The nematodes from soil were recovered by centrifugal-flotation method (JENKINS, 1964). The resultant aqueous suspension contained adults (males and immature females) and juveniles of *R. reniformis*. Nematodes within roots (eggs, juveniles, males and immature females) were recovered as described above for *R. reniformis* inoculum production. The nematodes were preserved alive at 10 ºC, and counted twice in a Peters' counting slide (1mL aliquot) at 100x magnification using a light microscope (Olympus CH2, Japan). The *R. reniformis* final population (Pf) was the sum of the population recovered from the soil (Pf soil) and the population from the roots (Pf Roots); thereafter, the reproduction rate (R=Pf/Pi) was estimated.

### Root-knot nematode (experiment 2)

Each seedling was inoculated with 1,000 specimens of *M. javanica* by pouring the inoculum in a 2-cm-hole made in the soil (Sep 9, 2021). The plants were maintained in a glasshouse until the evaluation (Nov 29, 2021 / 81 DAI), which procedure was similar to the described above for *R. reniformis*.

### Lesion nematode (experiment 3)

Each seedling was inoculated with 1,000 specimens of *P. brachyurus* by pouring the inoculum in a 2-cm-hole made in the soil (Sep 9, 2021). Seedlings of cotton 'DP-1730' were also inoculated aiming to assess the infectiveness of the inoculum. The plants were maintained in a glasshouse until the evaluation (Dec 11, 2021 / 93 DAI), which procedure was similar to the described below for *R. reniformis*.

### Experimental design

The trials were conducted in a completely randomized design (CRD), with three treatments (*P. foetida*, *P. edulis* and cotton/muskmelon) and seven replicates. Normality of the data was assesses using Shapiro-Wilk test, and when was not fulfilled, data was transformed. For experiment 1, the data obtained (R) were transformed using $\log_{10}(x+1)$, using the R package (R Core Team) and the mean values were compared by the Tukey honest test at the 5% significance level.

## Results and discussion

The results were presented in Table 1. The reproduction of *R. reniformis* and *P. brachyurus* on cotton, and of *M. javanica* on muskmelon, confirmed the infectiveness of the inocula. Although the reproduction of *M. javanica* on muskmelon 'Asturia' could be considered low (R=3.97), it was similar to the values usually obtained previously. For example, the R of 14 genotypes of muskmelon ranged from 0.23 to 4.24 in a greenhouse experiment (DINIZ et al., 2016).

The reniform nematode reproduced equally well on both *Passiflora* species. The nematode population increased more on *P. edulis* than on cotton, probably because the *R. reniformis* was isolated and maintained on *P. edulis* plants. In addition to *P. edulis*, three other passion fruit species (*P. cincinnata* Mast., *P. setacea* DC. and *P. alata* Curtis) are susceptible to *R. reniformis* (INOMOTO; FONSECA, 2020).

However, to our knowledge, the present result is the first report of showing the reproduction of the reniform nematode on *P. foetida*. Thus, producers of stinking passion flower and of passion fruit grafted on *P. foetida* ought to select carefully the nurseries and avoid fields infested with this nematode.

**Table 1.** Number of *Passiflora foetida*, *Passiflora edulis*, cotton and muskmelon evaluated plants (N), reproduction of *Rotylenchulus reniformis* (83 days after inoculation – DAI), *Meloidogyne javanica* (81 DAI) and *Pratylenchus brachyurus* (93 DAI) on *P. foetida*, *P. edulis* and standard hosts (cotton and muskmelon).

| Plants | N | Reproduction Rate (R) | | |
|---|---|---|---|---|
| | | ***R. reniformis*** | ***M. javanica*** | ***P. brachyurus*** |
| ***P. foetida*** | 7 | 16.27 ab | 0.00 a | 0.00 a |
| ***P. edulis*** | 7 | 23.04 a | 0.00 a | 0.01a |
| **Cotton 'DP-1730'** | 7 | 8.29 b | - | 18.21 b |
| **Muskmelon 'Asturia'** | 7 | - | 3.97 b | - |

Means followed by the same letter in column do not differ according to Tukey test at 5% significance. [1]Data were transformed using log10 (x+1) before performing the statistical analysis.

Conversely, both *P. foetida* and *P. edulis* proved to be immune or highly resistant to *M. javanica* and *P. brachyurus*. The high resistance of stinking passion flower to *M. javanica* confirmed results obtained by Sauer and Alexander (1979), which tested *P. foetida* to *M. javanica*; *M. incognita* and *M. hapla* and noticed only the reproduction of *M. incognita*. Some passion fruit species, as *P. nitida* Kunth., *P. ligularis* Juss. and *P. mucronata* Lam. are susceptible to *M. javanica*, but others, including sour passion fruit are immune or highly resistant (GARCIA et al., 2008; ROCHA et al., 2013; SHARMA et al., 2003). Based on our work and the available data, *M. javanica* is not a concerning pathogen to both stinking and sour passion fruit.

To our knowledge, the present results are the first work addressing the host status of these *Passiflora* species to *P. brachyurus*. Both *P. foetida* and *P. edulis* proved to be immune or highly resistant to this root lesion nematode. A survey carried out in an orchard the State of Amapá showed the occurrence of *P. brachyurus* on roots of murici (*Byrsonima* sp.) and soil around roots of murici and cupuaçu [*Theobroma grandiflorum* (Willd.ex Spreng. K. Schum.)], but not on the roots of an unidentified species of passion fruit and in soil around the roots (SANTOS; MARTINELLI, 2016).

The results about *M. javanica* and *P. brachyurus*, which are ubiquitous in Brazilian croplands, demonstrated that both *P. foetida* and *P. edulis* may be cultivated in infested areas with these nematodes. Conversely, *R. reniformis* may be a limiting factor for stinking passion flower rootstock implementation and for stinking passion flower cultivation.

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## References

BERNACCI, L. C.; CERVI, A. C.; MILWARD-DE-AZEVEDO, M. A.; NUNES, T. S.; IMIG, D. C.; MEZZONATO, A. C. **Passifloraceae in lista de espécies da flora do Brasil. Jardim Botânico do Rio de Janeiro**. Disponível em: <http://reflora.jbrj.gov.br/jabot/floradobrasil/FB182>. Acesso em: 22 mai. 2022.

BONFIM JUNIOR, M. F.; INOMOTO, M. M.; ARAÚJO FILHO, J. V. Phytonematodes infesting common bean fields in Brazil, and pathogenicity tests with *Pratylenchus brachyurus*. **Arquivos do Instituto Biológico**, São Paulo, v. 88, e00312020, p. 1-11, 2021.

CARVALHO, J. S.; SÃO-JOSÉ, A. R.; ARANTES, A. M.; SÃO-JOSÉ, A. R.; KURFIS, M. A. Survival of passion fruit species with or without partial root exposure on an area with history disease of *Fusarium solani*. **Agroecossistemas**, Belém, v.13, p.163-181, 2021.

CHAVES, R. C.; JUNQUEIRA, N. T. V.; MANICA, I.; PEIXOTO, J. R.; PEREIRA, A. V.; FIALHO, J. F. Enxertia de maracujazeiro-azedo em estacas herbáceas enraizadas de espécies de passifloras nativas. **Revista brasileira de fruticultura**, Jaboticabal, v. 26, n. 1, p. 120-123, 2004.

DARIVA, J. M.; XAVIER, A. A.; COSTA, M. R.; FERREIRA, R. C. R.; SOUSA, T. V. Variabilidade Genética de Isolados de *Fusarium solani* e *Fusarium oxysporum* f. sp. *passiflorae* associados ao Maracujazeiro. **Revista brasileira de fruticultura,** Jaboticabal, v. 37, n. 2, p. 377-386, 2015.

DEBIASI, H.; FRANCHINI, J. C.; DIAS, W. P.; RAMOS JÚNIOR, E. U.; BALBINOT JÚNIOR, A. A. Práticas culturais na entressafra da soja para o controle de *Pratylenchus brachyurus*. **Pesquisa agropecuária brasileira**, Brasília, v. 51, n. 10, p. 1720-1728, 2016.

DINIZ, G. M. M.; CANDIDO, W. S.; SOARES, R. S.; SANTOS, L. S.; MARÍN, M. V.; SOARES, P. L. M.; BRAZ, L. T. Reaction of melon genotypes to *Meloidogyne incognita* and *Meloidogyne javanica*. **Pesquisa agropecuária tropical**, Goiânia, v. 46, p. 111-115, 2016.

EISENBACK, J. D. ***Meloidogyne incognita* (root-knot nematode)**. Invasive Species Compendium. Wallingford, UK: CABI, 2020. Disponível em: <https://www.cabi.org/isc/datasheet/33245>. Acesso em: 18 jun. 2022.

FISCHER, I. H.; REZENDE, J. A. M. Doenças do Maracujazeiro. Ed. 2. Editora Ceres: **Manual de fitopatologia: doenças de plantas cultivadas**, 2016. p. 537-538.

GARCIA, M. J. D. M.; ALMEIDA, A. M.; WILCKEN, S. R. S.; FISCHER, I. H.; SAMPAIO, A. L. A.; JESUS, M.; FUMIS, T. Reação de Maracujazeiro Amarelo 'Afruvec' e 'Maguary' a *Meloidogyne spp*. **Revista arquivos do instituto biológico,** São Paulo, v. 75, n. 2, p. 235-238, 2008.

HILLOCKS, R. J. The effect of root-knot nematode on vascular resistance to *Fusarium oxysporum* f. sp. *vasinfectum* in the stems of cotton plants. **Annals of applied biology**, London, v. 107, p. 213-218, 1985.

INOMOTO, M. M.; FONSECA, A. B. Susceptibility of four passion fruit species to the reniform nematode. **Revista brasileira de fruticultura**, Jaboticabal, v. 42, n. 2, e-557, 2020.

JENKINS, W. R. A. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant disease reporter**, London, v. 48, n. 9, p. 692, 1964.

MÁRQUEZ, L. A. Y.; GOMES, C. B.; BELLÉ, C.; DALLAGNOL, L. J.; ARAÚJO FILHO, J. V. Unveiling the structure and distribution of plant-parasitic nematode communities in soybean fields in southern of the Brazil. **European journal of plant pathology**, Clayton, v. 160, p. 457-468, 2021.

MUNHOZ, C. F.; COSTA, Z. P.; CAUZ-SANTOS, L. A.; REÁTEGUI, A. C. E.; RODDE, N.; CAUET, S.; DORNELAS, M. C.; LEROY, P.; VARANI, A. M.; BERGES, H.; VIEIRA, M. L. C. A gene-rich fraction analysis of the *Passifora edulis* genome reveals highly conserved microsyntenic regions with two related Malpighiales species. **Scientific reports**, London, v. 8, n. 13024, p. 1-18, 2018.

PREISIGKE, S. C.; SILVA, L. P.; SERAFIM, M. E.; BRUCKNER, C. H.; ARAÚJO, K. L.; NEVES, L. G. Seleção precoce de espécies de *Passiflora* resistente a fusariose. **Summa phytopathologica**, Botucatu, v. 43, n. 4, p. 321-325, 2017.

ROCHA, L. S.; RIBEIRO, R. C. F.; XAVIER, A. A.; SILVA, F. J.; BRUCKNER, C. H. Reação de genótipos de maracujazeiro a *Meloidogyne incognita* raça 3 e *Meloidogyne javanica*. **Revista brasileira de fruticultura**, Jaboticabal, v. 35, n. 4, p. 1017-1024, 2013.

SANTOS, C. H. B.; NETO, A. J. C.; SOARES, T. L.; OLIVEIRA, E. J.; JESUS, O. N.; GIRARDI, E. A. Porta-enxertos e fixadores de enxerto para enxertia hipocotiledonar de maracujazeiro azedo. **Ciência rural**, Santa Maria, v. 46, n. 1, p. 30-35, 2016.

SANTOS, R. S.; MARTINELLI, P. R. P. Nematodes associated with five fruit trees in the state of Amapá, Brazil. Ciência rural**, Santa Maria, v. 46, n. 9**, **p. 1509-1513, 2016.**

SAUER, M. R.; ALEXANDER, D. McE. Root-knot resistance in *Passiflora foetida*. **Australasian plant pathology**, Clayton, p. 50-51, 1979.

SHARMA, R. D.; RITZINGER, C. H. S. P.; JUNQUEIRA, N. T. V.; ALVES, R. T. **Reprodução e patogenicidade de *Meloidogyne javanica* no híbrido EC-2-0 de maracujá-azedo**. Planaltina: Embrapa Cerrados, 2003. 12p.

SILVA, R. M.; AMBRÓSIO, M. M. Q.; AGUIAR, A. V. M.; FALEIRO, F. G.; CARDOSO, A. M. S.; MENDONÇA, V. Reaction of passion fruit cultivars in areas with fusariosis. **Summa phytopathologica**, Botucatu, v. 43, n. 2, p. 98-102, 2017.