University of São Paulo
"Luiz de Queiroz" College of Agriculture

Understanding the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* in grafted passion fruit seedlings

Aline Mayara Gonçalves Barros Silva

Thesis presented to obtain the degree of Doctor in Science.
Area: Plant Pathology

Piracicaba
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RESUMO

Compreendendo a interação entre *Meloidogyne incognita* e *Fusarium oxysporum* f. sp. *passiflorae* em mudas enxertadas de maracujazeiro

O nematoide-das-galhas, *Meloidogyne incognita*, e o fungo da fusariose, *Fusarium oxysporum* f. sp. *passiflorae* (FOP) causam danos significativos à cultura do maracujá, resultando em perdas econômicas. Uma estratégia para o manejo de patógenos de solo associados à cultura, é explorar a variabilidade genética das espécies silvestres de *Passiflora*. Dentre essas espécies, *P. nitida* é uma espécie potencial para ser utilizada como porta-enxerto, pois é resistente a FOP. Porém, trata-se de uma espécie extremamente suscetível a *M. incognita*. Logo, o primeiro trabalho foi realizado para avaliar o efeito de *M. incognita* no crescimento de *P. nitida* e quais as alterações anatômicas causadas por essa infecção. Três ensaios foram conduzidos em delineamento inteiramente casualizado (DIC) em períodos distintos e ao final dos ensaios, variáveis relacionadas ao crescimento vegetal e a reprodução do nematoide foram coletadas. Estes dados foram avaliados estatisticamente e as médias dos tratamentos foram comparadas por meio do teste de Tukey a 5% de significância. Na oportunidade, também foram coletadas amostras de raízes para análise histológica. Notou-se que devido a alta taxa de multiplicação desse nematoide em *P. nitida*, causando intensa formação de galhas nas raízes e reduzindo o crescimento das plantas, *P. nitida* não deve ser utilizada como porta-enxerto ou mesmo cultura principal em campos infestados por *M. incognita*. No segundo estudo, investigamos a interação entre *M. incognita* e FOP, inoculados individualmente ou em combinação, em mudas de *P. edulis* f. *flavicarpa* enxertadas em *P. nitida*. Dois ensaios foram realizados em um delineamento inteiramente casualizado (DIC) em diferentes épocas, com o objetivo de avaliar a patogenicidade de *M. incognita* e FOP nessas mudas enxertadas. Foram coletados dados relacionados ao crescimento vegetal, incidência do fungo, tamanho das lesões causadas pelo fungo e densidade populacional do nematoide. Esses dados foram analisados estatisticamente, e as médias dos tratamentos foram comparadas utilizando o teste de Tukey a um nível de significância de 5%. Os resultados demonstraram uma interação neutra entre os patógenos, pois, independentemente da presença do fungo, *P. nitida* apresentou alta suscetibilidade a *M. incognita*, resultando na formação de galhas nas raízes e redução no crescimento das plantas. Além disso, observou-se que, mesmo na presença do nematoide, o tamanho das lesões causadas por FOP não aumentou. Vale ressaltar que a resistência de *P. nitida* a FOP foi mantida, mesmo na coinfecção com o nematoide. Essas descobertas têm implicações relevantes para o uso de *P. nitida* como porta-enxerto no manejo de FOP.

Palavras-chave: *Passiflora nitida*, Fusariose, Nematoide-das-galhas, Interação

ABSTRACT

Understanding the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* in grafted passion fruit seedlings

The root-knot nematode, *Meloidogyne incognita*, and the fusariosis fungus, *Fusarium oxysporum* f. sp. *passiflorae* (FOP) cause significant damage to passion fruit crops, resulting in economic losses. One strategy for managing crop-associated soil pathogens is to explore the genetic variability of wild *Passiflora* species. Among these species, *P. nitida* is a potential species to be used as a rootstock, as it is resistant to FOP. However, it is a species extremely susceptible to *M. incognita*. Therefore, this is the first study to evaluate the effect of *M. incognita* on the growth of *P. nitida* and the anatomical changes caused by this infection. Three trials were conducted in a completely randomized design (CRD) in different periods, and at the end of trials, variables related to plant growth and nematode reproduction were collected. These data were statistically evaluated and treatment means were compared using the Tukey test at 5% significance level. On that occasion, root samples were also collected for histological analysis. It was observed that due to the high multiplication rate of this nematode in *P. nitida*, causing intense gall formation on roots and reducing plant growth, *P. nitida* should not be used as rootstock or even main crop in fields infested by *M. incognita*. In the second study, the interaction between *M. incognita* and FOP, inoculated individually or in combination in *P. edulis* f. *flavicarpa* seedlings grafted onto *P. nitida* was investigated. Two trials were carried out in a CRD, at different times, with the aim of evaluating the pathogenicity of *M. incognita* and FOP in these grafted seedlings. Data related to plant growth, fungus incidence, size of lesions caused by the fungus and nematode population density were collected. These data were statistically analyzed, and treatment means were compared using the Tukey test at 5% significance level. Results demonstrated neutral interaction between pathogens, because, regardless of presence of the fungus, *P. nitida* showed high susceptibility to *M. incognita*, resulting in the formation of galls on roots and reduced plant growth. Furthermore, it was observed that, even in the presence of the nematode, the size of lesions caused by FOP did not increase. It is noteworthy that the resistance of *P. nitida* to FOP was maintained, even in co-infection with the nematode. These findings have relevant implications for the use of *P. nitida* as rootstock in the FOP management.

Keywords: *Passiflora nitida*, Fusariosis, Root-knot nematodes, Interaction

1. GENERAL INTRODUCTION

Passion fruit tree (*Passiflora* spp.) is known for its excellent adaptation to tropical and subtropical climates. According to FAO data, global passion fruit production reached the mark of 1.5 million tons per year during the period from 2015 to 2017, with special emphasis on South America (Altendorf, 2018). Brazil, in particular, stands out as the largest producer, reaching estimated production of 683,993 tons (IBGE, 2021). Although Brazil dominates the world passion fruit production, productivity has remained stable in recent years (approximately 14 t/ha/year); however, the estimated crop productivity is 50 t/ha/year (Meletti et al., 2005). The low passion fruit productivity is largely attributed to the occurrence of biotic diseases, especially those induced by soil-associated fungi and nematodes.

An alternative to manage crop-associated soil pathogens is to explore the genetic variability present in wild *Passiflora* species (Meletti & Bruckner, 2001). Among them, *P. nitida* Kunth is a potential species, since it is considered rustic and tolerant to diseases, including bacteriosis, anthracnose and those caused by soil fungi, which makes it an interesting option for breeding programs that include interspecific hybridization or to be used as rootstock for the management of soil-associated diseases (Junqueira et al., 2005; Menezes et al., 1994; Pereira et al., 2019; Preisigke et al., 2017).

P. nitida is resistant to the main crop-associated soil fungi, especially *Fusarium oxysporum* f. sp. *passiflorae* W.L. Gordon 1954 (FOP) (Miguel-Wruck et al., 2021; Preisigke et al., 2017). FOP hyphae penetrate the passion fruit root system and, when they reach xylem vessels, obstruct the transport of water and essential nutrients to different plant parts. The primary symptom associated with infection by this fungus is the wilting of infected plants, accompanied by necrosis in conducting vessels that propagates upwards. In more severe cases, this condition can lead to the sudden death of infected plants (Ortiz & Hoyos-Carvajal, 2016; Silva et al., 2013).

FOP control is based on planting in sites with no history of the disease. Recently, there has been the possibility of recovering areas infested with this fungus by planting passion fruit seedlings grafted onto *P. nitida* (Semprebom et al., 2012), which is resistant to the fungus (Preisigke et al., 2017), since the abandonment of these areas is a common practice due to the presence of the disease. However, *P. nitida* is susceptible to the *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 nematode (Rocha et al., 2013).

M. incognita infection in passion fruit manifests through the formation of characteristic root galls, resulting in significant reduction in plant growth and development, in addition to

yellowing and atrophy of leaves. These symptoms have significant negative impact on crop productivity and longevity (Fischer & Rezende, 2008).

In view of the above, it is important to consider the potential impairment of *P. nitida* resistance to FOP in the presence of *M. incognita*, as it occurs in other pathosystems involving *F. oxysporum* and *Meloidogyne* species (Bertrand et al., 2000; Kassie, 2019). However, there are currently no studies showing whether resistance is maintained when soil is infested with *M. incognita*, which is extremely important, since several studies have demonstrated the existence of synergistic interaction between root-knot nematodes and *F. oxysporum* (France & Abawi, 1994; Sidhu & Webster, 1977). Therefore, the aim of this work was to evaluate the effect of *M. incognita* on the growth of *P. nitida*, to investigate the specific histopathological alterations induced by this nematode in this particular plant species and to characterize the interaction between *M. incognita* and FOP in sour passion fruit (*P. edulis* f. *flavicarpa* Deg.) seedlings grafted onto *P. nitida* in order to provide evidence about this interaction and its effects on plant resistance to the fungus.

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2. EFFECT OF THE SOUTHERN ROOT-KNOT NEMATODE ON THE GROWTH OF BREATH PASSION FRUIT

Abstract
Breath passion fruit (*Passiflora nitida*) is wild species widely distributed in Brazil with high potential, as its fruit has sweet taste and pleasant aroma. Furthermore, it has been studied as rootstock for sour passion fruit (*P. edulis*) in order to control wilting and collar rot caused by *Fusarium* spp. However, breath passion fruit is supposedly susceptible to the Southern root-knot nematode (*Meloidogyne incognita*), which is widespread in tropical crop fields. Two trials were carried out to assess the effect of *M. incognita* on the growth of *P. nitida* and a third trial was carried out to assess the anatomical changes induced by this nematode in *P. nitida*. In view of the high multiplication rate of this nematode in breath passion fruit, causing intense root galling and reducing plant growth, *P. nitida* should not be used as rootstock or even main culture in fields infested by *M. incognita*.

Keywords: Colar rot, Fusarium wilt, *Meloidogyne incognita*, *Passiflora edulis*, *Passiflora nitida*, Rootstock, Histopathology, Giant cells.

2.1. Introduction

Among passion fruit species, sour passion fruit (*Passiflora edulis* Sims) is the most cultivated worldwide and Brazil stands out as the largest passion fruit producer and consumer (IBGE, 2020). More than 500 of *Passiflora* species are known and most of them may be economic exploited due their culinary, medicinal, or ornamental properties (Faleiro et al., 2019). Breath passion fruit (*P. nitida* Kunth), also known as "suspiro" passion fruit, sigh passion fruit, bell apple or water lemon, is a wild species widely distributed in Brazil with high potential due to its sweet taste and pleasant aroma. Thus, breath passion fruit presents high potential for fresh consumption. Furthermore, in recent years, breath passion fruit (BPF) is an ever-increasing interest species due to its resistance to Fusarium wilt and collar rot (Carvalho et al., 2020; Miguel-Wruck et al., 2021; Preisigke et al., 2017; Rocha et al., 2021).

Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *passiflorae* W.L. Gordon 1954 (FOP), and collar rot, caused by *F. solani* (Mart.) Sacc. 1881, are major sour passion fruit diseases. Since both fungi form resistance structures (chlamydospores) that survive in the soil for a long period, their control is a challenge (Carvalho et al., 2015; Pereira et al., 2019; Pires et al., 2022; Rocha et al., 2021). Generally, once pathogens are established in a crop field, this area usually should no longer be cultivated with sour passion fruit. Grafting sour passion fruit onto breath passion fruit for *Fusarium* spp. control has been intensively

studied in Brazil as a promising strategy (Junqueira et al., 2006; Miguel-Wruck et al., 2021). However, BPF is possibly susceptible to the Southern root-knot nematode (SRNN), *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949. Two greenhouse trials have found conflicting results on susceptibility of BPF to SRNN. Although galls and egg masses were observed on BPF roots inoculated with 2,200 specimens of a SRNN population isolated from *Passiflora capsularis*, the nematode population density decreased 90% at the end of 62 days (Castro et al., 2010). Conversely, the population density of SRNN race 3 built up 15.7x, 90 days after the inoculation of 3,000 eggs (Rocha et al., 2013). Additionally, in a further experiment, all BPF plants inoculated with *M. incognita* race 3 (n=6) died during the experimental period of 180 days, supposedly due to the nematode infection (Rocha et al., 2021). However, control non-infected plants were not included, as the main objective of that assay was to evaluate the interaction between *F. solani* and *M. incognita*.

If in fact breath passion fruit is intolerant to SRRN, it should be not grown in infested fields. This would greatly restrict the use of breath passion fruit as a crop or rootstock, because *M. incognita* is extremely prevalent in tropical croplands (Eisenback, 2020).

In BPF, there is no histological documentation on alterations caused by infection by *Meloidogyne* species; therefore, this study was carried out to evaluate the effect of *M. incognita* on the growth of BPF and also to gather information about which histopathological changes are induced by this nematode in this plant species.

2.2. Material and methods

2.2.1. Effect of *M. incognita* on the growth of BPF (trial 1 and 2)

Two pot trials were conducted in greenhouse at the Laboratory of Nematology, located in the state of São Paulo, Brazil. The *M. incognita* isolate was collected from cotton (*Gossypium hirsutum* L.) roots in 2004 in Campo Verde (MT) and has been maintained in greenhouse, alternating cotton, bell pepper, common bean, corn, and tomato in order to preserve to infectiveness to different plants. Once a year, species identification was confirmed based on the perineal configuration of mature females (Kleynhans, 1986; Jepson, 1987). Immediately before obtaining the inoculum, the electrophoretic profile of the esterase isoenzyme was performed (Alfenas & Brune, 2006).

M. incognita inoculum for trials consisted of eggs and juveniles (J2) obtained from corn roots, which were ground with 1% sodium hypochlorite solution in a common kitchen blender at low speed for 1 minute. Then, the resulting suspension was poured through a sequence of three sieves (60 – 200 – 500 Mesh), removing sodium hypochlorite with running water. The

material retained on the 500 Mesh sieve (0.025mm aperture) was collected in beaker and nematodes were counted under compound light microscope (model CHS, Olympus Optical Co., Ltda., Tokyo Japan) at 100x magnification with the aid of a Peters' counting slide.

BPF seeds were supplied by Embrapa Cerrados in 2020. Gibberellic acid (GA_3) solution (1.000 $mg.L^{-1}$) and artificial light were used to break seed dormancy (Passos et al., 2004). Sowing was performed in tray containing autoclaved sandy (121°C/2h). After 72 days, plantlets were transplanted into plastic pots (12cm in diameter x 15cm in height) previously filled with 1,500 cm^3 of autoclaved sandy loam soil. Forty-two days after transplanting, 15 plants with similar size (18-20 cm height and 5-6 leaves) were chosen for the trial. Eight plants were kept non-inoculated and seven were inoculated with suspension containing the initial population density (Pi) of 10,000 specimens by pouring the inoculum into two 2-cm deep holes made close to the plant stem. Plants were kept in greenhouse for 60 days after inoculation (DAI). During this period, they were daily irrigated with tap water and received slow release NPK (15-9-12) fertilizer fortnightly (Osmocote®); pest or disease control were not necessary. The following variables were assessed: shoot height (cm), dry aerial part weight (g) and root fresh weight (g), number of nematodes per gram of root (Nema/g of root) and total number of nematodes in the soil. Nematodes were recovered from roots by the same procedure used to obtain the inoculum, while soil samples were processed by sucrose flotation method (Jenkins, 1964). The trial was repeated once, with smaller plants (12-15 cm height and 3-4 leaves) and longer experimental period (92 DAI evaluation).

Trials were carried out in a completely randomized design (CRD), with two treatments (inoculated plants; non-inoculated plants) and 7-8 replicates (trial 1) and 7 replicates (trial 2). Data were analyzed using the R package (r-project.org) and mean values were compared using Tukey's test at 5% significance level.

2.2.2. Interaction plant-nematode and histopathological analysis (trial 3)

Trial was carried out from September to April 2023 under laboratory and greenhouse conditions. Unlike trials 1 and 2, in this trial, sour passion fruit seedlings grafted onto BPF were used. "Embrapa Cerrados" provided the propagation material used to obtain BPF cuttings. Cuttings were treated with rooting solution and placed in trays filled with vermiculite for about 30 days under greenhouse conditions. Meanwhile, sour passion fruit seeds supplied by "Embrapa Cerrados" were sown in trays containing autoclaved sandy loam soil. After 30 days, the seedlings were top cleft grafted onto the rooted BPF cuttings. Grafted plants were then transplanted into plastic pots filled with autoclaved sandy-loam soil and kept in greenhouse for

30 days. Later, plants were divided into two treatment groups: (I) plants inoculated with *M. incognita* and (II) non-inoculated plants. The nematode inoculum consisted of *M. incognita* eggs and second-stage juveniles (J2) obtained from a pure population maintained in corn (*Zea mays* L.) roots. The nematode was inoculated into treatment I by applying 2,000 eggs + J2 into holes near the plant stems. Plants were then left in greenhouse for 105 days, with regular irrigation and fertilization. At the end of the experiment, the pathogenicity of *M. incognita* was evaluated based on various parameters such as dry aerial part weight (DAPW), shoot height (SH), root fresh weight (RFW) and number of nematodes per gram of root (Nema/g of root).

This trial was conducted in CRD with 6 to 8 replicates per treatment. Collected variables were submitted to normality test (Shapiro-Wilk) with 5% significance level and statistically analyzed using analysis of variance (ANOVA) and the RStudio software (versão 2022.02.3 Build 492).

For histopathological analysis, root samples were collected from plants distributed among the different treatments. Samples were washed to remove residues. Root fragments were excised and fixed in solution adapted from Karnovsky (1965), according to protocol proposed by Marques & Soares (2021). During fixation, samples were submitted to vacuum pump to remove air present in tissues. Then, samples were dehydrated using increasing series of ethanol concentrations (10, 20, 30, 40, 50, 60, 70, 90, 100%). After dehydration, samples were infiltrated in hydroxyethyl methacrylate resin (Leica Historesin®), according to manufacturer's instructions. Tissues were then cut into fragments with thickness of 5-7 µm using Leica RM 2235 rotary microtome. Sections were placed on glass slides and stained for different histological analyses. Toluidine blue (Sakai, 1973) was used for usual histological analyses. After staining, sections were examined using Zeiss Axioskop 2 microscope with a camera attached to capture images. This process was repeated four times for each treatment.

2.3. Results

2.3.1. Effects of SRRN infection on BPF plants

In trial 2, SRRN reached higher root population densities (1,315,695 specimens) than in trial 1 (183,093 specimens), supposedly due to longer experimental period. Thus, BPF is undoubtedly a good SRRN host, as previously reported by Sharma et al. (2005) and Rocha et al. (2013). However, soil population densities were very low (Tables 1 and 2). Thus, the effort to recover *Meloidogyne* specimens from soil seems to be not suitable for trials aiming to evaluate root-knot nematode reproduction, according to the authors' previous experience. In both trials, BPF plants inoculated with SRRN had lower shoot growth (Figures 1 and 2) and

lower shoot dry weight compared to non-inoculated ones. The fresh root weight of inoculated did not differ from non-inoculated ones (Tables 1 and 2); however, infected roots were very short and with numerous galls of variable sizes (Figure 3). Indeed, the formation of galls as a rule negatively interferes with the absorption and translocation of water and nutrients by roots, consequently reducing plant growth (Agrios, 2005).

Table 1. Effect of *Meloidogyne incognita* (trial 1) on the growth of *Passiflora nitida* plants, and final nematode density, 60 days after inoculation.

Treatments	N	Shoot height (cm)	Dry aerial part weight (g)	Root fresh weight (g)[1]	Nema/g of root	Number of nematodes in the soil
Control	8	58.14 b	7.76 b	15.75 a	-	
10,000 Mi	7	31.02 a	5.39 a	22.22 a	8,240	27
CV	-	37.03%	28.66%	12.59%	-	

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.

Table 2. Effect of *Meloidogyne incognita* (trial 2) on the growth of *Passiflora nitida* plants, and final nematode density, 92 days after inoculation.

Treatments	N	Shoot height (cm)	Dry aerial part weight (g)	Root fresh weight (g)	Nema/g of root	Number of nematodes in the soil
Control	7	103.36 b	12.76 b	32.08 a	-	
10,000 Mi	7	22.08 a	2.96 a	23.69 a	55,538	680
CV	-	37.84%	43.92%	33.81%	-	

Means followed by the same letter in column do not differ according to the Tukey test at 5% significance.

Figure 1. Evaluation of the effect of two treatments (inoculated plants with *Meloidogyne incognita*; non-inoculated plants with *M. incognita*) 60 days after inoculation (DAI) – trial 1. **A.** Plant inoculated with 10,000 specimens (right) and Uninoculated plant (left). **B.** Root system of the respective plants. Plant inoculated with 10,000 specimens (right), showing root galls, peeling and brown coloration, with tissue rot.

Figure 2. Evaluation of the effect of two treatments (inoculated plants with *Meloidogyne incognita*; non-inoculated plants with *M. incognita*) 92 days after inoculation (DAI) – trial 2. **A.** Plant inoculated with 10,000 specimens (right) and Uninoculated plant (left). **B.** Root system of the respective plants. Plant inoculated with 10,000 specimens (right) with root weight reduction and showing root galls.

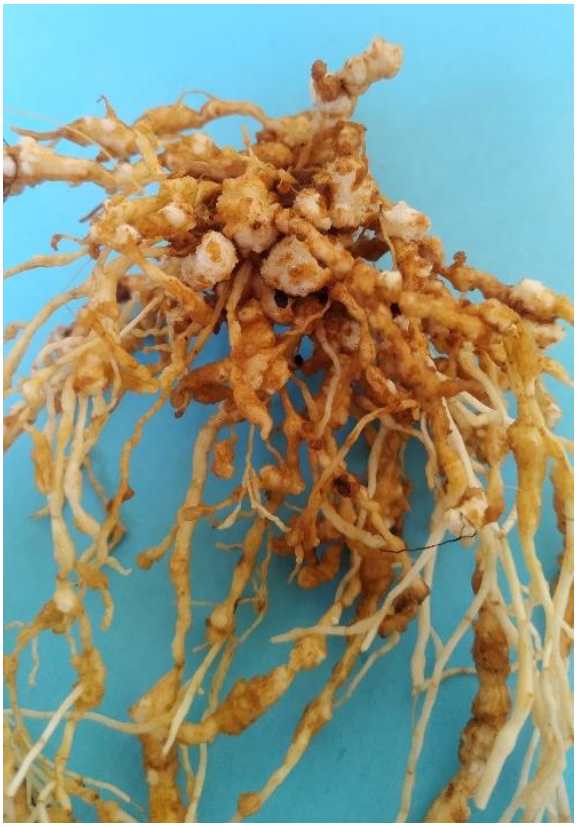

Figure 3. Approximation of the inoculated plants with *Meloidogyne incognita*, emphasizing the symptoms of the disease.

2.3.2. Interaction plant-nematode

In order to simulating a situation similar to field conditions, using BPF as rootstock, it was observed that the presence of *M. incognita* had significant impact on SH and DAPW variables that reflect the growth of the sour passion fruit cultivar (Table 3). The RFW of inoculated plants did not differ from non-inoculated plants (Table 3); however, numerous galls were found in the root system of BPF. The population density in the roots of this plant species was 260,395 specimens.

Table 3. Effect of *Meloidogyne incognita* (trial 3) on the growth of sour passion fruit seedlings grafted onto *Passiflora nitida*, and final nematode density, 105 days after inoculation.

Treatments	N	Shoot height (cm)	Dry aerial part weight (g)	Root fresh weight (g)	Nema/g of root
Control	6	172.8 b	13.52 b	43.93 a	-
2,000 Mi	8	134.6 a	6.86 a	41.87 a	6,219
CV	-	20.25%	17.08%	17.73%	-

Means followed by the same letter in column do not differ according to the Tukey test at 5% significance.

2.3.3. Histopathological analysis

When analyzing BPF roots inoculated with *M. incognita* through histopathological study, several anatomical alterations in the internal structure were observed. Cross sections of infected roots revealed severe nematode infestations, as shown in Figure 4C. Nematodes induced the formation of giant multinucleated cells with dense cytoplasm and walls thicker than normal in the stele region (Figure 4F). Furthermore, hypertrophy of the cortical parenchyma resulted in visible compression and, in some cases, led to the obliteration of xylem vascular elements, causing complete disorganization of the central cylinder (Figure 4D). These conditions were not observed in the roots of plants not inoculated with the nematode, since their cells presented organized and mononucleated architecture (Figures 4A; 4B). The natural presence of phenolic compounds in the roots of BPF was observed (Figure 4A); however, the concentration of these compounds became higher after infection with *M. incognita* (Figure 4E).

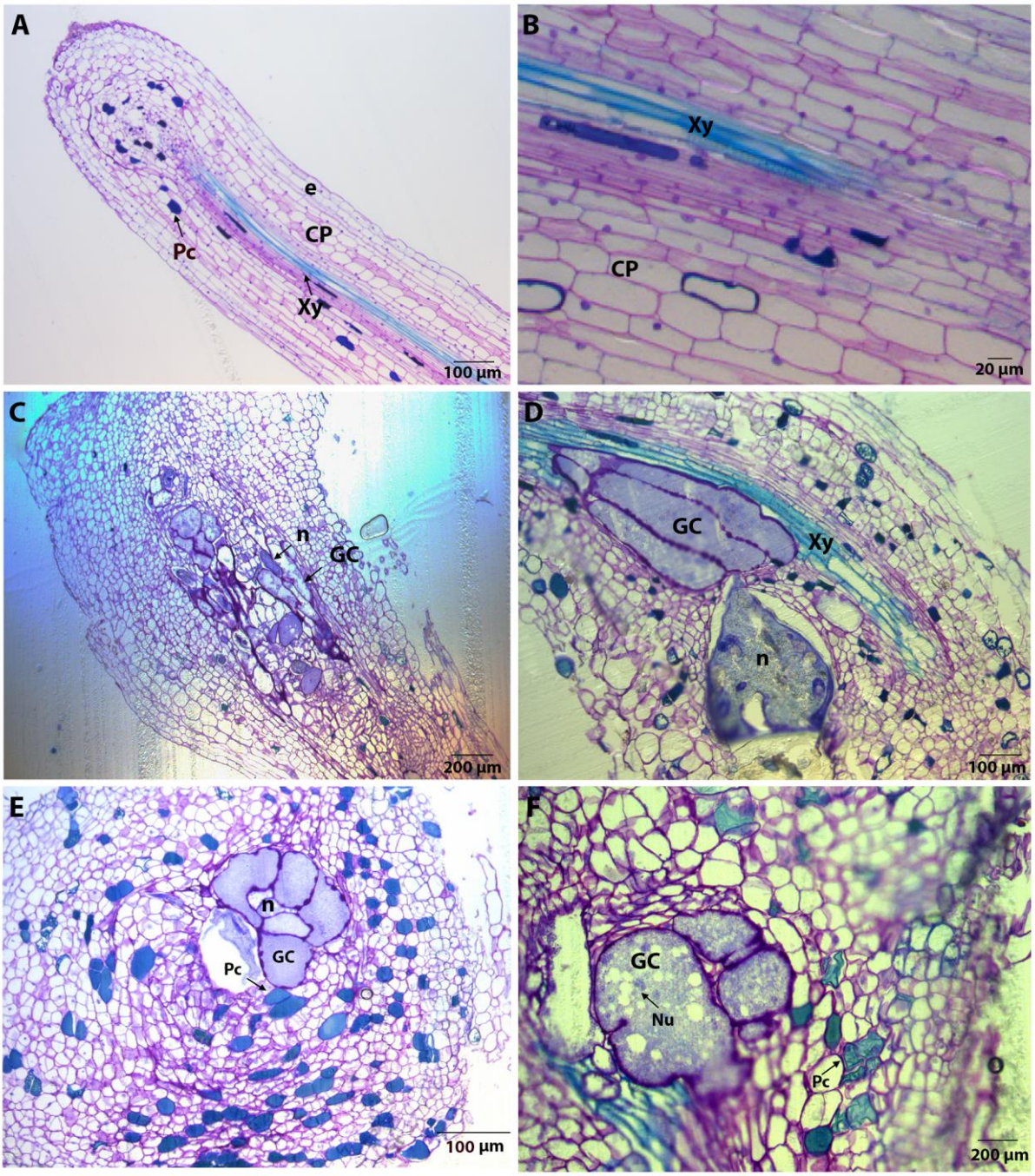

Figure 4. Histological characteristics of *Passiflora nitida* roots. **A. B.** Non-inoculated roots. **C.** Several giant cells formed near the stele region. **D.** Adult female and giant cells causing visible compression of the xylem vascular elements. **E.** High concentration of phenolic compounds is evidenced by the arrow. **F.** Cluster of multinucleated giant cells. CP: cortical parenchyma; e: epidermis; GC: giant cells; n: nematode; Nu: nucleus; Pc: phenolic compounds; Xy: xylem.

2.4. Discussion

Rocha et al. (2021) studied the reaction of ten passion fruit species, among them BPF, to the complex SRRN and *F. solani*. All six replicates inoculated with SRRN race 3 or the complex nematode + fungus died during the experimental period of 75 days. Therefore, the

nematode reproduction could not be assessed. Conversely, replicates inoculated with the fungus but not with the nematode survived, suggesting that BPF is resistant to *F. solani* but so intolerant to SRRN that all infected plants died. Despite the differences between Rocha et al. (2021) and the present work, namely the Pi inoculated (5,000 *versus* 10,000), the plant size at inoculation (3-6 leaves *versus* 3-4 / 5-6 leaves) and the effect on plants (death *versus* growth reduction), both are concurring the pathogenic potential of *M. incognita* to BPF, demonstrating that SRRN may be considered a major BPF pathogen. Some reports claimed that BPF is a promising rootstock for sour passion fruit aiming to control wilt and collar rot caused by *Fusarium* spp. However, its use, as rootstock or even main culture, should be avoided in fields infested with this nematode.

The present study presents, for the first time, a detailed description of the histopathological changes induced by *M. incognita* in BPF, which confirms the susceptibility of this *Passiflora* species to this nematode. The changes observed in plant roots reveal an adaptive response of the root system of BPF against infestation *by M. incognita*, characterized by the formation of feeding sites aimed at absorbing nutrients and supporting the development of this nematode.

The alterations observed in the anatomy of BPF roots, induced by *M. incognita*, constitute substantial evidence of the adverse effects caused by this infestation, causing damage to the integrity and normal functioning of the plant's root system. These changes compromise the effectiveness of the vascular system, as they negatively affect the transport of water and nutrients, which, in turn, has consequences on plant growth and development (Asmus et al., 2000). In the study carried out by Zucareli et al. (2020) with *P. alata*, *P. edulis*, *P. giberti* and *P. cincinnata*, it was observed that *M. incognita* caused more evident anatomical alterations in the roots of the last two species; thus, the presence of giant cells in the region of the central cylinder was observed, causing compression and partial obstructions of the xylem. In BPF, similar phenomena were also observed, corroborating these findings.

The accumulation of phenolic compounds observed in BPF roots infected with *M. incognita* is considered a host defense reaction to pathogen invasion (Lopes et al., 2020). However, information about the nature of such compounds, as well as the potential role of these compounds in plant defense is scarce.

However, it is essential to emphasize that additional studies are needed to deepen the understanding of biochemical responses triggered by BPF in response to infestation by *M. incognita*. Investigations in this direction can help identify possible resistance pathways or

management strategies that can minimize the adverse effects caused by this nematode in this plant species.

Therefore, it is evident that *M. incognita* represents a real threat to BPF cultivation, as some phytonematodes, namely the reniform-nematode (*Rotylenchulus reniformis* Linford & Oliveira 1940), the root-knot nematodes (*Meloidogyne* spp.) and the spiral nematode (*Helicotylenchus dihystera*) are widespread in Brazilian passion fruit orchards (Miguel-Wruck et al., 2021).

The Southern root-knot nematode does not hold the status of a pathogen of relevance for the sour passion fruit plant (Silva & Inomoto, 2022); thus, it would not pose a concern for sour passion fruit plants grown under ungrafted conditions. However, the presence of this nematode would become a problematic issue for sour passion fruit plants grafted onto BPF, given that *M. incognita* is highly pathogenic to BPF, inducing root galls and inhibiting shoot growth.

Furthermore, the susceptibility of wild passion fruit species to the phytonematodes more prevalent in passion fruit orchards (*Meloidogyne* spp., *R. reniformis* and *H. dihystera*) should be more intensively investigated.

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3. ASSESSING THE INTERACTION BETWEEN *Meloidogyne incognita* AND *Fusarium oxysporum* ON PASSION FRUIT

Abstract
Despite *Passiflora nitida* presenting resistance to *Fusarium oxysporum* f. sp. *passiflorae* (FOP), it is highly susceptible to nematodes, including *Meloidogyne incognita*. This study aims to assess and characterize the interaction between these two pathogens in *P. edulis* f. *flavicarpa* seedlings grafted onto *P. nitida*. As such, these grafted seedlings were subjected to individual and combined parasitism by FOP and *M. incognita* under controlled conditions, while non-inoculated plants were used as controls in experiments. The pathogenicity of both pathogens was evaluated based on variables related to plant growth, fungal incidence, size of lesions caused by the fungus, and nematode population density. Our results suggest a neutral interaction between these two pathogens, as regardless of presence of the fungus, *P. nitida* demonstrated high susceptibility to *M. incognita*, resulting in the formation of galls in the root system and plant growth reduction. Additionally, it was observed that although FOP caused lesions in the plant's collar region, the size of lesions did not increase due to co-infection with the nematode. It is noteworthy that the resistance of *P. nitida* to FOP was maintained, even in the presence of the nematode. These findings have important implications for the use of *P. nitida* as a rootstock in FOP management.

Keywords: *Passiflora nitida*, *Fusarium*, *Meloidogyne incognita*, interaction, pathogenicity, *P. edulis*, passion fruit.

3.1. Introduction

Passiflora nitida Kunth is one of the seventy passion fruit species recognized for the production of edible fruits (Coppens d'Eeckenbrugge et al., 2001; Cunha et al., 2002). In Colombia, this species is part of the passion fruit production chain; however, in Brazil, only species sweet passion fruit (*P. alata* Curtis) and sour passion fruit (*P. edulis* Sims) are grown on large commercial scale. Sour passion fruit (*P. edulis* f. *flavicarpa* Deg.) is the most cultivated species, as it occupies about 90% of the area destined to passion fruit cultivation in the country (Faleiro et al., 2019; Meletti et al., 2005). Despite its limited use in passion fruit crops in Brazil, *P. nitida* has notable potential to be used in breeding programs that include interspecific hybridization or as rootstock with the objective of controlling soil-associated phytopathogenic fungi (Junqueira et al., 2005; Pereira et al., 2019; Preisigke et al., 2017).

Among phytopathogenic fungi that *P. nitida* shows resistance, *Fusarium oxysporum* f. sp. *passiflorae* W.L. Gordon 1954 (FOP) stands out, as it is a pathogen widely reported in areas of sour passion fruit cultivation (Aiello et al., 2021). The symptoms of FOP infection can vary according to plant age, host variety and stage of the disease. Usually, the first signs of infection are wilting and yellowing of lower leaves, which gradually spread to upper leaves. As the

disease progresses, lesions spread to the stem, leading to obstruction of water and nutrient-conducting vessels, causing wilting and tissue death. In cases of severe attack, entire plants may wilt and die (Fischer et al., 2010; Fischer & Rezende, 2008; Melo et al., 2020).

Although *P. nitida* is resistant to FOP, it is considered a species highly susceptible to nematodes, such as the reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira 1940) and especially the root-knot nematode - *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 (Rocha et al., 2013; Sharma et al., 2005). Infection caused by *M. incognita* in this nematode species was studied by Rocha et al. (2013), where after 90 days, typical galls were observed on the root system of inoculated plants and the nematode population reproduced 15.7 times during this period.

The development of a disease in crop field is intrinsically linked to a complex interrelation between host, pathogen and the prevailing environmental conditions. In nature, it is uncommon, if not non-existent, for plants to be exposed to a single potential pathogen. This fact is especially true for pathogens found in soil, since there is a wide range of possible interactions with other microorganisms that occupy the same ecological niche (Back et al., 2002).

When the interaction between fungus and nematode is analyzed based on the symptomatology and observed damage, it is possible to characterize it in three different ways: synergistic, antagonistic and/or neutral interaction. In the synergistic interaction, there is enhancement of symptoms and/or damage caused by involved pathogens. This means that the presence of one pathogen can increase the activity or reproduction of the other pathogen, resulting in intensification of observed symptoms or damage. This synergistic interaction can be especially harmful to the host plant, as pathogens act together, increasing the incidence or severity of the disease. On the other hand, in the antagonistic interaction, there is reduction of symptoms and/or damage caused by involved pathogens, which means that the presence of one pathogen can inhibit or suppress the activity or reproduction of the other pathogen, resulting in decrease of observed symptoms or damage. This antagonistic interaction can be beneficial to the host plant, as it reduces the negative impact of pathogens, while in the neutral interaction, there is no enhancement of symptoms and/or damage caused by involved pathogens, which means that the presence of a pathogen does not increase the symptoms or damage caused by the other pathogen. Neutral interaction implies that pathogens act independently without significantly influencing each other (Aghale et al., 2017; Back et al., 2002; Mangeiro et al., 2022).

Studies have shown that there is higher incidence of wilts caused by *Fusarium* in the presence of *Meloidogyne* spp. associated with the root system; thus, the processes of penetration and establishment of the nematode feeding site help the fungus to establish in the tissues of the host plant (Francl & Wheeler, 1993). Thus, individually, these pathogens may have a limited impact, but the synergistic interaction between them results in more pronounced harmful effects (Jeffers & Roberts, 1993). This type of interaction has been reported in: coffee *x M. arabicida x F. oxysporum* (Bertrand et al., 2000); tomato *x Meloidogyne* spp. *x F. oxysporum* (Kassie, 2019); lentil *x M. javanica x F. oxysporum* (De et al., 2001); cotton *x M. incognita x Thielaviopsis basicola* (Walker et al., 2000) and passion fruit *x M. incognita x F. solani* (Fischer et al., 2010).

The first documented record of synergistic interaction between nematodes and fungi was performed by Atkinson (1892), who observed that the incidence of *Fusarium* wilt in cotton (caused by the fungus *F. oxysporum* f. sp. *vasinfectum*) was higher in the presence of root-knot nematodes (*Meloidogyne* spp.). Additional studies confirmed this interaction in field experiments in which soil was sterilized using ethylene dibromide or 1,3-dichloropropene. Under these conditions, the incidence of wilting in cotton plants was significantly reduced. Given that these chemicals have limited fungicidal activity, it could be inferred that they indirectly reduce infection by pathogens by decreasing nematode populations with which they interact (Newson & Martin, 1953; Smith, 1948).

Understanding the mechanisms involved with antagonistic interactions between specific fungi and nematodes present in the rhizosphere of plants offers a promising perspective for the development of plant defense strategies against pathogens present in the soil, such as *Meloidogyne* spp. (Sreenayana et al., 2022), which interactions can provide an effective control strategy (Kerry, 2000).

On the other hand, neutral interactions between pathogens occur when they colonize different plant tissues or regions, without causing significant physiological changes that indirectly affect the other pathogen. In this case, these pathogens coexist without positively or negatively affecting the associated culture. In recent study, Mangeiro et al. (2022) provided concrete evidence of neutral interactions between *Meloidogyne* species and soil fungi in passion fruit. These results contradict previous claims of synergy between plant nematodes and soil fungi in this specific crop. This finding has significant implications, as it challenges previous assumptions that the combined presence of the nematode and soil fungi would decrease passion fruit productivity.

Due to the potential of *P. nitida* to reduce the mortality of passion fruit plants due to their resistance to FOP (Ferreira et al., 2023; Miguel-Wruck et al., 2021), there is great interest in using this passion fruit species on a large scale as rootstock in the management of FOP. However, there are no studies demonstrating whether the resistance to FOP is maintained, since the crop field is infested with root-knot nematodes. Therefore, the aim of this study was to determine and characterize the interaction between *M. incognita* and FOP in *P. nitida*.

3.2. Material and Methods

Trials were carried out from February 2022 to September 2022 (trial 1) and from September to April 2023 (trial 2) under laboratory conditions and greenhouse conditions.

3.2.1. Interaction plant-nematode-fungus

3.2.1.1. Plant materials

P. nitida cuttings were obtained from propagation material supplied by "Embrapa Cerrados". These cuttings were collected from the middle region of branches of plant aged approximately 1 year to be used as rootstocks. To promote rooting, cuttings were immersed for 5 minutes in rooting solution (Forth Enraizador®) and then distributed in polyethylene trays with dimensions of 29x39.5x7cm (with capacity of 5000 cm3) filled with medium expanded vermiculite. The rooting process lasted about 30 days and was conducted under greenhouse conditions, where intermittent nebulization system was implemented.

Sour passion fruit (*P. edulis* cv. Gigante Amarelo) seeds, also supplied by "Embrapa Cerrados", were sown in polyethylene trays (29x39.5x7cm) containing autoclaved sandy loam soil (at 121ºC for 2 h). Thirty days after sowing, top cleft grafting of sour passion fruit seedlings was performed on rooted *P. nitida* cuttings, according to method proposed by Santos et al. (2011). These grafted plants were then transplanted into plastic pots previously filled with 500cm³ of autoclaved sandy-loam soil and remained in greenhouse for 30 days. Subsequently, they were distributed in one of the following treatments: (I) plants inoculated with *M. incognita* only; (II) plants inoculated with the nematode and, 60 days later, with FOP; (III) plants inoculated with FOP only and (IV) non-inoculated plants (negative control). The procedures described below were the same for both trials.

3.2.1.2. Inoculum preparation and inoculation

The *M. incognita* MT isolate inoculum, previously identified according to Silva & Inomoto (2023), consisted of eggs and second-stage juveniles (J2). These specimens were obtained from a pure population of the nematode maintained in corn roots for 60 days (*Zea mays*) under greenhouse conditions.

To obtain the nematode inoculum, corn roots were cleaned under running water and ground in domestic blender, along with 1% sodium hypochlorite solution, at low speed for 1 minute. Then, the mixture was carefully poured through a sequence of three overlapping sieves (60 Mesh / 200 Mesh / 500 Mesh) to remove excess sodium hypochlorite using tap water. The material retained on the 500 Mesh sieve was collected in beaker and calibrated with the aid of Peters counting slide under light microscope at 100x magnification.

After 30 days of transplanting the grafted seedlings, the nematode was inoculated in treatments I and II, where two 2-cm deep holes were performed close to the plant stem, in which 2,000 eggs + J2 were applied. These plants remained in greenhouse for 60 days.

The FOP isolate was collected from an area of passion fruit cultivation with plants showing symptoms of wilting in the municipality of Livramento de Nossa Senhora, Bahia, Brazil, which was provided by "Embrapa Mandioca e Fruticultura". To recover the isolate pathogenicity, it was inoculated into a sour passion fruit plant and, subsequently, this fungus was indirectly isolated.

In order to prepare the FOP inoculum, the recovered isolate was cultivated in plastic Petri dishes containing PDA at temperature of 25°C for 7 days. Then, to stimulate sporulation, FOP was transferred to Petri dishes containing Spezieller Nährstoffarmer Agar (SNA) and incubated at constant temperature of 25ºC for 14 days, with 12-hour photoperiod.

The spore suspension was prepared minutes before inoculation, according to protocol described by Silva et al. (2013). After preparation, the suspension concentration was adjusted to 10^6 macroconidia and/or chlamydospores/mL. Plants belonging to treatments II and III were inoculated with FOP 90 days after transplanting. Thus, seedlings were removed from the sterile soil where they were sown, and their roots were carefully washed with sterile water. Subsequently, the root system was immersed in the spore suspension for 5 minutes and then transplanted into plastic pots (12cm in diameter x 15cm in height) previously filled with 1,500 cm^3 of autoclaved sandy-loam soil. Plants from treatments I and IV were submitted to the same procedures, except that their roots were only soaked in sterile water. In both trials, all plants remained in greenhouse for another 100 days. Plants were daily irrigated with tap water, and

fertilization was carried out every two weeks using 3g of Osmocote® (N-P-K: 15-9-12). It was not necessary to carry out pest control during the experimental period.

3.2.1.3. Disease assessment

At the end of trials, the pathogenicity of *M. incognita* and FOP was evaluated based on several variables: (i) dry aerial part weight (DAPW), in g; shoot height (SH), in cm; and root fresh weight (RFW), in g; (ii) incidence of symptoms in plant shoot (Wilting Index-WI) according to the scale of scores modified from that proposed by Abawi & Barker (1984). In this scale, score 0 represents plant without wilting, score 1 indicates one or two wilted leaves, score 2 indicates that half of leaves are wilted and the beginning of yellowing, score 3 indicates that ¾ of leaves are wilted, yellowed and the beginning of necrosis, score 4 indicates that all leaves are wilted and the beginning of senescence of lower leaves, and score 5 represents dead plant; (iii) lesion length (LL) from the base of the plant, in cm and (iv) total nematodes recovered from roots (Nem).

In order to confirm Koch's postulate (1876), indirect FOP isolation was performed. For this, stem fragments were collected from the bordering area between healthy tissues and those with lesions close to the stem and roots of plants inoculated with *M. incognita* and FOP (treatment II) and with FOP only (treatment III). These fragments were disinfected in 70% alcohol for 30 seconds and then placed in 0.5% sodium hypochlorite solution for 15 seconds and rinsed three times in ADE. Subsequently, fragments were transferred to Petri dishes containing agar-water medium. After 7 days, colonies were morphologically analyzed to confirm FOP re-isolation.

The nematode population in roots was extracted according to the previously mentioned extraction method and the quantification of eggs and/or J2 was performed in Peters slide under optical microscope to determine the total amount of nematode recovered from roots (Nem).

3.2.1.4. Statistical analysis

Data were conducted in a completely randomized design (CRD): trial 1 with four treatments: (i) inoculation with *M. incognita* only; (ii) inoculation with *M. incognita* and, 60 days later, with FOP; (iii) inoculation with FOP only; and (iv) uninoculated control (negative control) and 8-9 replicates per treatment; trial 2 with the same four treatments, but with 6 replicates per treatment.

Plant growth variables (DAPW, SH and RFW), WI, LL and Nem were statistically analyzed through analysis of variance (ANOVA) using RStudio software (version 2022.02.3

Build 492). Means were compared by the Tukey's test at 5% significance level. To meet the assumptions of data normality, data obtained in trial 1 (DAPW, Nem and WI) and trial 2 (Nem) were transformed by applying the formula [$\sqrt[3]{(x+1)}$] before statistical analysis. Graphs representing plant growth variables were elaborated using Sigma Plot software (version 12.5).

3.3. Results

In trial 1, the nematode, both alone and in association with FOP, was found to have significant negative effect ($p < 0,05$) on DAPW and SH of passion fruit plants (Figure 1), which indicates that the presence of *M. incognita*, either alone or in association with FOP, resulted in statistically significant reduction in plant shoot growth and development (Figure 2A). On the other hand, the Tukey's test did not show significant differences ($p > 0,05$) between treatments for the RFW variable, as also shown in Figure 1. This suggests that, in terms of fresh root mass development rate, there was no statistically significant difference between treatments. However, it was observed that in the presence of *M. incognita* and FOP, plant roots showed less volume and many galls compared to control treatment (Figure 2B).

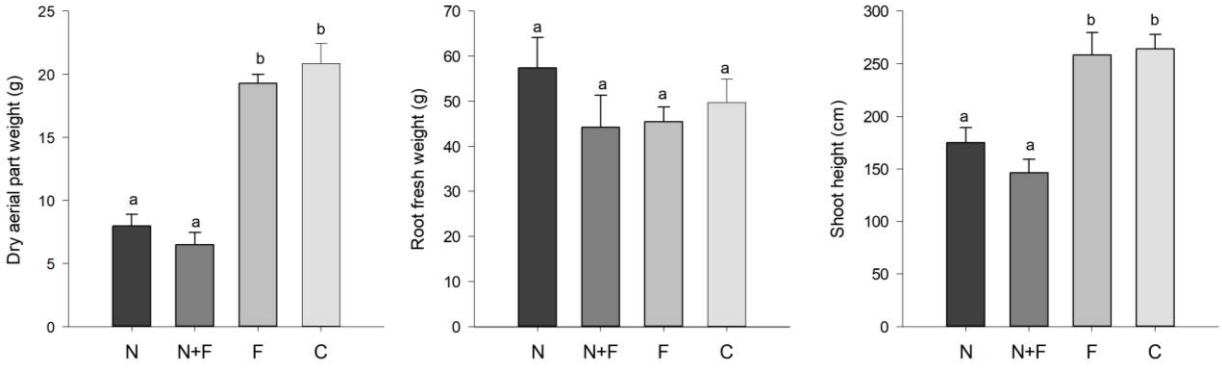

Figure 1. Analysis of the main effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* -FOP (0315 isolate) and their interaction on the growth parameters of sour passion fruit (*Passiflora edulis* f. *flavicarpa*) seedlings grafted onto *P. nitida* under greenhouse conditions (trial 1). N: plants inoculated with *M. incognita* only; N + F: plants inoculated with the nematode and, 60 days later, with FOP; F: plants inoculated with FOP only and e C: non-inoculated plants (negative control). Each value is the mean of 8-9 replicates. Means followed by diferent letter did not difer according to the Tukey test at 5% signifcance.

Figure 2. Evaluation of the effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* – FOP (0315 isolate) both alone and in combination (trial 1). **A.** Behavior of shoots of sour passion fruit (*Passiflora edulis* f. *flavicarpa*) plants grafted onto *P. nitida* at the end of the trial. **B.** Behavior of the root system of sour passion fruit plants grafted onto *P. nitida* at the end of the trial. N: plants inoculated with *M. incognita* only; N + F: plants inoculated with the nematode and, 60 days later, with FOP; F: plants inoculated with FOP only and e C: non-inoculated plants (negative control).

In this trial, when evaluating WI based on the scale of scores modified from that proposed by Abawi & Barker (1984), it was observed that there was no difference between the incidence of symptoms in plant shoots induced by FOP alone or in association with *M. incognita*. No plant death was observed after FOP inoculation.

FOP injured the stem region of all plants in trial 1, which ascended the stem by up to 50 mm, never exceeding the grafting point. The vascular tissue and the stem cortex region of plants inoculated with FOP showed reddish-brown lesions (Figure 3D), as described by Rooney-

Latham & Blomquist (2011). However, no statistical difference was observed in relation to the size of lesions produced by FOP in plants belonging to treatments II and III.

Figure 3. Evaluation of the pathogenicity of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* - FOP (0315 isolate) in combination. (trial 1) **A.** Healthy plant root system. **B.** *Meloidogyne incognita* induced gall symptoms and FOP-induced internal stem spot. **C.** Approximation of the plant root shown in B, emphasizing the coalescence of these galls, forming more elongated thickenings. **D.** Approximation of the plant root shown in B, emphasizing the infected stem with reddish-brown discoloration.

M. incognita reproduction was not affected by co-parasitism with FOP ($p > 0,05$), but the rootstock used (*P. nitida*) was highly susceptible to *M. incognita*, with root population densities for treatments I (366,186 specimens) and II (233,721 specimens).

In trial 2, DAPW was found to be significantly reduced ($p < 0,05$) in the presence of *M. incognita* alone or in association with FOP. However, SH did not show significant variation between treatments. Furthermore, it was observed that plants belonging to control treatment, that is, those that were not inoculated with any of the pathogens, had lower RFW compared to plants inoculated with *M. incognita* and FOP (as shown in Figure 4). This difference can be attributed to the presence of galls on the root system of plants inoculated with the two pathogens, as shown in Figure 5B.

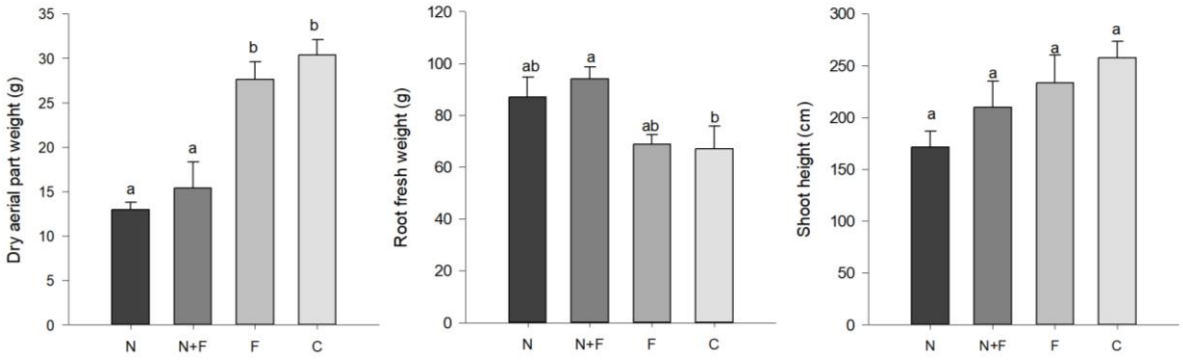

Figure 4. Analysis of the main effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* -FOP (0315 isolate) and their interaction on the growth parameters of sour passion fruit (*Passiflora edulis* f. *flavicarpa*) seedlings grafted onto *P. nitida* under greenhouse conditions (trial 2). N: plants inoculated with *M. incognita* only; N + F: plants inoculated with the nematode and, 60 days later, with FOP; F: plants inoculated with FOP only and e C: non-inoculated plants (negative control). Each value is the mean of six replicates. Means followed by diferent letter did not difer according to the Tukey test at 5% signifcance.

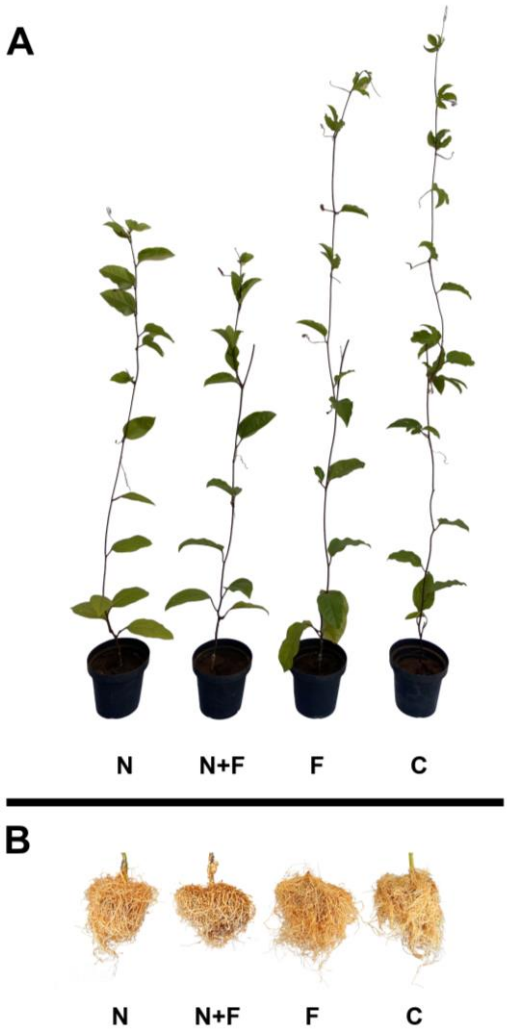

Figure 5. Evaluation of the effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *passiflorae* – FOP (0315 isolate) both alone and in combination (trial 2). **A.** Behavior of shoots of sour passion fruit (*Passiflora edulis* f. *flavicarpa*) plants grafted onto *P. nitida* at the end of

the trial. **B.** Behavior of the root system of sour passion fruit plants grafted onto *P. nitida* at the end of the trial. N: plants inoculated with *M. incognita* only; N + F: plants inoculated with the nematode and, 60 days later, with FOP; F: plants inoculated with FOP only and e C: non-inoculated plants (negative control).

When evaluating the wilting index (WI) of plants in trial 2, it was observed that all plants in treatments II and III had one or two wilted leaves, receiving score 1 according to the scale of scores modified from that proposed by Abawi & Barker (1984). This indicates that the presence of *M. incognita* and FOP caused some level of damage to plants, but no plant died during the experimental period, as well as in trial 1. It was found that FOP was able to cause lesions in the vascular tissue and in the root cortex of plants inoculated with this fungus alone or in association with the nematode. However, there was no variation in the size of lesions between treatments, which means that the presence of FOP affected plant tissue in a similar way, regardless of presence of the nematode.

As in the first trial, the results of this trial showed that *M. incognita* reproduction is not affected by the presence of FOP, which means that the presence of the fungus had no significant impact on nematode reproduction. However, the rootstock used, *P. nitida*, proved to be highly susceptible to *M. incognita*. Population densities were observed in the roots of 756,777 specimens in treatment I and 254,229 specimens in treatment II.

When reisolating FOP from stem and root fragments of plants inoculated separately with the fungus and those inoculated with the nematode and the fungus, pink colonies with cottony aerial mycelium were observed. In addition, the presence of globose chlamydospores (8,0-12,0 x 7,0-11,0 µm), multiseptate elliptical macroconidia (31-34 x 5,0-6,0 µm) and elliptical microconidia (5,0-8,0 x 2,5-4,0 µm) was observed. These characteristics are typical of FOP and indicate the presence of the fungus in the fragments of plants inoculated in trials 1 and 2 (Zheng et al., 1992).

3.4. Discussion

The results obtained in this study corroborate the findings of Mangeiro et al. (2022), who also observed neutral interactions between *M. incognita* and FOP. However, the passion fruit species used by these authors, *P. edulis* (cultivar UENF Rio Dourado), showed resistance to *M. incognita*, with maximum nematode reproduction factor (RF) value of 0.44. In contrast, this study revealed high average population density of *M. incognita* in *P. nitida* roots at the end of trials, regardless of presence of FOP. The infection of this nematode in this passion fruit species was characterized by the formation of galls of variable size and irregular shape along

the entire root system. As the infection progressed, these root galls coalesced, forming more elongated thickenings. These changes in the root system significantly compromised the plant's ability to absorb water and essential nutrients, affecting shoot development. This evidence the remarkable susceptibility of *P. nitida* to *M. incognita*, as also observed in the study by Rocha et al. (2021). In the experiment conducted by these authors, all *P. nitida* plants that were inoculated with *M. incognita* did not survive.

Under field conditions, Miguel-Wruck et al. (2021) evaluated the mortality rates and susceptibility of seedlings of a commercial sour passion fruit (*P. edulis* f. *flavicarpa* cv. Gigante Amarelo) grafted onto *P. nitida*. The experiment was conducted in two different experimental areas with history of fusariosis, called areas A and B. The results showed that in area A, the presence of *R. reniformis* was identified, while in area B, both *R. reniformis* and *Meloidogyne* spp. were identified. At the end of the experiment, the plant mortality rate was 6.25% in area A and 12.5% in area B. According to classification proposed in the study, in both areas, *P. nitida* rootstock was considered moderately resistant to fusariosis. The presence of *Meloidogyne* spp. in area B it did not affect the resistance of *P. nitida* to FOP, result similar to those observed in trials previously carried out.

In our study, although the fungus caused lesions in the stem region of the plant, the infection did not progress beyond the grafting point; therefore, no reflex symptoms induced by the fungus were observed in the shoots of inoculated plants in both trials. This is justified by the fact that *P. nitida* has proven resistance to FOP (Junqueira et al., 2005; Pereira et al., 2019; Preisigke et al., 2017).

The interaction between nematodes and phytopathogenic fungi, especially fungi that cause vascular wilting, is quite common (Bertrand et al., 2000; De et al., 2000; Kassie, 2019). However, in most cases, nematodes are not essential for the invasion, establishment and development of the disease caused by the fungus, especially in interactions that occur in plant roots. Instead, plant parasitic nematodes play an auxiliary role, increasing host susceptibility and consequently accelerating development and aggravating the severity of the disease caused by fungal pathogens (Khan, 1993). However, in this study, it was found that co-parasitism did not increase the susceptibility of *P. nitida* to FOP or *M. incognita*, so there was no additional impact on plant growth variables. Furthermore, there was no increase in FOP-induced lesions or in the incidence of plant wilt, as well as no increase in the nematode multiplication rate.

The susceptibility of *P. nitida* to *M. incognita* and the apparent damage caused by this nematode highlight the importance of considering this aspect when developing FOP-resistant cultivars in passion fruit breeding programs. Although the interaction between FOP and *M.*

incognita in this study was classified as neutral, it is crucial to be aware of the susceptibility of this species to the nematode to avoid future problems and ensure high productivity of the passion fruit crop.

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