Research Article

Meloidogyne incognita reduces the *Passiflora nitida* growth[1]

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ABSTRACT

Breath passion fruit (*Passiflora nitida*) has been used as rootstock for sour passion fruit (*P. edulis*) in order to control wilting and collar rot caused by *Fusarium* spp. However, it is supposedly susceptible to 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 a *P. nitida* accession, and a third one was performed to assess the effect on the growth of *P. edulis* grafted onto *P. nitida* and the anatomical changes induced by this nematode in *P. nitida*. In view of the high multiplication rate of this nematode in *P. nitida*, causing intense root galling and reducing plant growth of both *P. nitida* and *P. edulis*, *P. nitida* should not be used as rootstock, or even main crop, in *M. incognita*-infested fields.

KEYWORDS: *Passiflora edulis*, *Fusarium*, breath passion fruit, sour passion fruit, root-knot nematode.

RESUMO

Meloidogyne incognita reduz o crescimento de *Passiflora nitida*

O maracujá-suspiro (*Passiflora nitida*) tem sido utilizado como porta-enxerto para o maracujá-azedo (*P. edulis*), com o objetivo de controlar a murcha e a podridão do colo causadas por *Fusarium* spp. No entanto, acredita-se que ele seja suscetível ao nematoide-das-galhas (*Meloidogyne incognita*), que está amplamente distribuído em campos de cultivo tropical. Foram realizados dois ensaios para avaliar o efeito de *M. incognita* no crescimento de um acesso de *P. nitida*, e um terceiro ensaio foi conduzido para avaliar o efeito no crescimento de *P. edulis* enxertado em *P. nitida* e as alterações anatômicas induzidas por esse nematoide em *P. nitida*. Em vista da alta taxa de multiplicação desse nematoide em *P. nitida*, causando galhas intensas nas raízes e reduzindo o crescimento das plantas, tanto de *P. nitida* quanto de *P. edulis*, *P. nitida* não deve ser usado como porta-enxerto, ou mesmo cultura principal, em campos infestados por *M. incognita*.

PALAVRAS-CHAVE: *Passiflora edulis*, *Fusarium*, maracujá-suspiro, maracujá-azedo, nematoide-das-galhas.

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 *Passiflora* species are known, and most of them may be economically valuable due to their culinary, medicinal or ornamental properties (Faleiro et al. 2019). Breath passion fruit (*P. nitida* Kunth), also known as 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, *P. nitida* presents a high potential for fresh consumption. Furthermore, in recent years, *P. nitida* is an ever-increasing interest species due to its resistance to Fusarium wilt and collar rot (Preisigke et al. 2017, Carvalho et al. 2020, Miguel-Wruck et al. 2021, Rocha et al. 2021).

Fusarium wilt, caused by the *Fusarium oxysporum* f. sp. *passiflorae* W. L. Gordon 1954 fungus, and collar rot, caused by *F. solani* (Mart.) Sacc. 1881, are major *P. edulis* 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, Rocha et al. 2021, Pires et al. 2022). Generally, once pathogens are established in a crop

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field, the area should no longer be cultivated with *P. edulis*.

Grafting *P. edulis* onto *P. nitida* for *Fusarium* spp. control has been intensively studied in Brazil as a promising strategy (Junqueira et al. 2006, Miguel-Wruck et al. 2021). A noteworthy example is that of producers in the northern Mato Grosso state, especially in the municipality of Terra Nova do Norte. Until 2009, there were more than 300 *P. nitida* producers, of which, in the following two years, 90 % abandoned their activity due to Fusarium wilt. The interest in the use of *P. nitida* occurred due to the observation of spontaneous and healthy *P. nitida* plants in places infested by *F. oxysporum* (Faria 2019). However, *P. nitida* is possibly susceptible to the root-knot nematode *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949.

Two greenhouse trials have found conflicting results on the susceptibility of *P. nitida* to *M. incognita*. Although galls and egg masses were observed on *P. nitida* roots inoculated with 2,200 specimens of a *M. incognita* population isolated from *Passiflora capsularis*, the nematode population density decreased 90 % at the end of 62 days (Castro et al. 2010). Conversely, the *M. incognita* population density increased 12x at 50 days after the inoculation of 5,000 eggs (Sharma et al. 2005), and 15.7x at 90 days after the inoculation of 3,000 eggs (Rocha et al. 2013). Additionally, in a further experiment, all *P. nitida* plants inoculated with *M. incognita* race 3 (n = 6) died during the experimental period of 180 days, supposedly due to nematode infection (Rocha et al. 2021). However, non-infected control plants were not included, as the main objective was to evaluate the interaction between *F. solani* and *M. incognita*.

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

This study was carried out to evaluate the effect of *M. incognita* on the growth of *P. nitida* plants and *P. edulis* grafted onto *P. nitida*. There is no histological documentation on alterations caused by infection of *Meloidogyne* species in *P. nitida*; therefore, another objective was to gather information about which histopathological changes are induced by this nematode in this plant species.

MATERIAL AND METHODS

Two pot trials with *Passiflora nitida* plantlets were carried out from December 2020 to July 2021, under laboratory conditions, at the Universidade de São Paulo, São Paulo state, Brazil. *M. incognita* isolate was collected from cotton (*Gossypium hirsutum* L.) roots in 2004, at Campo Verde (Mato Grosso state, Brazil) and maintained under greenhouse conditions, alternating cotton, bell pepper, common bean, corn and tomato, in order to preserve its 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).

The inocula 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 min. Then, the resulting suspension was poured through a sequence of three sieves (60, 200 and 500 mesh, corresponding to 0.250, 0.075 and 0.025 mm opening, respectively), removing the sodium hypochlorite with running water. The material retained on the 500-mesh sieve was collected in a beaker and nematodes were counted under a compound light microscope (model CHS, Olympus Optical Co., Ltda., Tokyo Japan) at 100x magnification, with the aid of a Peters' counting slide.

P. nitida seeds of an accession from the northern Mato Grosso state were supplied by the Embrapa Cerrados in 2020. A 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 a tray containing autoclaved sand (121 ºC/2 h). After 72 days, the plantlets were transplanted into plastic pots (12 cm in diameter x 15 cm in height) previously filled with 1,500 cm^3 of autoclaved sandy loam soil. At 42 days after transplanting, 15 plants with similar size (18-20 cm in 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 of 10,000 specimens by pouring the inoculum into two 2-cm deep holes made close to the plant stem. The plants were kept in a greenhouse for 60 days after inoculation (DAI). During this period, they were daily irrigated with tap

water and received a slow release NPK (15-9-12) fertilizer fortnightly (Osmocote®). Pest or disease control was not necessary.

The following variables were assessed: shoot height, shoot dry weight, root fresh weight, number of nematodes per gram of root and total number of nematodes in the soil. The nematodes were recovered from roots by the same procedure used to obtain the inoculum, while the soil samples were processed by the sucrose flotation method (Jenkins 1964). The trial was repeated once, with smaller plants (12-15 cm in height and 3-4 leaves) and a longer experimental period (92 DAI evaluation).

The trials were carried out in a completely randomized design, with two treatments (inoculated plants; non-inoculated plants) and 7-8 replicates (trial 1) and 7 replicates (trial 2). The data were analyzed with the R package and the mean values compared using the Tukey test at 5 % of significance.

A third trial was carried out with *P. edulis* grafted onto *P. nitida* plants obtained from 10-cm long cuttings treated with rooting solution (Forth Enraizador®) and placed in trays filled with expanded vermiculite for about 30 days, under greenhouse conditions. *P. edulis* 'BRS Gigante Amarelo' ('BRS GA-1') seeds were provided by the Embrapa Cerrados and sown in trays containing autoclaved sandy loam soil. After 30 days, the seedlings were top cleft grafted onto rooted *P. nitida* cuttings. The grafted plants were then transplanted into plastic pots filled with autoclaved sandy loam soil and kept in the greenhouse for 30 days until inoculation. The population density of 10,000 specimens of *M. incognita* caused a very intense reduction of *P. nitida* growth in trials 1 and 2. Therefore, eight plants were inoculated with a lower *M. incognita* density (2,000 eggs + J2) and six were kept free from nematodes. Subsequently, the plants were maintained in the greenhouse for 105 days, with regular irrigation and fertilization.

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

RESULTS AND DISCUSSION

The root-knot nematode reached higher root population densities (1,315,695 specimens) in trial 2 than in trial 1 (183,093 specimens), supposedly due to the longer experimental period. However, soil population densities were very low (Tables 1 and 2).

Thus, the effort to recover *Meloidogyne* specimens from the soil seems to be not suitable for trials aiming to evaluate the root-knot nematode reproduction, in accordance with the authors' previous experience. In both trials, *P. nitida* plants inoculated with *M. incognita* had smaller vines than non-inoculated ones (Figures 1 and 2). The root weight of inoculated plants did not differ from non-inoculated ones (Tables 1 and 2); however, infected

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

Treatments	N	SH (cm)	SDW (g)	RFW (g)[1]	N/g	NS	Pf/Pi
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	18.31
CV (%)	-	37.03	28.66	12.59	-		

* Means followed by the same letter in the column do not differ according to the Tukey test at 5 % of significance. [1] Data transformed using log10 (x + 1) before performing the statistical analysis. N: number of replicates; SH: shoot height; SDW: shoot dry weight; RFW: root fresh weight; N/g: number of nematodes per gram of root; NS: total number of nematodes in the soil; Pf/Pi: reproduction factor.

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

Treatments	N	SH (cm)	SDW (g)	RFW (g)	N/g	NS	Pf/Pi
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	131.57
CV (%)	-	37.84	43.92	33.81	-		

* Means followed by the same letter in the column do not differ according to the Tukey test at 5 % of significance. N: number of replicates; SH: shoot height; SDW: shoot dry weight; RFW: root fresh weight; N/g: number of nematodes per gram of root; NS: total number of nematodes in the soil; Pf/Pi: reproduction factor.

Figure 1. Effect of *Meloidogyne incognita* on *Passiflora nitida*, at 60 days after inoculation, for trial 1. A) Vine of *P. nitida* plant inoculated with 10,000 specimens (left) and non-inoculated (right); B) *P. nitida* roots infected with *M. incognita* (right) showing root galls, peeling and brown coloration, with tissue rot.

Figure 2. Effect of *Meloidogyne incognita* on *Passiflora nitida*, at 92 days after inoculation, for trial 2. A) Vine of *P. nitida* plant inoculated with 10,000 specimens (left) and non-inoculated (right); B) *P. nitida* roots infected with *M. incognita* showing root galls (right).

roots were very short and with numerous galls of variable sizes (Figure 3). In fact, 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), as observed in the *P. nitida* shoots in trials 1 and 2 (Tables 1 and 2; Figures 1 and 2).

P. nitida rootstocks infected with *M. incognita* provided smaller *P. edulis* vines than non-infected ones (Table 3). As in trials 1 and 2, the fresh weight of roots infected with *M. incognita* did not differ from non-infected ones (Table 3); however, infected roots were very short and exhibited numerous galls and hosted high population densities (average of 260,395 specimens per plant).

The root cells of plants not inoculated with the nematode presented an organized and mononucleated architecture (Figures 4A and 4B). A natural presence of phenolic compounds in *P. nitida* roots was observed (Figure 4A); however, the concentration of these compounds became higher after the infection with *M. incognita* (Figure 4E).

The cross-section of *P. nitida* roots inoculated with *M. incognita* shows several histopathological changes. 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 cells resulted in a complete disorganization of the central cylinder (Figure 4D).

P. nitida is undoubtedly a good *M. incognita* host, as previously reported by Sharma et al. (2005) and Rocha et al. (2013), but contrary to reports of Castro et al. (2010). Perhaps this difference is related to *P. nitida* diversity, as it occurs spontaneously in many Brazilian biomes.

Rocha et al. (2021) studied the reaction of ten passion fruit species, among them *P. nitida*, to the *M. incognita* and *F. solani* complex. All six replicates inoculated with *M. incognita* race 3 or the nematode + fungus complex 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 *P. nitida* is resistant to *F. solani*, but intolerant to *M. incognita*, in which all infected plants died. Despite differences between the results obtained by Rocha et al. (2021) and those of the present study, namely the initial population density (5,000 *versus* 10,000 or 2,000), plant size at inoculation (3-6 leaves *versus* 3-4/5-6 leaves/grafted plants) and the effect on plants (death *versus* growth reduction), both are concurring the pathogenic potential of *M. incognita* to *P. nitida*, demonstrating that *M. incognita* may be considered a major *P. nitida* pathogen, reducing

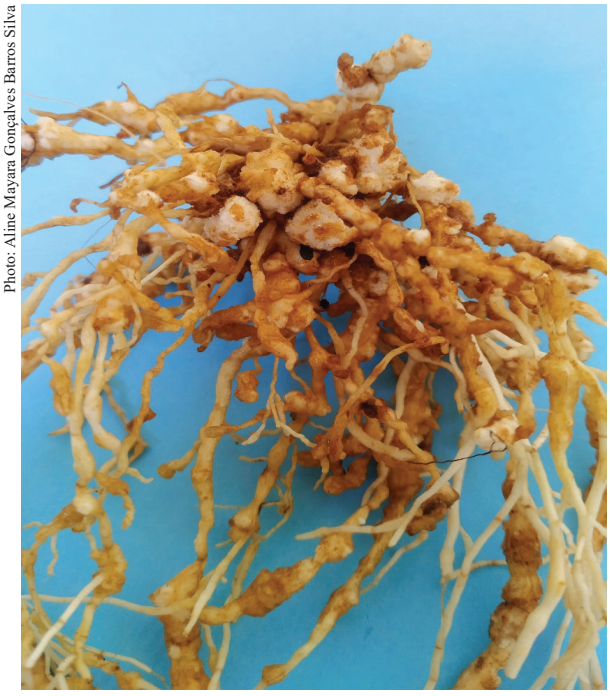

Photo: Aline Mayara Gonçalves Barros Silva

Figure 3. Example of plant inoculated with *Meloidogyne incognita*, emphasizing root galls.

Table 3. Effect of *Meloidogyne incognita* (trial 3) on the growth of *Passiflora edulis* grafted onto *P. nitida* and final nematode density at 105 days after inoculation.

Treatments	N	SH (cm)	SDW (g)	RFW (g)	N/g	Pf/Pi
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	130.19
CV (%)	-	20.25	17.08	17.73	-	-

* Means followed by the same letter in the column do not differ according to the Tukey test at 5 % of significance. N: number of replicates; SH: shoot height; SDW: shoot dry weight; RFW: root fresh weight; N/g: number of nematodes per gram of root; Pf/Pi: reproduction factor.

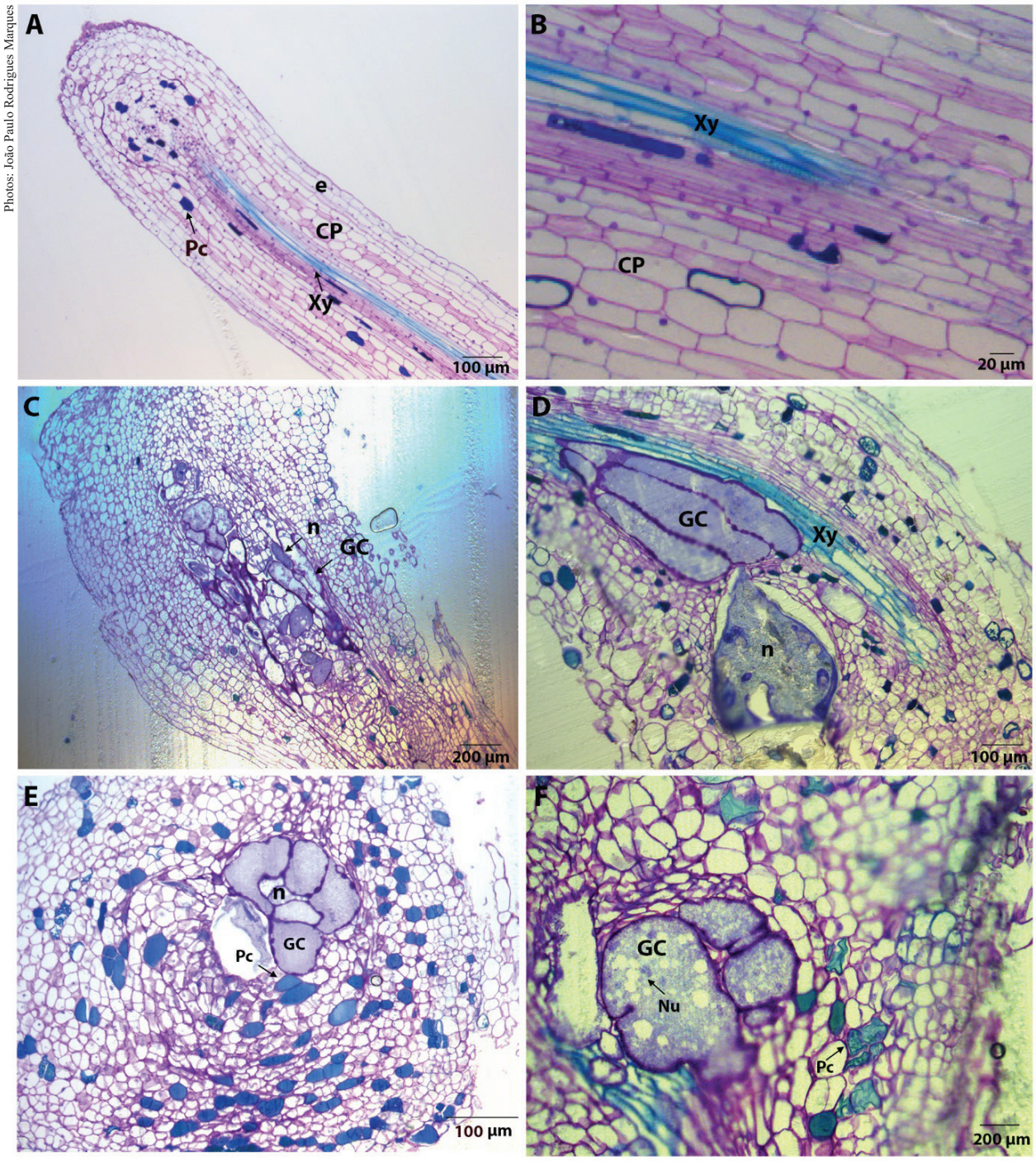

Figure 4. Histological characteristics of *Passiflora nitida* roots. A and B) non-inoculated roots; C) several giant cells formed near the stele region; D) adult female and giant cells causing visible compression of the xylem vascular elements; E) high concentration of phenolic compounds is evidenced 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.

the growth of *P. nitida* plants and *P. edulis* plants grafted onto *P. nitida*. Some reports claimed that *P. nitida* is a promising rootstock for *P. edulis* aiming to control wilt and collar rot caused by *Fusarium* spp. (Junqueira et al. 2006, Faria 2019, Miguel-Wruck et al. 2021). However, its use as rootstock, or even as main crop, 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 *P. nitida*, which confirms the susceptibility of this *Passiflora* species to this nematode. The changes observed in plant roots reveal an adaptive response of the *P. nitida* root system 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 *P. nitida* roots, induced by *M. incognita*, constitute substantial evidence of the adverse effects from 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, have 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 a complete disorganization of the central cylinder. In *P. nitida*, similar phenomena were also observed, corroborating these findings.

The accumulation of phenolic compounds observed in *P. nitida* 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. It is essential to emphasize that additional studies are needed to deepen the understanding of biochemical responses triggered by *P. nitida* in response to infestation by *M. incognita*. Investigations in this direction can help to identify possible resistance pathways or management strategies that can minimize the adverse effects caused by this nematode on this plant species.

The present results evidenced that *M. incognita* represents a real threat to *P. nitida* cultivation, as some phytonematodes, namely reniform-nematode (*Rotylenchulus reniformis* Linford & Oliveira 1940), root-knot nematode (*Meloidogyne* spp.) and spiral nematode (*Helicotylenchus dihystera*), are widespread in Brazilian passion fruit orchards (Miguel-Wruck et al. 2021).

CONCLUSIONS

Meloidogyne incognita does not hold the status of a pathogen of relevance for the *Passiflora edulis* plant; thus, it would not pose a concern for *P. edulis* plants grown under ungrafted conditions. However, the presence of this nematode has become a problematic issue for *P. edulis* plants grafted onto *P. nitida*, given that *M. incognita* is highly pathogenic to *P. nitida*, at least to some accessions of this species, inducing root galls and inhibiting shoot growth.

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